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Tuberculosis and Respiratory Diseases in Paediatric and in Adolescent

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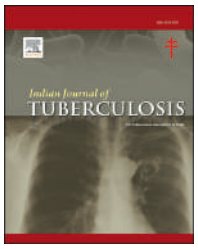

Editorial

Global epidemiologic aspects of tuberculosis in children

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Tuberculosis in children reflects the recent extent of failure of the tuberculosis programs to prevent transmission of *Mycobacterium tuberculosis*. Whether and how swift elimination of tuberculosis can be achieved depends on the magnitude of the reduction of transmission emanating from newly emerging sources of *M. tuberculosis* that program interventions achieve with case finding and chemotherapy. Newly emerging infectious cases have the potential to transmit tubercle bacilli to any person in a community and thus potentially creating secondary cases. One cannot be certain whether a new tuberculosis case in an adult is the result of direct progression from recent transmission or that of reactivation of a remotely acquired infection. In contrast, a new tuberculosis case in a child under the age of 5 years has by definition resulted from tubercle bacilli that have been transmitted within the past 5 years, be they drug-susceptible or drug-resistant.[1]

The utmost epidemiologic importance of *M. tuberculosis* transmission to children and resulting disease manifestations is thus blatantly obvious. Measuring latent infection and disease accurately meets however often almost insurmountable difficulties. Determining the extent to which *M. tuberculosis* has been transmitted relies either on the tuberculin skin test, a test fraught with poor specificity[2] or an interferon-gamma release assay imposing even larger logistic and cost issues for the gain made in specificity.

Enumerating clinically manifest tuberculosis in children suffers epidemiologically from the lack of a clear case definition. While bacteriologic confirmation in adults can be successfully sought and found, tuberculosis in children is usually paucibacillary and even sophisticated diagnostic laboratory services can identify the causative agent in only a minority of cases.[3,4] Where such services are unavailable, diagnosis largely rests on clinical judgment. This is often difficult to quantify for objective epidemiologic case ascertainment.

Finally, in addition to latent infection and morbidity, case fatality must be quantified. While the general assessment is that childhood tuberculosis is often mild and even self-limited, it can nevertheless be severe, life-threatening and fatal.

In the following an attempt shall be made to review the epidemiologic evidence from surveillance and estimates from mathematical modelling to describe the magnitude of tuberculosis in children in various settings and globally.

1. Transmission of *M. tuberculosis*, latent infection, and risk of infection

The "Tuberculosis Roadmap" towards ending tuberculosis in children of the World Health Organization quoted the estimate that each year 7.5 million children aged below 15 years become infected with *M. tuberculosis*.[5] This number is the median model estimate from the work of Dodd and colleagues on tuberculosis among children in 22 high-burden countries.[6] Perhaps the largest body of data on relatively recent estimated prevalence of tuberculous infection among children comes from India. Under the leadership of the National Tuberculosis Institute in Bangalore, tuberculin prevalence surveys were conducted systematically in children in four of the six zones of India to calculate the average annual risk of infection.[7] The annual risk of infection is the algebraically derived probability required to result in the observed infection prevalence to approximate the estimated annual incidence of latent tuberculous infection.[8] Depending on the zone in India, the estimates for this annual risk of latent infection varied from about

1 to 2%.[7] This is very high compared to industrialized countries where it has long become virtually unmeasurably small.[9–11] It is relatively low compared for instance to South Africa with up to 4% or Zambia with up to 2.5% annual risk of infection.[12] The India finding is of comparable magnitude with the 6.3% prevalence of tuberculous infection in pre-school children recently found in Sichuan province, China.[13] In Mongolia, a survey with an interferon-gamma release assay was done in 18 schools among almost 10,000 children aged 9.4 years.[14] This perhaps largest ever conducted interferon-gamma release assay survey to measure latent infection prevalence in a low-income country gave an estimated annual risk of latent tuberculous infection of 1.1%. Notably, 14% of the examined test-positive children actually had clinically manifest tuberculosis.[15] Knowledge of the risk of acquisition of latent tuberculosis infection is a highly informative indicator to pinpoint settings in which tuberculosis control is not yet succeeding in successfully curtailing transmission of *M. tuberculosis*. However, the logistic and epidemiologic interpretation difficulties in conducting and analyzing a prevalence survey to find something that is rare even where it is relatively frequent are such an impediment that it has basically lost appeal to the extent that it is now only exceptionally being done.

2. Clinically manifest tuberculosis in children

The probability of becoming latently infected increases with age, perhaps not steadily but modified by how exposure risk varies for the growing child due to the widening of the circle of an increasing number of adult contacts with whom the child interacts. It is known that the risk of progression from latent tuberculous infection to manifest tuberculosis is far from being a constant ratio through childhood.[16] Simplified, the youngest children have the highest risk for disease progression; in primary school this risk reaches an all-time low, and with puberty the risk begins to rise to an all-time high in late adolescence and among young adults.[11] These data have been reviewed and extended to provide supplemental relevant clinical and immunologic information.[17]

In 2022, tuberculosis in children accounted for 11% of the total case incidence estimated by the World Health Organization.[18] Tuberculosis in children and adolescents is substantially under-reported and under-notified as has been reconfirmed by a recent systematic review.[19] While there is an intrinsic problem with diagnosis even when the best efforts and techniques are at disposal, the paucibacillary nature of childhood tuberculosis alone cannot explain the large variation in under-notification that the authors found.[19] National tuberculosis programs need to be alert to differences in reporting childhood cases between their jurisdictions, rather than just being satisfied with a diffuse sense that tuberculosis in children is likely under-reported. In the extreme, if there are jurisdictions reporting (virtually) zero child cases, yet in others the proportion is sizeable, then strengthening supervision would surely be indicated to evaluate possible causes of such differences. Similarly, if no extrapulmonary cases among children are reported (if cases are reported stratified by age and sex), then something is very obviously not going as it should and remedial action is indicated.

In extension of Dodd's 22 high-burden countries analysis,[6] the analysis of Yerramsetti estimated the burden of pediatric tuberculosis in countries representing more than 99% of the global tuberculosis burden.[20] For 2019, they estimated 1 million incident pediatric case, almost half of whom were among children aged 0–4 years. Importantly, the case detection ratio improved substantially over the study period between 2013 and 2019, notably in the two most populous world regions, i.e. the Western Pacific and South-East Asia.

Authors from the World Health Organization with collaborators report that only about 36% of the estimated incident cases among children are actually treated for tuberculosis.[21] They propose means of action to improve both international and national surveillance. Age disaggregation of tuberculosis cases is obviously a critical requirement to inform about the magnitude of the problem to begin with. The authors furthermore suggest that special emphasis should be put on specific research projects on the most deadly form, tuberculous meningitis.[21]

3. Death from and with pediatric tuberculosis

In the period from 1930 to 1946 without any chemotherapy available, 21.5% of 980 children admitted with primary tuberculosis to Bellevue hospital in New York City died from their tuberculosis.[22] When streptomycin and para-aminosalicylic acid were introduced, fatality dropped to 5.0% and when isoniazid was added to that regimen, it dropped further to 1.5%. In contrast to uncomplicated primary tuberculosis, case fatality from meningeal tuberculosis had been 100% in the pre-chemotherapy era,[22] with a disease duration from symptom onset to death ranging from one to 63 days among 167 affected children.[23]

A systematic review and meta-analysis showed that children with tuberculosis in the pre-chemotherapy era had a pooled case fatality ratio of 21.9%.[24] It was substantially higher in children aged 0–4 years (43.6%) than in those aged 5–14 years (14.9%). In the recent chemotherapy era, it had dropped to 0.9%.[24] The detailed World Health Organization Global Tuberculosis Report 2016 shows the large variation in the case fatality ratio from or with pediatric tuberculosis between the regions.[25,26] It ranged from a low 2% in Europe to 34% in Africa.

Of 1380 pediatric patients admitted to the National Institute of Tuberculosis & Respiratory Diseases, Delhi, India, 74 (5.4%) of children died, 68 (92%) of whom had pulmonary tuberculosis.[27] In a prospective study of 100 patients with tuberculous meningitis admitted in 2018 and 2019 in Mumbai, India, a quarter had died by the end of in-patient care.[28]

When The Union[29] and the World Health Organization[30,31] helped building up national tuberculosis control programs, the major emphasis was on control and reduction of transmission in the community. Coherently, treatment outcome was thus evaluated among notified incident sputum smear-positive cases to ensure a high proportion of treatment success among the most important transmitters of bacilli in the community. Furthermore, because data were aggregated from

paper recording systems, a stratification by age was solely required for the description of case finding activities of sputum smear-positive cases but not for the outcome of treatment amongst such cases. Of course, all forms of tuberculosis had to be diagnosed, treated, and notified, but to reduce the administrative burden for frontline health care workers, they were spared to report detailed demographics (age, sex, specific extrapulmonary sites). This has only recently changed in parallel with the development of electronic reporting systems.[32] The World Health Organization updated its guidance for national tuberculosis programs on the management of tuberculosis in children in 2014.[33] Among many substantial changes there was also a particular emphasis on case definitions for childhood tuberculosis with clear guidelines how pediatric tuberculosis has to be recorded and reported. Of particular relevance is here that outcome analysis is now required for children analogous to the standards used for adult tuberculosis patients.[33] However, the 2018 World Health Organization Roadmap reports that despite of the progress that has been made, there is a substantial gap of failing to report outcomes for the age groups 0–4 and 5–14 years. Thus, in the context of this review, it is not surprising that there are only scarce routine program data available allowing ascertainment of the frequency of death with tuberculosis among children. Data from research projects or clinical studies from referral centers remain a selection-biased source of information and must be taken with a grain of salt when attempting to generalize.

The 2013 World Health Organization "Roadmap" called for zero deaths.[34] Not an easy task if one is virtually clueless about the true magnitude of the problem. Nevertheless, childhood tuberculosis treatment outcome data were analyzed in 13 locations in Africa and Asia in programs run by Médecins Sans Frontières.[35] Among HIV-infected children, 12.9% died compared with 5.7% among HIV-negative children. Unsurprisingly, the proportion who died was highest among those with meningeal tuberculosis (18%) and lowest among those with peripheral lymphatic tuberculosis (4.6%).

A very large study on over 40,000 tuberculosis cases among children has been reported (covering 2010–2017 notifications) from the electronic database from the national tuberculosis program of China.[36] As the authors note, childhood tuberculosis is seriously under-reported in China, with just about 1% of all cases reported among children. Thus, this analysis addressed perhaps only a tenth of outcomes among all pediatric cases. Of these, 6.1% had an unsuccessful treatment outcome, highest in those under the age of 5 years (12.2%) and lowest among teens (5.0%). A total of 0.4% died (with tuberculosis), with the highest risk among those below age 5 years (1.0%) and lowest among the 10- to 14-year-old (0.3%). No detailed information on site of disease was available.

While the treatment outcome of children with tuberculosis is favorable in a very high proportion of all cases of tuberculosis thus coming under treatment and observation, children also suffer from sequelae as summarized in a systematic review.[37] Pulmonary tuberculosis may leave radiologic residuals. Disabling deformities may remain after musculoskeletal or cutaneous tuberculosis and tuberculous meningitis may leave a substantial proportion of children physically and/or mentally disabled.[37]

4. Implications for national tuberculosis control programs

Epidemiologically, tuberculosis in children is the most important indicator for the extent to which interruption of recent transmission has failed. While it is clear that tuberculosis among children is seriously under-notified, the true extent of dysfunctional surveillance remains unknown. The absolute magnitude of the pediatric tuberculosis burden cannot be known, it's intrinsic to the clinical and diagnostic characteristic of childhood tuberculosis. However, national tuberculosis programs can know relative differences in reported proportions of childhood cases among all tuberculosis cases in their jurisdiction. Such differences are least likely attributable to true epidemiologic differences and more likely due to other, programmatic factors. This gives a superb leverage to supervision and for potential remedial action in those areas that have relatively low notifications. Where the proportion of childhood cases among all tuberculosis cases is below the expected average, adherence to the principle of consistently conducting contact investigations should likely be the first step for evaluation.[38] In any setting, a clinical examination of all children under the age of 5 years living under the same roof with a newly discovered adult index case with microbiologically confirmed pulmonary tuberculosis must be the mandatory minimum and be carried out in an as large proportion as feasible. This will by necessity identify cases of childhood tuberculosis in any setting and additionally identify healthy children for whom preventive therapy is indicated.[39–41] Contact investigations are indeed a "damage control strategy" and they will miss transmissions by the index case that have occurred outside the household. Nevertheless, one must not argue about the impossible but tackle first the blatantly feasible and imperatively indicated.

Tuberculosis in children is of extraordinary importance, not just from a humanitarian and ethical perspective but notably relevant for any elimination strategy. If we do not put supervisory resources into improving known surveillance deficiencies then it is difficult to see how any tuberculosis elimination strategy can be realistic, given that the key to every tuberculosis control effort lies in knowing about how successful recent transmission is being controlled.

Conflict of interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Pediatric tuberculosis – A diagnostic Dilemma

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1. View point

In India, 6–7 % of all tuberculosis (TB) cases reported under National TB Elimination Programme (NTEP) belongs to pediatric age group.[1] Although there are variations in cases reported from different states due to health seeking behavior, burden of disease and availability of pediatricians and diagnostic facilities available. Nikshay data for the year 2021 shows, there were one lakh pediatric TB cases in the 0–14 years age group and 1.4 lakh among 15–18 years age groups,[2] probably due to more chances of exposure in older children. Because of majority of pediatric TB cases are not confirmed microbiologically and are at Extra pulmonary sites, this may be under estimate of burden of pediatric TB.[3]

In addition, many pediatric TB cases are being treated in the private sector, outside the NTEP. Adults comprise the maximum proportion of TB cases in any country as adulthood life span is much longer than pediatric. In India, TB control is not one of the strategies for childhood survival (which usually focuses on premature birth, parental asphyxia and injuries, pneumonia and diarrhea – as they are the cause of highest mortality rate in the age group). Many times TB in children is reported as pneumonia because of similar presentation, so the exact contribution of childhood TB is not known and 'under -5-mortality'. Autopsy studies in low and middle income countries have suggested, the childhood TB contributes to most deaths in under five age group.

In principle, the diagnosis remains same in pediatric age group and children, but because of host characteristics and type of disease, there are challenges in establishing diagnosis of TB in children. In adults, the disease is usually infectious and can be diagnosed by sputum testing. In children, disease is usually in the form that sputum examinations is not helpful, more ever, young children do not produce sputum but swallow it. The alternative methods to collect sputum samples (induced sputum, gastric lavage or broncho. alveolar lavage requires an invasive process and professional skill. This all makes diagnosis in children challenging and difficult to decentralize diagnostic services, which is a priority in TB Elimination Programme. Molecular tests (Gene-expert, Truenaat) give a confirmed diagnosis provided a good sample is collected. Further, clinical presentation and symptoms suggestive of pulmonary tuberculosis overlaps with other disease. So many times chest radiography is used as a screening test. Additionally, radiography increases the sensitivity of the molecular tests, which are very costly, thus making these tests cost-effective.

In addition, new molecular tests not only detects *M. tuberculosis* but also gives rifampicin resistance status, which means we can diagnose, MDR TB upfront. This paves the way for universal Drug Sensitivity testing (U-DST), which is now considered the preferred strategy under NTEP. The only point to take care is to have a good quality biological specimen, which is a challenge in children.

For treatment of TB in this age group, there has been significant improvement such as like – Dosage of anti TB drugs has been revised upwards to achieve optimal drug levels, introduction of flavoured dispersible fixed drug combinations to achieve compliance, six weight bands to titer optimum dose and avoid drug toxicity, and adding ethambutol in continuation phase so to take care of undetected isoniazid resistance.[4]

Drug resistance TB is another challenging problem in children. Upfront molecular tests for all presumptive TB patients on a good quality biological sample can help us catch these cases at the earliest. Treatment of MDR-TB disease in children in changing rapidly and all oral regimens, which include newer and repurposed drugs, have led to a shorter and safer regimen.[5]

Although rapid molecular tests are available but getting a good quality biological sample in children is still not feasible in peripheral areas especially when some invasive procedure like bronchoscopy or biopsy of a tissue is required. We may think of some scoring method for diagnosis in this age group, which is convenient to apply in field areas and by general health system doctors to diagnosis pediatric TB. As of now, because of TB elimination programme, incidence is decreasing in adults. As per Global TB Report data, the incidence of TB in India for the year 2021 is 210 per lakh population as compared to 256 per lakh population in 2015.[6] There has been 18 % decline which is 7% points better than global average of 11 %. It will have effect on incidence of pediatric TB cases, but if we are not able to do early diagnosis in this age group, we may not to able to sustain advantage of decrease in incidence

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in adults.

Separate guidelines for managing pediatric TB were developed by RNTCP and NITRD (then LRS Institute) way back in year 2004.[7] At that time diagnosis was based on X-ray chest and tuberculin test, in cases where sputum sample was not available in children. Treatment was given intermittent. The guidelines have been revised many times and the present guidelines[8] of 2022 recommends use of rapid molecular tests to rule out MDR TB at the time of diagnosis (integrated diagnostic algorithm) and treatment with dispersible HRZ tablet with Ethambutol tablet in drug sensitive TB and use of Bedaquiline and Delanamide, if MDR TB is detected. Bedaquiline is recommended for children above 5 years and those weighing more than 15 Kgs. Delanamide, although WHO recommends in 3–5 years, but in India regulatory approval for this age group is still awaited.

To conclude, the latest diagnostic algorithm, introduction of molecular diagnostics, revision in treatment guidelines and ensuring drug adherence have all made management of TB and MDR TB in this age group easy and specific. The only few points, we must keep in mind like, pediatric tuberculosis is still being missed in both public and private sector, a high index of suspicion and optimal utilization of molecular tests on a good quality sample in the key to success. Present day treatment modalities and adherence strategies can help us overcome this problem in near future.[9]

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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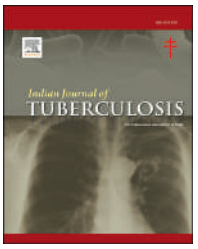

Review article

Latent TB in Indian pediatric population: An update on evidence gaps and research needs

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Pediatric

ABSTRACT

The main aim of this article is to review various studies conducted in relation to diagnosis, treatment and management of Latent TB Infection (LTBI) in under-five children, thus highlighting research gaps and further scope of improvements with respect to Indian context. The methodology involved literature review of various online review articles and research papers along with current published guidelines for LTBI management by World Health Organization (WHO) and National tuberculosis Elimination Program (NTEP). There is a dearth of statistically significant data regarding prevalence of LTBI among under-five children in India. LTBI prevalence in Indian adults has been reported between 21 and 48%. The exact prevalence of pediatric LTBI in India is still not clear, however, as per few studies, the LTBI prevalence ranges around 40% and 22% in adolescent followed by under-5 population. Studies to fill in the research gap of scarcity of prevalence data, regarding pediatric LTBI in high TB burden areas of India, is a pivotal step to curb the global pandemic of TB disease. There is a massive undervaluation of the true burden of childhood LTBI as the influence of environmental reservoir in childhood LTBI and TB are not accounted for in pediatric LTBI regimens. Also, there is no substantiate amount of data that highlights the other aspects of LTBI in pediatric population, like awareness regarding LTBI condition and other physiological adverse effects of LTBI in pediatric population, which have been often observed in under-five children suffering from LTBI.

1. Background

India reports nearly one fourth of the global TB burden.[1,2] Pediatric tuberculosis contributes to 27% of the global cases and 8–20% of TB-related deaths in India.[3] Nearly five percent of the new cases in India are reported among children.[4–6] However, there is an underestimation of the true burden of TB in pediatric population as sputum microscopy, smear-positive TB is reported only in 0.6%–3.6% of children (<14 years old).[7]

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The recognition of TB in vulnerable populations like under-five children is crucial as it will also help policy makers to take appropriate actions. This is of utmost importance as the End TB Strategy by WHO targets for reduction in TB deaths by 90% and incidence rates of TB by 80% till the year 2030. Early detection and treatment are important pillars to prevent mortality and limit morbidity of TB including prevention of multidrug-resistant (MDR) TB.[8] Early detection of Tuberculosis primarily involves diagnosis of Latent TB infection (LTBI) as it does not manifest any clinical indicators of TB disease and is not contagious. However, it has 5–10% lifetime risk of reactivation of TB with the majority developing active TB disease within five years after initial infection.[9]

LTBI is described as a sustained immune response to *Mycobacterium tuberculosis* (MTB) specific antigenic stimulation without evidence of clinically manifested active TB disease.[10] It denotes the presence of a specific immune response to MTB complex (MTC) antigens in asymptomatic healthy individuals.[11] LTBI begins with invasion of bacteria in the host alveolar cells where it is easily phagocytosed by alveolar macrophages. The bacteria thus encounter this first line of defense by the host immune system. If it can survive this environment, it actively replicates which ultimately results in progression of active TB disease with the classical clinical manifestations.[12,13] LTBI is a stage where the MTB bacterium ceases its multiplication even though being viable.[14] Hence, as the bacterium is not dividing actively, the patients remain asymptomatic, rendering the condition non-transmissible. The exact reasons and mechanisms are still not understood, but many models have been proposed to address the LTBI condition which state nutritional deprivation in the microenvironment of the entrapped bacterium, progressing anaerobic conditions in the microenvironment and stressing stimulants within the macrophage phagolysosome as the plausible reasons.[13] The risk of developing active TB disease post exposure or infection is mainly controlled by the immunological status of the host. Therefore, identification and treatment of LTBI can achieve up to 80–90% reduction in active TB resulting from reactivation of LTBI.[15]

Nearly 67 million children can have latent TB[16] and are at higher possibility for progression to active TB disease with more potential for disseminated disease than adults.[17] Under-five children have the highest risk of progression with development of disseminated and central nervous system TB with majority progressing to TB disease within 2–12 months of initial infection.[18,19]

1.1. *Diagnosis of LTBI*

Tuberculin Skin Test (TST), which is used currently to screen LTBI, is tested by measuring the skin induration against intradermally injected purified protein derivative (PPD). The observed skin induration is measured within the time period of 48–72 h after injection. However, it may lead to false positive results in view of cross-reaction due to Bacillus Calmette-Guerin (BCG) vaccination or immune reaction triggered by non-tuberculous Mycobacteria (NTM).[20–23] Also, there is a probability of false negative results in earlier phase as positive result of TST requires around 3–6 weeks after exposure.[24]

A few years back, an alternative of new *in-vitro* tests for cellular immunity has emerged in the form of T-cell based interferon gamma (IFN-γ) release assays (IGRAs). While TST reflects delayed hypersensitivity to *Mycobacterial* antigen, IGRA is a laboratory test for lymphocyte recognition and response to MTB, which can be used to detect primary as well as LTBI.[25] IGRA tests are based on delayed type hyper sensitive reaction by lymphocytes towards specific antigens of MTB like ESAT-6 (early secretary target-6) and CFP-10 (culture filtrate protein-10).[24] It is based on the certainty that interferon gamma (INF-γ), a cytokine known to be involved in various immunological responses, is released by lymphocytes on stimulation by antigens. Hence, by detecting and measuring its release after lymphocytes are exposed to antigens specific to MTB, the intensity of its response can be indirectly extrapolated to determine whether the person was exposed to this bacterium. IGRA test was developed to overcome the limitations shown by tuberculin or MT test.

IGRAs that are found commercially are QuantiFERON (QFT)-TB In-tube test (Qiagen, Valencia, CA, USA) test which is based on Enzyme-linked immunosorbent assay (ELISA) assay, and the T-spot test (Oxford Immunotec, UK) which is based on Enzyme-linked immunosorbent spot (ELISPOT) assay. In 2015 a new variant of In-tube IGRA test, IGRA Gold In-tube test was introduced. In 2016, a newer version, IGRA Gold Plus, was introduced which facilitated more specific responses from different lymphocytes.[20] In a recent study conducted in Japan, which made a comparative analysis between QFT-TB Gold Plus, QFT-TB T-spot and QFT-TB Gold in-tube tests, all the three methods of IGRA were found to identify active TB infection with similar accuracy, which was much accurate than that of traditional method of diagnosis result. Even though similar, the specificity and accuracy of QFT-TB Gold Plus test was comparatively higher than QFT-TB T-spot and QFT-TB Gold In-tube test.[20] This can be chalked up to the attribute that QFT-TB Gold Plus provides additional analysis which also facilitates in distinguishing the release of IFN-γ from CD8+ T cells and CD4+ T cells resulting in increased specificity and further helping in analyzing how recent the exposure to antigens of MTB. Various other published reports also consider QFT-TB Gold Plus to be a more reliable marker than TST with higher sensitivity as well as specificity and a better correspondence with exposure to MTB as per various published reports.[26,27]

The diagnostic utility of both IGRA by QFT-GIT and TST is reported in health-sector workers and in at-risk groups in high TB endemic countries.[28–30] However, both TST and IGRA have reduced sensitivity in immunocompromised patients. Despite being commercially available in India, it is not being routinely used to screen LTBI in populations at risk. The priority indicators for monitoring implementation of End TB Strategy includes investigation of people with history of contact with active TB patients and LTBI treatment among contact children and HIV positive people. According to the End TB Strategy of WHO, treatment of LTBI would significantly reduce global TB burden,[31] thereby making LTBI management utterly crucial. The current review looks into the prevalence of LTBI and will give insights regarding its relevance diagnostic point of view in pediatric population.

2. Methodology

Literature search and its retrieval were carried using online databases like pubmed, researchgate and search engines like Google. Related scientific manuscripts from the year 2000 till date were filtered out using keywords like pediatric latent TB, latent TB prevalence and latent TB management. The long-standing experience of the authors gained from actively working in pediatric and TB research helped in pinpointing the deficits in pediatric latent TB research. Around 80 references were reviewed, which consists of more than 50 published studies with respect to LTBI research. The ones which were associated with pediatric and juvenile population and whose demographics were closely related to that of India were incorporated for this article. Along with these, the recent guidelines on LTBI Management by WHO and RNTCP open to the public domain were also referred so as to identify what measures can be employed in order to fill the existing research gaps.

3. Findings

3.1. *General prevalence of LTBI*

The diagnosis of LTBI is targeted to vulnerable groups which include people having any form of physical contact with active TB patients, undernourished and underweight populations and, immune-compromised patients. An independent review of 88 consolidated case studies from 36 countries across the globe by Cohen et al, has reported a global LTBI prevalence of 24.8%. They had concluded that roughly one-fourth of the world is infected latently with MTB.[32] Houben et al also came to a similar conclusion by mathematically calculating the global LTBI prevalence.[16]

On basis of both Tuberculin skin test and IGRA in-tube test, a general LTBI prevalence of 45.0% was reported among the age group of 5–40 years old population in South Africa. By combining both of the testing methods, they were able to infer and conclude that both the types of screening methods measure a different aspect of immune response towards MTB, thus making both methods essential to in diagnosing LTBI.[33] Birch et al also expressed that applying both the diagnostic methods gave a better demographics to the population.[34]

With respect to India, from a study conducted in high endemic TB zone from Nagpur, an LTBI prevalence of 48% was observed via IGRA – GIT assay.[35] Kinikar et al conducted a cohort study to determine the prevalence of LTBI among medical students and nurses having a median age of 25 years in India. The overall LTBI prevalence was found to be around to be around 21–22.5% using both TST and IGRA-GIT methods.[36]

3.2. *Overview of worldwide studies on pediatric LTBI*

Among children, various studies have been reported concerning the prevalence of LTBI, Table 1 represents characteristics of the studies conducted so far including pediatric age group.

In a cross-sectional study conducted in Matlosana, South Africa, the prevalence of LTBI among 7 years old school going children was found to be higher than the 5 years old school going population. The prevalence of 5 years old population was found to be around 15.1%. Another study reported in a smaller township of Cape Town, also reported a similar prevalence among 5 years old population residing high burden TB areas. However, a major drawback for this study is only IGRA results were considered for initial screening of children and the children turning out to be positive were then traced back to determine whether they were having contact with any active TB patients.[37]

In a retrospective study conducted in the Republic of Macedonia, among the 73 cases that were studied, most frequent cases of LTBI was observed in patients belonging to the age group of 5–14 years.[38] When further assessed within this age group, children below the age of 5 are more surrounded by household environment where they are constantly around adults. Household active TB contact has been known to be a major source of spread of LTBI. Hence, this pediatric population is even more susceptible to LTBI.

3.3. *Studies done so far in with respect to pediatric prevalence of LTBI in India*

There are no exact estimates regarding prevalence of pediatric LTBI in India, however, WHO data suggests around 3.5 Lakh children do require LTBI treatment.[39] Benachinmardi et al chose 77 subjects ($2.5 \leq$ age ≤ 18 years) who had contact history with active TB patients and their LTBI status was determined using both TST and IGRA. The prevalence of LTBI was found to be around 40% using IGRA and 22% using TST, with the positive percentage being higher in adolescents followed by under-five children.[40] As only subjects having history of contact were included in the study and small sample size, the determined prevalence cannot be correlated with the actual prevalence of pediatric LTBI in India.

An investigation conducted by Dorjee et al, exhibited high prevalence of active TB among Tibetan refugee children and adolescents (median age 13 years). Instead of diagnosis of LTBI, the TBI (TB infection) was detected using MTB growth indicator tubes and was found to be 18% among school children. As 46 confirmed active TB cases were detected out of 5391 school children, including one MDR TB case, the LTBI treatment was directed to 90% school children of the respective school.[41]

4. Discussion

4.1. *Research gaps due to missed pediatric populations*

The epidemiology of TB in pediatric population is not well understood in India as though studies have reported significant prevalence of Tuberculosis in children. The pediatric LTBI diagnosis is targeted towards children who have a history of contact with a patient suffering from active TB disease. On the contrary, infectious TB bacilli droplets are known to survive in the environment for more than six months.[42] Hence, there is an underestimation of the true burden of childhood TB. True

Table 1 – Important attributes of relevant studies conducted so far including pediatric age group.

Country and author	Age group	Sample size	LTBI prevalence	TST prevalence	IGRA prevalence
United Arabs, Emirates; Al Mekaini, 2014[36]	1–19 years (mean age 8.7 years)	669	0.45%	–	0.66%
Matlosana, South Africa; Lebina L, 2015[37]	5 years 7 years	2105	15.1% 19.7%	–	16.6%
Bangladesh; Hossain, S. 2013[38]	5–9 years 10–14 years	17,718	12.4% 22.6%	16.7%	–
China; Chen, P.C. 2008[39]	6–14 years	2504	9.3%	–	9.3%
Malaysia; Wong J. Y, 2020[40]	13 years	430	12.8%	11.3%	15.4%
Brazil; Perez-Porcuna M. T. 2016[41]	Upto 6 years	105	23.9%	41.2%	50.0%

prevalence of pediatric LTBI can be determined by mass community screening of children, especially residing in areas with poor sanitation. Hence, statistically significant data from community screening is instrumental for accurate analysis of prevalence of pediatric LTBI.

Most of the prevalent studies done either based on routine screening of schools and health dispensaries, or children having contact history with patients diagnosed with active TB. No parallel forms of study were found by the authors who individually assessed both the categories of under-five populations and also gave a cohort analysis on both the populations.

However, the authors were unable to find literature that specifically targeted undernourished under-5 population. Also, the authors were not able to find many longitudinal studies for under-5 children that highlighted how the IGRA negative children who turned out to be TST positive were followed up.

4.2. *Significance of IGRA in LTBI diagnosis*

Traditional LTBI diagnosis is highly dependent on TST test. However, the results of this diagnostic test may not always be reliable. There is a high chance of underweight children showing a false negative result,[43] and they are one of the most vulnerable groups in many LTBI preventive programs. Also, as previously mentioned, there are many other false results associated with TST test. Hence, in such a case, IGRA test becomes especially important in determining the LTBI status with respect to young children. In an independent study by Benachimardi et al, from Karnataka, IGRA was found to be more accurate in detecting LTBI in children.[40] But this study was limited to children having household contact with active TB patients. Another comparative study in China by Wook Yun et al, IGRA test again was demonstrated to be more accurate and useful in diagnosing LTBI among under-five children, majorly because they observed lack of decline in INF-Gamma levels in under-five children, hence making IGRA a better diagnostic marker.[44]

IGRA test requires the host body to regain a memory of interaction with MTB. One of the possible setbacks of IGRA is false negative results which may happen if the infection is very recent. The chances of this scenario in under-five pediatric population is all the more higher. Hence, longitudinal studies with repetition of IGRA, especially when the child is IGRA negative + TST positive is required, to determine the influence of this aspect on the prevalence of LTBI. Also studies on repetition of IGRA for borderline results is crucial in determining the true prevalence.[45]

A newer TB skin test, the C-TB test, is similar to the TST. However, it contains the purified antigens CFP10 and ESAT-6.[46] This may eventually prove to be more convenient for screening LTBI especially among under-five age group. However, evidence on its operational feasibility is required before its programmatic implementation.

4.3. *Adverse effects or other physiological implications of LTBI in pediatric population*

Pediatric TB is one of the major causes of deaths in children around the globe. Each year around 70,000 children die of TB infection.[47] This number can very well be brought down by use of preventive approaches which includes diagnosing and treating LTBI, thereby significantly lowering the overall number of active TB cases. There is an insufficient amount of statistical data which highlights other effects of LTBI in pediatric population like stunted growth, low body weight, and many other physiological parameters, which have been observed in my cases. This aspect of effect of LTBI must be investigated further as it may aid in making policies more streamlined for preventive LTBI treatment.

4.4. *General receptiveness regarding LTBI*

Treatment of LTBI primarily involves use of single antibiotic against MTB for 6–9 months or use of two antibiotics for about 3 months. Serious adverse events such as Isoniazid associated hepatotoxicity are rare with currently recommended dosage of anti TB treatment and nausea or vomiting are also reported uncommon.[48,49] Lengthy treatments of LTBI and a general lack of awareness regarding the severity of this condition often results in a noncompliant attitude towards the prescribed treatment, thereby leading to failure of LTBI treatment.[11] Hence a holistic approach is required towards LTBI

screening as well treatment to ensure successful results, especially in high TB burden areas. Such approaches include close monitoring of subjects undergoing LTBI treatment. Urine testing kits which detect metabolites of isoniazid and orange discoloration due to presence of rifampicin can be also employed for the same purpose.[11] Ensuring completion of LTBI treatment will also contribute in minimizing the rising number of MDR-TB cases in pediatric population.

One of the advantages of community screening approaches for determining pediatric LTBI prevalence is to also analyze the receptiveness of parents or guardians towards the method of diagnosis, general awareness and willingness towards the treatment. The overall pliability of parents or guardians is an important factor in ensuring success in LTBI management.

5. Conclusion

Determining the exact prevalence of LTBI in pediatric population becomes crucial. One of the best ways in determining the true and accurate prevalence of pediatric LTBI is through community screening. This approach will not only help in finding the prevalence but also help in understanding the general perspective of people towards pediatric LTBI and other aspects of this disease in pediatric well-being. Such a data is necessary, especially in high burden TB areas in India like urban slums, in order to make policies more streamlined to eradicate LTBI from under-five children, thereby climbing one step closer to the goal of significant reduction of TB deaths and overall cases of TB disease by the year 2030.

Both TST and IGRA tests are required to assess and filter out true latently infected patients. Also, studies on repeat IGRA and factors influencing IGRA test are required to understand the reliability of the test with respect to Indian pediatric population. However, it is important to note that although treatment of LTBI is an integral component of End TB strategy, routine screening is recommended only among in high-risk groups in high TB burden countries like India. Hence, possibility of reinfection after LTBI treatment should also to be considered for which follow up studies assessing conversion and reversion rates of IGRA would probably guide in formulating repeat screening and treatment strategies for such children.

Conflicts of interest

The authors have none to declare.

Author's contribution

Dr. Suchitra Surve and Kajal Naukariya were involved in the consolidation of all the studies that were used in making in this article. The discussions and suggestions made in the manuscript are based on the first-hand experience of Dr. Suchitra Surve and Dr. Ira Shah who are actively engaged in pediatric tuberculosis research. The first draft was prepared by inputs from all the authors. The final draft was prepared by the knowledgeable and experienced input of Dr. Ira Shah.

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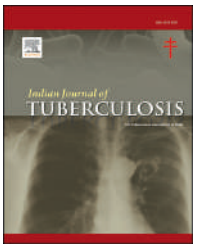

Review article

An overview of the BCG vaccine and its future scope

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ABSTRACT

Despite intense elimination efforts, tuberculosis (TB) still poses a threat to world health, disproportionately affecting less developed and poorer countries. The Bacillus Calmette-Guérin (BCG) vaccine, the only anti-TB authorized vaccine can partially stop TB infection and transmission, however, its effectiveness ranges from 0 to 80%. As a result, there is an urgent need for a more potent TB vaccination given the widespread incidence of the disease. Enhancing BCG's effectiveness is also important due to the lack of other licensed vaccinations. Recently, fascinating research into BCG revaccination techniques by modulating its mode of action i.e., intravenous (IV) BCG delivery has yielded good clinical outcomes showing it still has a place in current vaccination regimens. We must thus go over the recent evidence that suggests trained immunity, and BCG vaccination techniques and describe how the vaccination confers protection against bacteria that cause both TB and non-tuberculosis. This review of the literature offers an updated summary and viewpoints on BCG-based TB immunization regimens (how it affects granulocytes at the epigenetic and hematopoietic stem cell levels which may be related to its efficacy), and also examines how the existing vaccine is being modified to be more effective, which may serve as an inspiration for future studies on the development of TB vaccines.

1. Introduction

The bacterial infection *Mycobacterium tuberculosis* (*M. tb*), which causes tuberculosis (TB), is thought to have caused infection in more than one-fourth of the world's population and is still the biggest reason of mortality from a single illness worldwide despite being practically eliminated in affluent nations. Regardless of the presence of a recommended antibiotic administration to treat TB, problems with medication accessibility, the growth of MDR-TB and Extensive Multidrug-resistant TB (XMDR-TB), and other factors all contribute to TB's continued status as a worldwide public health liability.[1] In regions with low TB prevalence, disease prevention is mostly managed by good hygiene and screening, with immunization neither necessary nor advised. Just in regions with a high TB load is the BCG vaccination routinely administered. The wide variations in the vaccine's effectiveness, which varies from 0%

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to 80% for the control of TB, are a contributing factor in the variance in recommendations.[2]

Since 1921, BCG, a suppressed strain of *Mycobacterium Bovis* (*M.bovis*), has been the sole TB vaccine that has received approval for clinical use.[3] While most nations with a relatively low range of occurrence pursue particular vaccination techniques to target high-risk individuals, countries with high rates of tuberculosis continue to promote universal BCG vaccination.[4] In 2020, 154 nations stated that the BCG vaccine is a required component of children's immunization programmes, with 53 of those reporting coverage rates of more than 95%.[5] However, prior research indicated that BCG may only offer a negligible level of protection, if any, over TB in human populations.[6] As per the anticipation, around 9.9 million people are infected with approximately 1.43 million expected fatalities from the illness in 2020, the most recent WHO data on worldwide TB is still startling.[4]

Earlier, creating a vaccine more efficient than BCG was the primary focus but in recent years, vaccine assessment is still a very lengthy, expensive, and high-risk enterprise.[7] Techniques for enhancing current vaccinations by altering immunization regimens or schedules are more economical than creating whole new vaccines. As a result, innovative BCG vaccination methods are being evolved. The effectiveness of these in treating *M. tb* infection has been encouraging. Millions of individuals have been safeguarded from TB due to BCG, which has been utilized as an efficient technique for TB control for the past 100 years.[8] New and amazing immunological benefits have been shown by enhancing BCG vaccination methods. The belief that BCG will be more successful against TB has been revived as a result.[9]

To give direction and inspiration for future research this review article provides a brief outline of the BCG vaccine's historical development. We also talk about the vaccine's potential mode of action. Hematopoietic stem cells appear to undergo epigenetically remodeling as a result of the vaccination. Finally, we talk about the efforts being made to modify the vaccine's mode of administration and quickly outline the several strategies being used to either improve the effectiveness of the present vaccination or create a new one to replace BCG.

2. Intradermal route for BCG vaccine administration

In TB-endemic regions, it is well known that BCG is given through the intradermal (ID) method immediately after childbirth.[6] Although this inoculation approach is simple to use and induces a robust systemic immunity, it only partially protects against MTB in both human and animal models.[10] Additionally, this technique can result in positive tuberculin skin test (TST) findings; nevertheless, it has been demonstrated that the TST's positive conversion rate has no bearing on the effectiveness of BCG immunization.[11] A thorough analysis of the shortcomings of ID BCG vaccination may offer helpful insight for enhancing BCG immune strategy shown in Fig. 1.

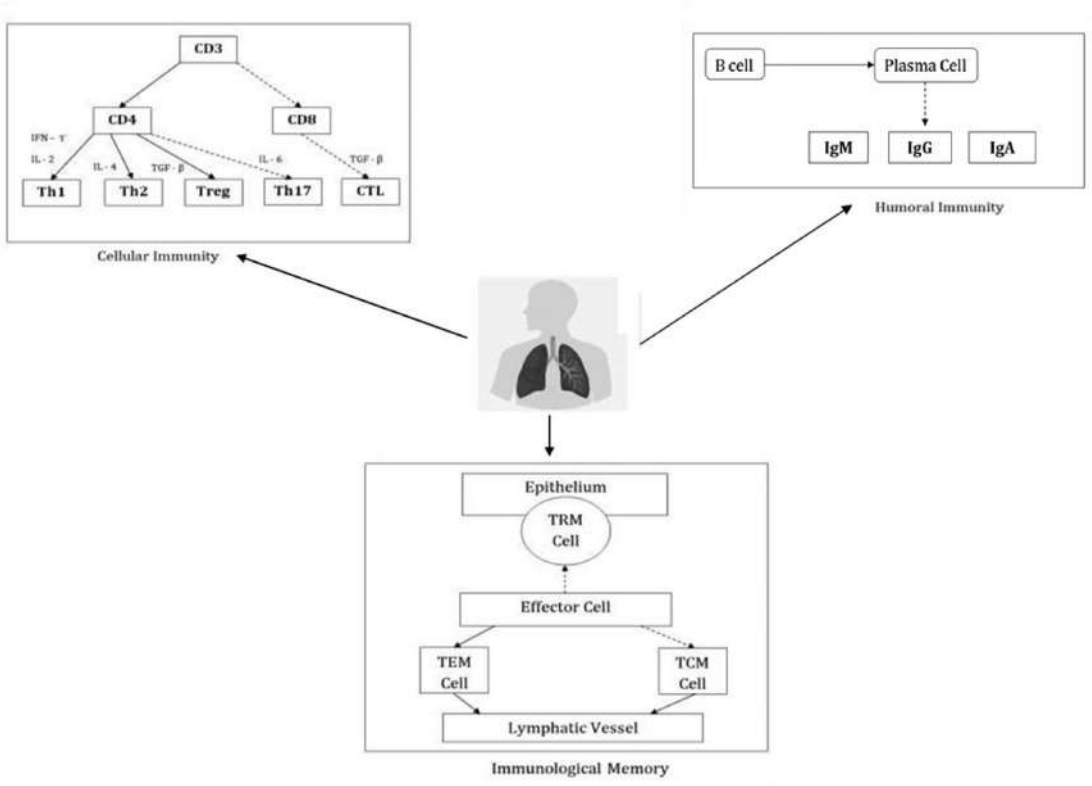

Fig. 1 – The body's reaction to the intradermal BCG vaccine.

Although the ID BCG vaccine can trigger a potent adaptive immune response in humans, this immunological response is insufficient to provide long-term protection against MTB infection because.

(a) Fighting off MTB infection requires effective cellular immunity. The Th2 and Treg immunological responses would suppress the T helper 1 (Th1) immune response, which is quite strong after ID BCG immunization. Additionally, the Th17 and $CD8^+$ immunological responses elicited by ID BCG immunization are subpar.
(b) The number of antibodies generated by the ID BCG vaccine is relatively little, or perhaps virtually imperceptible, despite recent findings suggesting that humoral immune responses serve an essential part in defense against MTB.
(c) Although the ID BCG vaccine can cause a significant increase in effector memory T (TEM) cells, resident memory T (TRM) cells and central memory T (TCM) cells make up a modest portion of the memory immune responses. It would be challenging to react promptly to the presence of pathogens and vaccine-prompted immunity would not be maintained for a long time with such a configuration of memory cells.

The effectiveness of ID BCG vaccination in eliciting T cell immunological responses may play a significant role in BCG immunization against TB. First off, it is unable to effectively stimulate $CD4^+$ and $CD8^+$ T cells.[12–15] The host's ability to fight off MTB infection depends on the Airway Luminal T (ALT) cells, but ID BCG immunization can just stimulate a tiny number of these cells which remain lacking for a minimum of 10 days following MTB infection in a TB mouse model.[12,13] In a model of infection in Guinea pigs, early ID BCG vaccination can result in a significant number of antigen (Ag)-specific lipopeptide-reactive CD4 + T cells in peripheral blood mononuclear cells (PBMCs), but this is not accompanied by a functional diversity that would prevent granuloma development.[14] Regarding CD8 + T cells, BCG may significantly activate Ag-specific CD8 + T cells, but it is ineffective in delivering antigen to the areas of T cell activation.[15] Second, the ID BCG vaccine has a poor ability to stimulate T helper 1 (Th1) and Th17 cells. Although ID BCG vaccination can cause a strong Th1 immune response, it is also adversely controlled by the Th2 and regulatory T cell (Treg) responses and does not offer enough protection. Research on newborn BCG vaccination came to the same outcome.[16] In response to BCG stimulation, human cord blood mononuclear cells specifically generated the Th2-type cytokines IL-10 and IL-5, and the amount of IL-10 was greater than that of 10-week-old unvaccinated newborns.[16] Additionally, babies who got BCG had a weaker lymphoproliferative and Th1 immune response than infants who were given BCG at the age of 10 weeks. Th17 cells can promote the production of CXCR3 chemokine ligands 9 (CXCL9), 10 (CXCL10), and 11 (CXCL11), which attract CD4 + T cells and induce them to produce interferon (IFN)-, therefore limiting the proliferation of M. tb. Interleukin (IL)-17 also contributes significantly to the prevention of M. tb infection by activating CXCL13, which promotes neutrophil migration to an infection site for pathogen control. The ID BCG immunization, however, is unable to stimulate adequate Th17 immune responses.[17] Thirdly, the immune system gradually becomes paralyzed as M. tb infection proceeds due to the functional fading away of BCG-induced CD4 + T cells and later CD8 + T cells. After infection, $CD4^+$ and $CD8^+$ T cells become exhausted, and this is linked to mitochondrial malfunction, the production of inhibitory receptors including PD1 and TIM3, as well as T cell immunoglobulin and mucin domain-containing protein 3 (TIM3).[18] To increase the efficiency of BCG, it is required to inhibit or counteract these complicated alerts regarding T cell fatigue and keep up a reservoir of certain self-renewing T cells that can facilitate persistent confinement.

Even though Mtb is an intracellular microbe, B cells and antibodies are crucial for preventing Mtb infection. In the mouse model of aerosol infection, total immunoglobulin (Ig), obtained from healthcare workers exposed to Mtb in a TB-particularized hospital, can provide immunity against Mtb infection. In Mtb-infected Balb/c mice, the lung burden of monoclonal antibodies targeting Mtb phosphate transporter component PstS1 obtained from active tuberculosis infection (ATBI) patients may be reduced by 50%. By neutralizing Mtb, improving phagocytosis, activating inflammasomes, and engaging cytotoxic natural killer (NK) cells, antibodies may help to guard against Mtb. While other investigations suggested the opposite outcomes, several studies have shown that ID BCG vaccination not only had the potential to create Mtb-determined antibodies but also that antibody concentration would grow somewhat but dramatically with a rise in dosage and immunization intervals.[19] These findings point to the heterogeneity of BCG-mediated humoral immunity, which may be a result of various BCG strains, the health of the recipients of the vaccination, the sheer volume of recipients, and the methods used for diagnostics.[19] However, it cannot be disputed that the ID BCG vaccine induces extremely modest antibody levels (Fig. 1(b)). BCG just notably elicited specific antibodies against lipoarabinomannan (LAM), according to further examination of the antigenic targets for those antibodies developed by the vaccination. The antibody generated by ID BCG vaccination is insufficient to restrict the invasion of Mtb, most likely because of low antibody levels, even though the antibody targeting LAM has been demonstrated to inhibit the development of Mtb.[20] Additionally, it was noted that the isotypes of anti-Mtb antibodies were closely related to their inhibitory efficacy. IgG antibodies facilitated host cell infection, but IgA antibodies that target Mtb surface antigens might conciliate the inhibitory consequence of Mtb absorption irrespective of Fc alpha receptor expression. The ID BCG immunization does not, however, generate enough IgA to be effective. Therefore, while developing novel BCG vaccination techniques, humoral immunity must be considered to enhance the immunological effects of BCG.

Another significant factor in the immunological failure of the ID BCG vaccination is the poor production of tissue-resident memory T (TRM) cells and central memory T (TCM) cells. Instead of the T effector memory (TEM) cells, the long-term memory response is primarily influenced by the size of TCM cells. TCM cells are produced in the lung after ID BCG vaccination far less frequently than TEM cells.[21] Furthermore, chronic exposure to environmental mycobacteria eventually depletes the host's TCM cells, which results in a reduction of

$CD4^+$ T cells that can produce IL-2 and an increase in KLRG1+ terminally differentiated T cells. When subjected to Mtb, TRM cells, also known as the local immune defense experts, can recognize contaminated cells and can act fast before the host recruits circulating memory T cells. By destroying contaminated macrophages and inducing preventive natural and acquired immune responses by releasing IFN-γ, TNF-α, and IL-2, $CD8^+$ TRM cells can limit the entrance of Mtb into lung tissue. When these cytokines are suppressed, the immune system's defensive responses are eliminated. Even though Intradermal BCG immunization can also cause TB-specific lung TRM cells, these cells are not very common. Additionally, TRM cells in the lungs are unstable, leading to a progressive decrease in defense. Although ID BCG vaccination in mice models might produce antigen-specific $CD4^+$ TRM cells in lung parenchyma for a minimum of twelve months, this long period is yet insufficient for vaccine defense.[22] Thus, to achieve a high degree of immunity against Mtb infection, the BCG vaccination method must be improved to produce both TRM cells and circulating memory T cells, particularly TCM cells.

3. Alternative vaccination routes for BCG administration

There has been a strong drive to reformulate the delivery route of intradermal BCG leading to enhanced immunogenicity and immunoprotection level to a certain extent.

4. Oral administration

Oral administration is more widely used, less expensive, simpler to administer, and needs no medical expertise. Direct mucosal contact with the vaccination is thought to be highly immunogenic, but developing vaccines in forms that can endure the stomach's particularly harsh mucosal conditions has restricted their usage.[23]

BCG was created in 1921 by Calmette and Guerin and was first given orally. Additionally, oral BCG vaccination was given to newborns in Brazil up until 1976, and several studies have shown its safety thus there is a resurgence of interest in oral BCG due to the poor ID BCG immunization. The mucosa is frequently the initial site of interaction with Mtb because it set foot in the host through infectious aerosols. Mucosal immunity can cause a unique immunity in the mucosa-associated lymphoid tissue (MALT), which is crucial for preventing Mtb infection after which a systemic immune response is produced by the particular immune cells in MALTs being distributed in every part of the body.[24] Furthermore, from the mucosal tissue, specific dendritic cells move to these lymph nodes giving B and T cells mucosal homing abilities that allow them to respond to Mtb quickly.[24] Therefore, mucosal administration can quickly trigger both local and systemic immune reactions. Additionally, mucosal immunity can generate particular secretory antibodies in the mucosa to facilitate the anti-Mtb actions. In addition to being simpler to administer, oral vaccination is also relatively safe than other mucosal immunization techniques[25] as it can successfully pass through the intestinal epithelium and tonsils of neonates while inducing a particular immunity in the MALTs.

Additionally, oral BCG might serve as a booster shot. A combined ID and oral BCG vaccination strategy may generate the ideal mix of mucosal and systemic immune responses necessary for resistance to TB infection and disease development in healthy persons. Besides, no significant safety risks had been identified with the combination ID and oral BCG immunization strategy. Oral BCG caused a greater mucosal secretory IgA (sIgA) response and a larger frequency of mucosal cytotoxic T cells, whereas ID BCG immunization more potently induced a systemic Th1 response.[25] As a result, combining the two vaccination approaches might be taken into account in clinical practice to increase the efficacy of BCG. However, oral vaccination techniques are now used for fewer vaccines in clinical settings because they bypass the first-pass effect of the body, which lowers medication bioavailability and places a functional strain on the liver and kidneys. When BCG was first administered, it had to be taken many times to have the intended protective effect. However, overly high dosages often result in mucosal tolerance, preventing the elicitation of an immunological response. Therefore, using strong adjuvants is important to increase the immunogenicity and stability of BCG. Modern-day BCG is frequently wrapped in novel materials that promote mucosal absorption and boost the protective immunogenicity of the vaccine.[26]

5. Intravenous administration

The intravenous (IV) route of administration has triggered the greatest immune response of all the available administration methods. The IV formulation of BCG caused a 5x rise in entire lymphocytes, notably T cells, in bronchoalveolar lavage (BAL) samples when administered to Macaques Monkeys as opposed to oral or ID administration.[27] Additionally, cytokine concentrations traditionally linked to TB infection (IFN, IL-2, TNF, and IL-17) were 100 times higher after IV treatment than after ID, and these levels persisted longer than they did after ID dispensation. In M. tb-specific IgG, IgA, and IgM titer levels rose more quickly and for an extended period after IV treatment than they did after ID injection, indicating that the immune response may be further potent in thwarting TB.[27]

It is difficult to comprehend that BCG, a 100-year-old vaccine, has an incredibly high degree of immunity against TB after switching immunization routes. Importantly, the small number of evaluated clinical safety indicators revealed that IV BCG would be well accepted in NHP, indicating that this vaccination method might have promising potential for use in humans. Because it is difficult to utilize IV injection for mass vaccination, it is noted that it is now employed for pharmacological therapy and is seldom used for immunization. IV vaccination, however, has demonstrated remarkable immunological benefits in the prevention of several illnesses. According to a recent study, IV vaccination led to a larger percentage of TCF1+ PD-1+ CD8 + T cells and a stronger antitumor response than subcutaneous immunization. An additional investigation of the malaria prevention vaccine PfSPZ revealed that when compared to subcutaneous and ID delivery, IV immunization provided greater immunogenicity and

preventive role in people.[28] Similarly, in preparation for using this vaccination approach on a little number of speculative individuals, several clinical trials have started in Europe, Africa the United States, and some other locations.[28] A non-replicating sporeworm vaccine, nevertheless, is PfSPZ.[28] According to a recent study, a mouse model of the COVID-19 mRNA vaccination may result in acute myopericarditis.

Regarding BCG, even though this method of delivery has been utilized in humans in the past to treat cancer, a more thorough study is still required to ascertain the security and efficiency of injecting bacterial pathogens with the capacity for reproduction into human blood. Even while the route of administration of BCG may sound appealing, it has limited use since it necessitates additional medical expertise and healthcare professional training than an ID or oral delivery. Patients could object to having a drip since IV bags are difficult to stock and dispatch. Before making the necessary expenditures on IV vaccines, additional research must be done to determine how much more effective IV delivery is over the present ID regimen. Larger needles also increase the chance of problems.

6. Intranasal administration

Intranasal (IN) vaccinations are other typical methods of delivering BCG to the mucosa. When tried compared to oral administration, this technique of immunization does not call for as much vaccine, and thanks to improved delivery technology, aerosol spray vaccination is more practical and appealing. Additionally, Mtb often infects hosts through the respiratory system, indicating that the IT and IN BCG vaccines are quite efficient in triggering protective immunity. The primary elements of BCG effectiveness are effector T cells and TRM cells, both of which are produced in significant quantities by these vaccination techniques in the lung airway. In addition to producing IFN-γ and TNF-α, two cytokines that serve as both decisive factors of protective immunity against TB and draw $CD4^+$ T cells and B cells to the Mtb-infected region to boost local immunity, the airway-resident $CD8^+$ T cells display classic TRM features. In contrast, a combination of T-bet + effectors and regulatory T cells that express Foxp3+ may be seen in the airway-resident CD4 + T cells. In addition, compared to $CD4^+$ T cells generated by BCG delivered intradermally, those generated by BCG in this method also have a particular cellular profile.[29] Lung parenchyma and bronchoalveolar lavage fluid (BALF) include Ag-specific CD4 + T cells that exhibit a PD-1+ KLRG1- phenotype. By enhancing the homing impact, these cells can improve the local immunological function at the infection site. Due to this characteristic, lung parenchyma instead of the pulmonary vasculature can be used to isolate these cells.[29] Due to the homing effect, CD4 + T cells from the lung parenchyma have more control over Mtb infection than those from the pulmonary vascular system. In addition to these immune cells, the fluid from the human alveoli also includes hydrolytic enzymes that can aid BCG in better controlling Mtb in the mice infection model. The technical features of BCG mucosal vaccination, such as oral or aerosol administration, immunological dose, immune adjuvant mechanism, etc., require more study. BCG mucosal immunization is seen to have a promising future overall.

7. Scope of tuberculosis vaccination

The next generation of TB prophylactic immunizations has also undergone extensive investigation due to the broad extent of recorded efficiency of the BCG vaccine. Apart from BCG transformation, there seem to be three distinct vaccine evolution strategies: the production of a brand-new TB vaccine, the development of a unique recombinant vaccine procured from the current BCG vaccine, and the generation of a booster shot to strengthen an already-effective BCG shot.

8. Production of a brand-new TB vaccine

a. Vaccine based on distinct mycobacterium species

The extremely antigenic variants of the Proline-Glutamate/Proline-Proline-Glutamate (PE/PPE) type of proteins that *Mycobacterium indicus pranii* (*M. pranii*) shares with *M. tb* show that *M. tb* immunity can be effectively conferred.[30] Animals subcutaneously injected with heat-killed *M. pranii* had vigorous Th1 reaction with increased production of IL-12 and IFN-y cytokines concurrent with the ingress of $CD4^+$ and $CD8^+$ T cells in the lungs. The *M. pranii*-vaccinated mass showed significantly lower lung pathology and bacterial load along with improved survivorship as a result.[30] It's interesting to note that *M. pranii* can be supplied by the mucosal route, which increases the localization of $CD4^+$ and $CD8^+$ T cells in the lungs, dendritic cell stimulation and enlargement, and bone marrow-acquired dendritic cell (BMDC) migration by upregulating CCR7.[30]

Mycobacterium vaccae is a different mycobacterium species that's been used for medical treatments (*M. vaccae*). *M. vaccae* has repeatedly shown its effectiveness over *M. tb* in trials, potentially by generating a Th1-biased reaction whilst reducing Th2.[31] However, 3 and 5-dose regimens of *M. vaccae* were quite well endured with few side effects and offered immunity against *M. tb* in fit and HIV-infected participants.[32] In later human studies, *M. vaccae* failed to produce defensive advantages as a single dosage administration, although. Notably, neither regimen showed a change in HIV viral load or the conversion of PPD skin tests.[32] However, it is regularly used with immunotherapy in human studies, significantly boosting TB immunotherapy with a smear(sputum) clearance rate of 68% compared to 23.1% in the placebo group. As a consequence, *M. vaccae* is a highly effective anti-TB vaccine that is now through phase III of clinical studies.

b. Vaccine based on a fusion protein formation

Chen et al. first introduced and demonstrated the effectiveness of the AEC/BC02 vaccine in decreasing the bacterial load in guinea pigs. The AEC/BC02 vaccine is a novel vaccine created by fusing the *M. tb*-specific antigens Ag85B and ESAT-6/CFP-10 (AEC) and facilitated by BCG CpG and aluminum salt (BC02).[33]

Another well-known subunit vaccine is H1/IC31, which is manufactured using the IC31 adjuvant method and consists of the leucine-rich peptide ESAT6 and the oligodeoxynucleotide ODN1a. Regardless of their BCG level, history of *M. tb* infection, HIV estimation, or other conditions, studies have shown that a two-dose regimen of the H1/IC31 vaccination is harmless for grown-ups.

The subunit vaccine M72/AS01E uses the AS01 adjuvant system and combines the mycobacterial antigens M. tb32A and M. tb39A. In addition to HIV-infected and *M. tb*-infected adults, several studies have reported the clinical safety profile of the vaccination and its long-lasting polyfunctional CD4 + T cells that express IFN-γ, IL-2, and TNF- among BCG-vaccinated neonates.[34]

c. Vaccine based on inserting antigenic protein into a viral vector

Much work has gone into developing a novel vaccine that uses a viral vector in addition to weakening related organisms to trigger an immune response. Vaccine against cytomegalovirus and tuberculosis for rhesus macaques (RhMCV/Tb) includes vectors that exhibit 9 distinct *M. tb* proteins.[35] Antigen-85A (Ag85A) was the highest immunogenic of the nine proteins, able to activate and sustain high-frequency T cell responses, particularly the effector memory phenotype $CD8^+$ and $CD4^+$ T cells. When tried to compare the control group and BCG-vaccinated, the total illness and bacterial load were noticeably reduced in the RhCMV/TB-vaccinated group.[35] Therefore, a single dose of the RhCMV/TB vaccine is sufficient to elicit a strong immunological response and give protection against *M. tb*.

ChadOx1/PPE15 is a vaccine made out of a chimpanzee adenovirus bearing a mycobacterial antigen-encoding vector, similar to the RhCMV/TB vaccine.[36] PPE15, the most immunogenic of the expressed antigens, confers considerable protective immunity, which is seen in a decreased M. tb bacterial burden. However, the ChadOx1/PPE15 vaccine's ability to provide protection is dependent on how it is administered. While intramuscular dispensing stimulated the CX3CR1+ KLRG1+ phenotype, which is primarily detected in blood vessels and is unable to migrate to contaminated lung tissue, intratracheal dispensation caused distinctions of lung parenchymal naive $CD4^+$ and $CD8^+$ T cells into the protective CXCR3+ KLRG1-phenotype. Notably, delivery of the ChadOx1/PPE15 vaccine to mice that had previously received the BCG vaccine stimulated an immune response that was stronger than that of animals that had only received the BCG vaccine, as indicated by increased concentration of $CD4^+$ cells after immunization. In contrast, mice who had only experienced the ChadOx1/PPE15 vaccine and had not received the BCG vaccine before showed a strong CD8 + T cell response.[36] However, both immunization programmes were able to offer more protection than the corresponding control group.

The third type of vaccination that may be used in addition to BCG immunization is subunit vaccines. In this type of immunization, immunogenic antigens are isolated from fungi, bacteria, or viruses and fused to a non-immunogenic subordinate. The most effective subunit vaccines for tuberculosis are H1/IC31, AEC/BC02, RUTI, and M72/AS01E.

9. Developing a vaccine derived from a pre-existing BCG strain

Recombinant BCG vaccinations, which use genetically altered parental BCG strains, are increasingly supported by scientific research. Four recombinant BCG vaccines are being researched right now to take the place of the parental BCG strain.

a. An attenuated *M. bovis* BCG vaccination with a gene knockout variation that produces the zinc metalloprotease Zmp1 is called BCG-Zmp1, which is still in the preclinical stage. When collated to mice immunized with the wild-type BCG strain, Johansen et al. found that mice immunized with the zmp1-deficient BCG strain were able to exhibit an acute immune reaction via amplification of antigen-specific T cells and an increase in the release of cytokines, especially IFN-γ. Comparing the vaccine's security and effectiveness to BCG-vaccinated and non-vaccinated control groups, as determined by bacterial load in the spleen and lungs, was another research done on guinea pig models.[37] Additionally, immunocompromised mice who received the BCG-Zmp1 vaccine lived much longer than mice that received the BCG vaccine alone.[37] As a result, when collated with conventional BCG immunization in mouse models, the BCG-Zmp1 vaccine affords greater security against *M. tb* because of its elevated immunogenicity with enhanced safety profile.

b. SapM:TnBCG, which has a SapM gene excision from the original *M. bovis* BCG strain, is now undergoing preclinical studies. The SapM gene encodes excreted acid phosphatase, which is crucial for the pathogenesis of *M. tb* because it predominantly prevents host macrophage development and lysosome-phagosome fusion.[38] Mice immunized with SapM:TnBCG has an additional potent Th1 immune response than mice immunized with parental BCG, along with a decrease in bacterial load and an improvement in extended existence.[38]

c. CysVac2, a recombinant BCG vaccine that expresses a fusion protein combining the antigen Ag85B and CysD, a protein produced amidst chronic *M. tb* infection, is a preclinical vaccine candidate. After giving mice the CysVac2 vaccine, macrophages, neutrophils, and DCs flocked to the shot site in large numbers. The emptying lymph node and the spleen both had an expansion in the quantity of Ag85B-specific CD4 + T cells.[39] Furthermore, relative to BCG-vaccinated and unvaccinated control groups, there were more IFN-secreting cells in the CysVac2-vaccinated animals. Therefore, the CysVac2 vaccination considerably decreased the lung bacterial burden while conferring better resistance toward M. tb infection.[39]

d. d. A recombinant BCG vaccination called VPM1002 substitutes the urease C gene in BCG with the listeriolysin O encoding gene (hly) of Listeria monocytogenes.[40] The stimulation of autophagy, antigen presentation, immune system stimulation, and apoptosis are all made possible by Hly gene expression in BCG, which also makes it easier for the release of mycobacterial DNA and antigens from the cytosol. In animal models, including SCID and guinea pigs, healthy mice, and neonatal rabbits, the safety profile of VPM1002 was equivalent to that of BCG. In actuality, in mice that have received the VPM1002 vaccine, the VPM1002 strain is barely

lethal and does not spread to the lungs. Additionally, as collated to the BCG control group, the VPM1002 immunization showed extraordinary protective effectiveness with a substantial Th1 response and bacterial load reduction.[41] Both single-dose and three-dose regimens of VPM1002 were tolerated rightly in a phase I study and stimulated significant numbers of polyfunctional T cells that co-expressed TNF-γ, IFN-α, and IL-2 against *M. Tb*. In a similar vein, a phase II experiment found that VPM1002 and BCG were equally safe and effective for babies. A phase III study of post-exposure vaccination with VPM1002 is now being conducted in India because VPM1002 is successful in eradicating *M. tb* in M. tb-exposed mice.[41]

10. Developing BCG vaccine along with booster vaccine

In the last few decades, researchers have looked at the idea of BCG re-vaccination as a booster in BCG-primed populations, with varying degrees of success. According to one research, BCG re-vaccination enhanced the size of the immune response with powerful, multifunctional BCG-specific CD4 + T cells, but it did not affect the CD4 + T cells' response rate.[42] Furthermore, BCG revaccination offers a 45.4% effectiveness against *M. tb* infection, according to Nemes et al. On the other hand, two sizable randomized studies failed to find any further advantages of BCG revaccination against TB.[43] The regional variety and mutation of the BCG strains, which might result in various reactions and efficacies, may be the cause of these contradictory findings. However, in teenagers who had received their first dose of BCG at birth, priming with the vaccine caused minimal to severe injection site responses that were transient and went away without any lasting effects. The creation of novel booster vaccines originating either from a fusion protein, viral vector, or new bacterial species has resulted from more recent efforts to increase the effectiveness of first immunization.

Due to its innate ability to trigger a dynamic immunological response, **adenovirus type 5 (Ad5)** is employed as a vector to produce the mycobacterial antigen Ag85A to suppress *M. tb* infection. There is convincing proof that the BCG-prime Ad5Ag85A-boost regimen is more successful than BCG alone at treating TB in mice, cows, goats, and cattle. This caused a considerable rise in the frequency of Ag85A-specific CD4 + T cells, which provided better TB protection in the bovine model. It's interesting to note that immunological responses to Ad5Ag85A vaccination vary on the route of delivery. Hardly any protection against pulmonary *M. tb* in the mouse model, the intramuscular approach produced strong Ag85A-specific T-cell responses in the lung interstitial and spleen.[44] As a result, the intranasal approach improved protection after a pulmonary challenge by eliciting a more substantial Cellular response in the lungs.[44]

Another recombinant adenovirus carrying *M. tb* antigens is called **Ad5-CEAB**. When compared to BCG alone, the BCG-prime Ad5-CEAB-boost regimen dramatically potentiated the antigen-specific T cell responses in mice with an increase in the anti-mycobacterial cytokines IFN-γ, TNF-α, and IL-2.[45] As a result, it could be promising in terms of offering *M. tb* resistance in the BCG-vaccinated group.

A TLR4 agonist and glucopyranosyl lipid adjuvant were mixed with four mycobacterial antigens to create the subunit vaccine **ID93/GLA-SE**, which was then emulsified in an oil-and-water solution. Prior research on mice, nonhuman primates, and other species had shown the vaccination to be safe and effective. The capacity of ID93/GLA-SE to stimulate the development of CD4 + T cells into polyfunctional CD4 + T cells double releasing CD154+ IFN-γ+ or CD154+ TNF-α+ cytokines in mice and TB naive people[46] has been the subject of several research. In light of this, ID93/GLA-SE by itself may dramatically lower the bacterial load, improve survival in mice, and greatly boost antibody responses that in turn drive NK cell degranulation/stimulation and THP1 monocyte-mediated antibody-dependent phagocytosis in humans.[46] TST's integrity was preserved in ID93/GLA-SE vaccinated specimens, contrary to the BCG vaccination, keeping its usefulness in determining prospective *M. tb* exposure.[47]

The **Ad35-TBS** vaccine (AERAS-402), a recombinant adenovirus 35 vector carrying a distinct collection of *M. tb* antigens, evoked potent $CD8^+$ and $CD4^+$ T-cell responses in a dose-dependent manner in mouse models, just as the aforementioned vaccination. A stronger and more effective T-cell response was also generated by intramuscular Ad35-TBS than by intranasal vaccination.[48] However, compared to unvaccinated mice, both immunization methods resulted in improvements in lung histology.[48]

Ag85B, ESAT-6, and Rv2660c fusion protein are the components of the new subunit vaccine **H56:IC31**, which also contains the IC31 adjuvant system.[49] Numerous investigations have shown that this vaccination is immunogenic and effective in lowering bacterial load and lung disease in BCG-vaccinated mice[49] and nonhuman primates. H56:IC31 has a good development of antigen-specific polyfunctional CD4 + T cells that express IFN-γ, TNF-α, and IL-2, and it has a tolerable safety profile.[50]

Ag85A and ESAT6-CFP10 coupled with a dextran-binding domain fixed on dextran, combined with an adjuvant system incorporating a DEAE-dextran core and the TLR9 agonist, CpG oligodeoxynucleotide, make up the complex vaccination known as **GaMtbvac**.[51] GaMtbvac has strong immunogenicity and can produce significant levels of IFN-γ and antigen-specific antibodies. Compared to non-vaccinated mice, GaMtbvac-vaccinated animals showed considerably decreased bacterial burdens in the lungs and spleen, demonstrating excellent illness management.[51] The efficacy and safety of heterologous regimens were evaluated in human adults who had received the BCG vaccine.[52] GaMtbvac adverse effects were seen as being modest, momentary, and self-resolving.

Investigations are being conducted into the use of **DAR-901**, an inactivated whole-cell mycobacterial vaccine made from the SRL172 strain53. Due to the increase of IFN-γ production, DAR-901 offers a greater defense against *M. tb* collated to just BCG in both murine models[53] and adult humans.[54] Nevertheless, research examining the vaccine's effectiveness yielded contradictory findings. In contrast, von Reyn et al. showed that healthy persons with past BCG

vaccination may be exposed to 1 mg of DAR-901 and have both cellular and humoral responses, along with significant IFN-γ generation in the attendance of *M. tb* lysate. Therefore, the booster can be used as a preventive vaccination without preventing *M. tb* screening from continuing.[54]

H4**IC31**, also known as AERAS-404, is made up of a TB10.4 and Ag85B fusion protein that has been supplemented with a combination of ODN1a, an oligodeoxynucleotide high in leucine.[55] HC: IC31 was demonstrated to be secure and sufficient against *M. tb* challenges, with a notable decrease in bacterial load among mouse models.[55] IFN-γ, TNF-α, and IL-2.98 were co-expressed by antigen-specific polyfunctional $CD4^+$ T cells, which provided protection. With greater effectiveness at a low dosage, H4:IC31 exhibits potential as a BCG booster.[56]

The live-attenuated *M. tb* vaccine **MTBVAC** has two genetic deletions that significantly reduce the pathogenicity of mycobacteria: fadD26 and phoP. When tested on guinea pigs and mice with impaired immune systems, MTBVAC was pronounced safe.[57] Congruently, MTBVAC's safety in newborn mice was suggested by Aguilo et al. who found that it did not affect growth and development.[58] Furthermore, when given subcutaneously to adults and children, M. TBVAC exhibited a comparable safety profile to BCG. Overall, as compared to BCG vaccination, M. TBVAC gives more protection in mouse models.[58] Furthermore, when collated to BCG immunization, the initial phase I study in people showed superior safety, comparable immunogenicity, and a larger polyfunctional CD4 + T cell response.[59] Overall, the data point to MTBVAC conferring more immunity than BCG both when given alone and much more so when given in the BCG-MTBVAC prime-boost regimen.

11. Conclusion

Mtb is a very "robust" and "tricky" intracellular pathogen that has incredibly effective immune evasion mechanisms and can stay with infected hosts for a very long period. Thus, TB vaccines should be able to gently alter the intricate regulatory signals brought on by *M. tuberculosis*, achieve an exquisite balance between inflammatory and regulatory immune responses, and sustain potent memory immunological reactions over an extended period. For the global Mtb control plan to be effective, the BCG immunization programme must be continually updated.

The immunological impact of BCG can be enhanced by either altering the vaccine method or depending on the prime-boost immune approach. Although conducting clinical trials is not without its difficulties, one of the main problems is the scarcity of precise and trustworthy immunological markers. Thus, while doing TB vaccine research, the sample size must be as broad as feasible and the scope of vaccination evaluation should be as broad as possible.

In many parts of the world, BCG immunization is still important throughout childhood. Although its efficacy has long been questioned, more recent studies using stricter controls and settings have provided light on how vaccination strain changes may impact the immune system after delivery. We may be able to schedule a better and regulate vaccination-induced immune responses if we pay closer observation to the strain of the BCG vaccine that is used and any potential epigenetic alterations it causes. This is because the procedure of action of the BCG vaccine is still being studied. The invention of the next generation of vaccines and its appendages, as well as inventive techniques to reconstitute an old vaccination into a fresh, more effective form, are the results of further attempts to completely eradicate the disease. Promises to be a developing field of study that might result in a vaccination that is more reliable and efficient.

Conflicts of interest

The authors have no conflict of interests.

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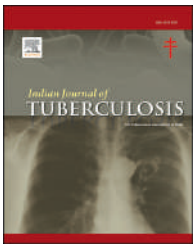

Review article

Adolescent tuberculosis in the ICU

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ABSTRACT

TB is a major concern in the paediatric age group, especially in India. More than 3.33 lakh children between 0 and 14 years of age are affected by TB. Adolescent tuberculosis has been a neglected area and this age group accounts for about 800,000 cases of tuberculosis (TB) cases every year. Information regarding adolescent tuberculosis patient requiring ICU admission/care is very scanty (unlike adult tuberculosis), and the authors believe that the mode of ICU presentation and challenges in adolescents would almost be the same as in adults, although the outcome is generally expected to be better in the adolescent population in view of lesser comorbidities when compared to adults. ARDS, multiorgan dysfunction and meningitis are the most common reasons for admission to ICU. Critically ill patients with TB carry a high mortality and the increased mortality is likely due to multiorgan dysfunction, nosocomial infections and sepsis. Advanced disease with chronic undernourishment influences not just morbidity but mortality as well. Further, the heavy financial burden incurred for ICU care in TB patients with poor expected outcome is a major concern since TB occurs predominantly in low socio-economic populations.

1. Introduction

In India, TB is a major concern in the pediatric age group. It is estimated that about 3.33 lakh children between 0 and 14 years of age are affected by TB with pulmonary TB being the most common.[1] The best period with the least incidence is mid-childhood when compared to the initial/early childhood or adolescent period. The definition of adolescence and hence adolescent tuberculosis has been highly vague and variable in the medical literature. Adolescence has been defined as the age between 10 and 24 years in view of the fact that numerous developmental processes will still keep going between 18 and 24 years.[2] The adolescent period has further been divided into young adolescents (10–14 years), older adolescents (15–19 years) and young adults (20–24 years). There is an increased incidence and prevalence of tuberculosis infection as well as disease in adolescents due to various reasons. The probable reasons cited include increased social contact, significant hormonal changes and associated immune system changes.[3] Despite the above facts, adolescent tuberculosis has been a neglected area and this age group accounts for about 800,000 cases of tuberculosis (TB) cases every year.[3]

The clinical presentation is variable as transition occurs from childhood form of TB to adult TB in adolescence.[4] The burden of multidrug resistant TB is also significant in this age group. An estimated 5.4% of new cases and 36% of previously treated cases have MDR TB in this age group as per WHO data.[5]

Tuberculosis in adolescent age groups is different in terms of presentation and social impact when compared to adults;

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and adolescents have higher bacillary load and can disseminate bacilli similar to adults and thus early identification and treatment is very important to curtail the spread of TB.[1] It is seen in various studies that the risk of MTB infection is maximum between 12 and 24 years age group and also a higher risk of progression to disease in adolescents.[6,7] Unfortunately, inadequate care facilities for adolescents especially in regions which have a high prevalence of tuberculosis is a big challenge and the issue needs to be tacked urgently.

It is seen that adolescents with pulmonary tuberculosis commonly have bacteriologically confirmed disease, however paucibacillary disease does not rule out tuberculosis and also is seen commonly. As transmission of TB in adolescents is very common, contact tracing is very crucial. The treatment of tuberculosis in adolescents is similar to adults.[8] Non-adherence to treatment is another major challenge in adolescents. In a study from South Africa, about 41% of patients had poor compliance to antitubercular medications.[9]

2. Adolescent tuberculosis patient in ICU

Despite an extensive search in the medical literature, information regarding adolescent tuberculosis patient requiring ICU admission/care is very scanty (unlike adult tuberculosis), and the authors believe that the mode of ICU presentation and challenges in adolescents would almost be the same as in adults, although the outcome is generally expected to be better in the adolescent population in view of less comorbidities when compared to adults.

Most of the patients with tuberculosis are treated as outpatients and only few of them may need hospitalization due to complications of TB like pneumothorax, empyema, consolidation, ARDS, hypoxemia due to extensive lung parenchymal involvement, severe extra-pulmonary TB, and other complications like hemoptysis and thromboembolism. Some of them may get admitted with complications of drug therapies like ATT induced hepatitis and severe hypersensitivity reactions. On an average about 3.4% who are admitted to hospital are transferred to intensive care unit.[10] In a single center study from India, only 1.7% of patients had active tuberculosis.[11] It needs to be remembered that the criteria for ICU admission could vary between country to country, center to center, government versus private facilities, and also depends on the affordability of the patient.

ARDS, multiorgan dysfunction and meningitis are the most common reasons for admission to ICU.[10] Unfortunately, the patients with tuberculosis admitted to ICU have a poor outcome and also have a high mortality rate (60%). The most common causes of increased death are multiorgan dysfunction, nosocomial infections and sepsis.[12]

There are certain unique challenges faced by treating intensivists in the management of tuberculosis patients which could be diagnostic and/or treatment related. The challenge could be diagnostic in which case represents an unconfirmed or suspected tuberculosis patient who gets admitted to ICU with advanced organ failures where the patient's life needs to be supported and stabilized before going onto any diagnostic evaluation or procedures, with the latter aspects often getting delayed.

Delay in confirmation of diagnosis of tuberculosis in the ICU setting can lead to delayed initiation of treatment and thus poor outcomes. Hence treatment should be initiated based on presumptive diagnosis derived from initial history, evaluation and imaging findings.[13-16] We should also note that critical illness itself should not be an indication for drug resistant tuberculosis and standard first line agents should be started. Second line agents should only be considered if history or TB NAAT is suggestive of drug resistant TB.[17]

Miliary tuberculosis often produces scanty sputum and invasive diagnostic procedures like bronchoscopy/BAL and trans bronchial lung biopsy may be required for accurate diagnosis. However, the above interventions itself could worsen the pre-existing respiratory failure pushing the self-ventilating patient into early intubation and mechanical ventilation with increased risk for ventilator associated events. The diagnosis of patients presenting with tuberculous meningitis is often a diagnostic challenge whenever the CSF is negative for TB PCR since the sensitivity varies between 75 and 100% though the specificity is 94–100%. The sensitivity of Xpert MTB/RIF in other specimens (e.g. pleural fluid, lymph node, gastric lavage etc) are also low which makes a big challenge in negative predictability. Many patients could present with an ongoing active pulmonary tuberculosis associated with pre-existing cor-pulmonale secondary to the fibrotic/bronchiectatic sequelae related to previous tuberculous disease hence adversely affecting the outcome. Increased incidence of MDR/XDR tuberculosis in pulmonary and extra-pulmonary tuberculosis patients getting admitted in the ICU increases the morbidity and mortality when compared with non- MDR/XDR disease. Association of tuberculosis with immunocompromised states including HIV infection often presents with disseminated disease which has been known to increase the mortality making the ICU management of such patients extremely challenging. Treatment MDR tuberculosis not only needs modified expanded drug regimen risking increased incidence of side effects and drug interactions. Also, there is an ongoing concern regarding alteration of pharmacokinetics of anti-tubercular drugs (eg rifampicin) in critically ill patients.[18] There is significant alteration of pharmacokinetics of ATT in critically ill patients.[19] The possible reasons are crushing and administration of TB medications via nasogastric tube, alteration of enteral absorption due to circulatory shock, not able to feed via enteral route due to intestinal obstruction, peritonitis or in postoperative patients who are not feed enterally.

This can be addressed by using anti TB drugs via parenteral route until enteral feeding and absorption is well established. The challenge here will be the unavailability of first line agents as intravenous preparations. Various combinations of second line injectable agents have been tried,[20] certain combinations have also used injectable linezolid which has good antitubercular activity when injectable rifampicin is unavailable.[21]

Many patients presenting to the ICU have advanced disease with chronic undernourishment which influences the morbidity as well as mortality. Finally, the heavy financial burden incurred for ICU care in TB patients with poor expected outcome is a major concern since TB occurs predominantly in low socio-economic populations.

Let us understand a few critical TB associated conditions in ICU.

3. Pulmonary tuberculosis

It is the most common cause of ICU admission and most of the deterioration is due to TB associated ARDS.[22] Patients most often present with classical symptoms of tuberculosis like fever, cough, weight loss and worsening respiratory failure and respiratory distress. Radiological evaluation can show bilateral infiltrates, cavities and apical involvement with similar pictures in adolescents as well as adults.

Patients with pulmonary tuberculosis are managed in negative pressure isolation facilities in the ICUs in view of potential for air-borne spread. Any patient with diagnosed or suspected tuberculosis with potential of transmission should be placed in an airborne infection isolation room with a negative pressure (0.01-in water gauge]) of at least 2 Pa lower than the surroundings with a minimum of 12 air changes per hour. HEPA filters must be used if air is recirculated from these isolation rooms.[23] Additional precautions must be taken in patients who are intubated. Generally HME filters are placed at the expiratory limb of the ventilatory circuit to provide additional filtration of airborne particles.[23]

In addition to the above, the staff involved in care of such patients should use a properly fitting N 95 masks/respirators when entering the isolation rooms. All aerosol generating procedures including endotracheal intubation must be done with maximum precautions. Aerosols can be generated during suctioning, nebulisation, non-invasive ventilation, bronchoscopy, other procedures like intubation and tracheostomy.[24] Generally, a TB patient will have the capacity of transmission or will be considered infectious till 14 days of effective treatment.[23]

Trial of non-invasive mechanical ventilation or High Flow Nasal Cannula can be tried in patients with better utilization of the former especially in patients who are having underlying obstructive airway disease or increased work of breathing before going ahead with intubation and mechanical ventilation. There is a theoretical concern regarding increased transmission risk to health care workers in view of potential for increased aerosol generation in non-invasive ventilatory settings though the concern has not been proven in clinical settings.[25] Invasive mechanical ventilatory management consists of lung protective strategies, PEEP optimization & prone ventilation in severe ARDS along with appropriate antitubercular therapy. There are reports of successful outcomes (Extra Corporeal Membrane Oxygenation) ECMOs in patients with TB induced ARDS.[26,27] However, the overall outcomes are poorer in such patients and carry higher mortality on mechanical ventilation.[28,29]

Tuberculous broncho-pleural fistula (BPF) is another challenging scenario to the intensivist who manages such patients with respiratory failure on mechanical ventilation especially with large leaks in excess of 500 ml per breath. The commonest clinical scenario is when a cavitary parenchymal tuberculosis is complicated with tension pneumothorax/pyopneumothorax requiring mechanical ventilatory support. Such persistent air leaks are also not uncommon following lung resections in the management of MDR/XDR tuberculosis. The BPF results in under-expansion of the ipsilateral lung, along with incomplete recruitment, barotrauma and volutrauma to the opposite lung, and failure to maintain PEEP. Consistent leak with loss of tidal volume causes systemic hypoxemia and respiratory acidosis. There are step wise approaches in the management of BPF on mechanically ventilation[30] which may include bronchoscopic interventions, lung protective strategies ventilatory strategies ipsilateral lung ventilation (ILV), High Frequency Jet Ventilation (HFJV) and High Frequency Jet Oscillation (HFJO), VATS assisted or surgical repair with or without support with ExtraCorporeal Membrane Oxygenation (ECMO). Except in dire emergencies, most thoracic surgeons prefer to wait at least for a few weeks (once the patient is put on ATT) before performing an open resection and repair due to the feared complication of persistence of leak in active tuberculosis (even after repair). Spontaneously healing and closure of small or medium sized fistula may happen in few cases if the lung could optimally be supported without further complications on mechanical ventilator or ECMO.

4. Tubercular meningitis

Even though TB meningitis occurs less frequently compared to pulmonary tuberculosis in the ICU setting, it accounts for about 6–18% of ICU admissions due to TB[31] and carries higher mortality of about 60% and can have neurological disability in 25% of patients.[32,33] In adolescent age group, it can occur as a part of disseminated tuberculosis. It presents with classical features of meningitis fever, headache, seizures also associated with weight loss and loss of appetite. They can also present with other features like decreased sensorium due to increased intracranial pressure, hydrocephalus, tuberculomas, basal arachnoiditis or cerebral infarction. Diagnosis is using a combination of imaging and CSF analysis. Treatment includes antitubercular treatment and corticosteroids. Intubation and mechanical will be required in patients with low GCS, refractory seizures or respiratory failure secondary to underlying disease or aspiration. ICP monitoring can be done in patients who have complications like hydrocephalus by measuring the Optic Nerve Sheath Diameter (ONSD) for the upper limit is taken as 5 mm when measured 3 mm behind the globe.

5. Haemoptysis

An occasional patient with active pulmonary tuberculosis or post TB sequelae can present with moderate to severe (or life threatening) hemoptysis and can get admitted to the ICU for dynamic monitoring and treatment intervention. The vast majority of massive hemoptysis in pulmonary TB originates in the bronchial arteries, and less than 10% are pulmonary arterial in origin. Rasmussen's aneurysm is pseudoaneurysmal dilation of a pulmonary artery adjacent to a cavity which could result in life threatening bleeding into the cavity. Apart from routine supportive care, bronchial artery embolization is a well-accepted first line minimally invasive intervention which involves embolization of the culprit vessel

with polyvinyl alcohol (300–600 micrometer), glue, coils, or gelatin sponge. Though there is significant achievement of haemostasis following the procedure, recurrence could occur in up to 50% of the cases.

6. Adrenal insufficiency

It can occur in 6–10% of patients with active tuberculosis.[34] Adrenal involvement from tuberculosis can occur due to direct involvement of the gland by tuberculosis or secondary to hematogenous spread. Tuberculosis is the second most common etiology for chronic adrenal insufficiency being second only to autoimmune cause. Moreover, tuberculosis patients can develop functional adrenal insufficiency while on rifampicin which enhances the metabolism of cortisol. Adrenal insufficiency can also contribute to refractory hypotension in patients with tuberculosis. Treatment in acute adrenal insufficiency includes IV hydrocortisone 100 mg 6–8 hourly (or a continuous infusion).

Tuberculosis in ICU can also present with multiorgan dysfunction syndrome and septic shock which most often occurs due to secondary bacterial infections.

Other presentations include pericardial effusion causing cardiac tamponade, airway obstruction in laryngeal TB, disseminated intravascular coagulation (DIC), and seizures caused by tuberculomas in the brain. Importantly, TB patients may also experience acute liver failure due to hepatotoxic drugs and rarely acute renal failure, mainly rifampicin-induced.[35]

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6.1. *TB with HLH*

Tuberculosis is one of the commonest causes of secondary hemophagocytic lymphohistiocytosis (HLH).[36] In a study done by Padhi et al,[37] more than 70 cases of TB with HLH has been reported.[37] H score can be used to identify HLH. A high index of suspicion is needed in patients who present with cytopenias and jaundice. The options for treatment include treating the primary cause (TB) and sometimes additional immunosuppressants can be used.[36,37] TB associated HLH generally carries a very high mortality despite identification and appropriate treatment (50%).

7. Final word on diagnostics

Standard diagnostic modalities for TB include sputum Ziel Niehlson staining, among others. However, the gold standard remains culture based drug susceptibility testing (which takes over a month to perform - this again depends on the bacterial burden).

Detection of AFB in conventional smear requires about 10,000 bacilli per mL. More concentrated samples offer better yield to testing thereby improving sensitivity to almost 90%.[38]

Molecular tests for TB have faster turnaround time than older culture-based DST with results being made available within hours to days. The most commonly employed ones are Nucleic Acid Amplifiation Tests (NAAT). Some NAA tests can detect genes that code for drug resistance as well. These are very likely as sensitive and specific as ZN Staining in the diagnosis of TB. Currently available GenXpert kits have been developed to detect multi and extensively drug resistant variants also. (Xpert MTB/XDR).[39]

Newer techniques such as pyrosequencing, Sanger sequencing, and next-generation sequencing are different methods of sequence based testing, which identify genetic mutations responsible for drug resistance and are shown to have greater accuracy than the currently used probe assays.[40]

Despite all of these, diagnosis of TB still remains challenging and exerts a significant strain on already difficult resources.

Thus, tuberculosis in general and adolescent tuberculosis in particular, remains a very important concern and often a neglected disease. The challenges in identification and addressing concerns like partial adherence or non-adherence is very important. Early diagnosis is paramount and rapid diagnostic tests are the need of the hour. Patients who present to ICU with TB even though rare needs special attention as it carries a higher mortality.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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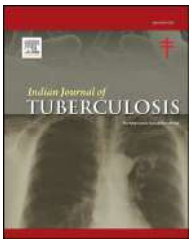

Review article

Stool CBNAAT: Alternative tool in the diagnosis of pulmonary tuberculosis in children

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ABSTRACT

Tuberculosis (TB) remains a significant public health concern, especially in children. The World Health Organization now provides estimates on pediatric TB cases and deaths, underscoring the urgency of addressing this issue. In India, childhood TB contributes significantly to the global burden, with a notable gap between reported cases and estimated incidence. Diagnosing pulmonary TB in children presents challenges, primarily due to difficulties in obtaining suitable respiratory specimens. Rapid tests like Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) have shown promise in enhancing diagnostic sensitivity. Recent research suggests that stool samples offer a non-invasive alternative for diagnosing pulmonary TB in children, with good diagnostic accuracy observed for stool CBNAAT. Furthermore, stool CBNAAT results demonstrate high agreement with gastric aspirate CBNAAT in TB diagnosis. Various stool processing methods, such as centrifugation, filtration, and sedimentation, have shown improved results for CBNAAT testing. However, it is crucial to standardize these methods to ensure consistent and comparable outcomes. Integrating stool CBNAAT into existing diagnostic algorithms for pediatric TB can enhance accuracy and efficiency in diagnosis. When implementing these algorithms, local resources, epidemiological context, and healthcare settings should be taken into account. Stool CBNAAT holds promise for microbiological confirmation of pediatric pulmonary TB, especially in resource-limited settings where obtaining representative respiratory specimens is challenging. Further comparative studies and standardization of stool processing methods are necessary to determine the most suitable approach in different contexts. By doing so, we can make significant strides in improving TB diagnosis and management in children.

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1. Introduction

Tuberculosis (TB) poses a significant public health concern, particularly among children. National tuberculosis programs have historically regarded TB in children (those under 15 years old) as a relatively minor issue, and several factors contribute to this perception. One such reason is the lower bacterial load of TB in children, which results in a reduced risk of disease transmission. However, this circumstance also makes it challenging to identify and treat TB in children, leading to a lower priority in addressing the problem.

Starting in 2012, the World Health Organization (WHO) started publishing estimates concerning pediatric TB cases and related deaths. Since 2020, the data has been further categorized by age groups: 0–4 years, 5–9 years, 10–14 years, and 15–19 years.[1] The Global Tuberculosis Report now includes information on drug resistance profiles and treatment outcomes. The estimated mortality rate for children with TB who do not receive specific treatment is 22%. However, this rate rises significantly to 43% for children younger than 5 years old. In contrast, among treated children, the mortality rate drops to a significantly lower percentage of 0.9%.

In India, it is estimated that around 3.06 lakh children (aged 0–14 years) contract TB annually, comprising approximately 11% of the total reported TB cases to the National Tuberculosis Elimination Program (NTEP).[2] Childhood TB in India constitutes about 31% of the global burden related to the disease. However, despite this significant burden, children only make up 6–7% of all the patients treated under NTEP every year, resulting in a gap of 4–5% between the total estimated incidence and the actual cases notified.

The diagnosis of pulmonary TB in young children is challenging as they seldom expectorate spontaneously and hence obtaining an appropriate specimen from the lower respiratory tract is difficult. In addition, pulmonary TB in children is often paucibacillary as children are less likely to form cavitary lesions which contain the bacilli.

Without microbiological confirmation, both over and under diagnosis is very common. Also, with the increasing number of cases of MDR TB, microbiological confirmation of diagnosis at the outset will help in identifying resistant cases early in the course of management. In view of the lower sensitivity of sputum smear and culture in children, WHO in 2013, made a conditional recommendation for the use of rapid molecular testing, that is, Cartridge Based Nucleic Acid Amplification Test (CBNAAT) as an initial test for rapid and accurate diagnosis with microbiological confirmation. Previous studies have shown better sensitivity of CBNAAT as compared to sputum smear microscopy. These studies mainly performed CBNAAT on gastric aspirate, induced sputum, broncho alveolar lavage etc. Invasive procedures are required to obtain these samples. In the resource limited primary care settings with a dearth of trained staff, obtaining such specimens is often difficult. Children tend to swallow sputum when they cough rather than expectorate and Mycobacterial DNA has been shown to survive the harsh digestive environment of the gastrointestinal tract. Hence, stool is a potential specimen for diagnosis of pulmonary TB in children as it can be collected non-invasively. If found to be effective in detection of cases of TB, it would greatly improve the microbiological confirmation rates, especially in areas where obtaining gastric aspirate and induced sputum is not feasible.

Thus, the development of more accurate estimates of pediatric TB cases, better diagnostic facilities targeting children with paucibacillary disease and difficult to get samples, represents an urgent need for public policy programming and resource allocation and, consequently, reduction of TB morbidity and mortality among children.

2. Stool CBNAAT in diagnosing TB

Stool CBNAAT is a new diagnostic test that has been shown to be effective in detecting TB in children. CBNAAT stands for cartridge-based nucleic acid amplification test. This test is a rapid and sensitive test that can detect the presence of *M. tuberculosis* DNA in stool samples. Stool CBNAAT is easy to collect and can be performed in a variety of settings. The WHO consolidated guidelines on the management of TB in children and adolescents published in 2022 recommend that in children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and rifampicin-resistance detection in sputum, gastric aspirate, nasopharyngeal aspirate and stool rather than smear microscopy/culture and phenotypic DST.[3]

In a study, conducted by one of the authors and his colleagues, published in the Indian Journal of Tuberculosis in 2022, the diagnostic accuracy of stool CBNAAT was evaluated in children with suspected TB.[4] The study included 75 children, aged 6 months to 12 years, who were presumptive cases of pulmonary TB. Gastric aspirate and stool samples were obtained from all participants. CBNAAT was performed on both samples and Gastric aspirate culture on MGIT was considered as the gold standard. The results of the study showed that stool CBNAAT had a sensitivity of 73% and a specificity of 97%.

Recent comprehensive evaluations of the utilization of CBNAAT for detecting *Mycobacterium tuberculosis* in stool samples revealed combined sensitivities ranging from 50% (95% confidence interval [CI]: 44–56) to 67% (95% CI: 52–79), and combined specificities ranging from 98% (95% CI: 96–99) to 99% (95% CI: 98–99) when compared to a microbiological reference standard.[3,5–9] Some experts have suggested that using two specimens, preferably of different types, may improve the likelihood of obtaining a bacterial diagnosis of tuberculosis due to the incomplete sensitivity of individual specimen types. However, the WHO has not yet issued any specific recommendations regarding this matter. Further research and evidence are likely needed to establish standardized guidelines for using multiple specimens in TB diagnosis.

3. Stool processing methods

There have been various approaches outlined for processing stool samples for CBNAAT testing.[10–17] These methods differ in terms of the techniques employed to suspend the stool (such as manual shaking or mechanical shaking using a

vortex) and to separate *Mycobacterium tuberculosis* bacilli from stool debris (including centrifugation, filtration, and sedimentation). As a result, the methods vary in terms of complexity, labour requirements, time commitment, and the equipment, supplies, and infrastructure needed.

Some authors have described relatively straightforward methods for stool processing that do not require centrifugation, such as the approaches proposed by Banada et al., Walters et al., and Andriyoko et al.[12,14,15] Other simple methods include the optimized sucrose flotation (OSF) method developed by the TB-Speed consortium and the simple one-step (SOS) method developed by the KNCV Tuberculosis Foundation (KNCV).[16,17]

As there are no standardized instructions from the manufacturers regarding the processing of stool specimens for CBNAAT testing, different studies have employed various methods for specimen processing. This variability in processing methods may have contributed to the inconsistent sensitivities reported across these studies. In a recent meta-analysis, researchers conducted a subgroup analysis based on the use of centrifugation.[6] The pooled sensitivity of studies that utilized sample centrifugation was found to be higher, with a sensitivity of 0.68 (with a 95% confidence interval of 0.60–0.76), compared to studies that did not use centrifugation. The latter group showed a sensitivity of 0.35 (with a 95% confidence interval of 0.28–0.43). This suggests that employing centrifugation as part of the specimen processing method may improve the sensitivity of CBNAAT testing on stool samples.

Due to significant variations in stool processing methods and study designs, as well as the resulting heterogeneity among pooled sensitivity and specificity values reported in the four systematic reviews, there is a need for standardized comparative studies.[6–9] These studies would help establish a consistent framework for evaluating and comparing the different methods used for stool processing. By addressing the existing heterogeneity, such studies would provide more reliable and comparable data on sensitivity and specificity values for these methods.

Jasumback et al. conducted a study in which they applied four different stool processing methods to stool samples that were intentionally spiked with varying concentrations of Mycobacterium bovis bacille Calmette-Guerin (BCG).[18] Walters et al. utilized a method that involved centrifugation, resulting in a higher frequency of BCG detection at lower concentrations.[19] Specifically, in 5 out of 5 replicate samples, BCG was detected at a concentration of 10^3 colony-forming units (cfu)/mL, and in 3 out of 5 replicate samples at a concentration of 10^2 cfu/mL. In comparison, the method employed by Banada et al. resulted in BCG detection in 3 out of 5 replicate samples at a concentration of 10^3 cfu/mL, and in 1 out of 5 replicate samples at a concentration of 10^2 cfu/mL. The centrifuge-free swab-based method used by Walters et al. resulted in BCG detection in 1 out of 5 replicate samples at a concentration of 10^3 cfu/mL, and in 0 out of 5 replicate samples at a concentration of 10^2 cfu/mL. The SOS stool method yielded BCG detection in 3 out of 5 replicate samples at a concentration of 10^3 cfu/mL, and in 1 out of 5 replicate samples at a concentration of 10^2 cfu/mL. However, the sample sizes were too small to conduct statistical analyses. The SOS stool method was deemed the most suitable for low-resource settings due to its low error rate, short processing time, and minimal requirements in terms of biosafety precautions and laboratory equipment.[18] However, yield of centrifugation method was found to be higher demonstrating better sensitivity.

In an in vitro study conducted by the TB-Speed consortium in collaboration with KNCV, two stool processing methods were compared: the two-step method described by Andriyoko et al. and the SOS stool processing method.[17] The comparison involved using stool samples with varying consistencies that were spiked with different concentrations of *M. tuberculosis*. The SOS stool method demonstrated higher sensitivity compared to the two-step method. This difference was attributed to the additional dilution step used in the two-step method, which potentially affected the detection of *M. tuberculosis*.

Additionally, two parallel studies led by the Foundation for Innovative New Diagnostics (FIND) and the TB-Speed consortium assessed three stool processing methods simultaneously.[20] These studies were conducted at referral laboratories in Africa and Asia. The evaluated methods included the disposable stool processing kit (SPK), which was optimized based on the methods described by Banada et al. and Walters et al., as well as the optimized sucrose flotation (OSF) method and the SOS stool processing method.

The studies conducted by FIND and the TB-Speed consortium also examined the user acceptability and feasibility of the stool processing methods, particularly focusing on their applicability for TB diagnosis in children.[20] The findings indicated that stool as a sample for TB diagnosis was well accepted by users. All evaluated methods were deemed easy to process by laboratory staff at the reference level, receiving high median ease-of-use scores. However, most users believed that these methods could not be performed by non-laboratory staff, such as nurses and healthcare workers, in primary healthcare settings that lack laboratory facilities. Among the assessed methods, the SOS stool method emerged as the preferred choice. This preference stemmed from the fact that the SOS stool method does not require additional equipment or supplies compared to sputum-based Xpert testing.[20]

Table 1 summarizes the results of some recent studies using different stool processing methods.

4. Integration of stool CBNAAT into existing diagnostic algorithms

Integration of Stool Xpert MTB/RIF into existing diagnostic algorithms for pediatric TB can enhance the accuracy and efficiency of diagnosis. The integration should consider the unique characteristics of pediatric TB, such as the challenges in obtaining respiratory samples and the need for rapid diagnosis to ensure timely initiation of treatment. Here are some considerations for integrating Stool Xpert MTB/RIF into pediatric TB diagnostic algorithms.

1. Screening and Clinical Evaluation: Stool Xpert MTB/RIF can be included as an initial screening test for children

Table 1 – Summary of some recent studies arranged chronologically which used different methods of stool processing for detection of Tuberculosis using CBNAAT.

Authors; Year [Reference No.]	Country	Sample Size	Stool Processing Method Includes				Reference Standard		Stool Xpert MTB/RIF performance	
			Dilution in	Vortexing	Centrifugation	Filtration	Assay	Sample	Sensitivity in % (95%CI)	Specificity in % (95%CI)
Agarwal et al.[4]; 2022	India	75	PBS and XSR	Yes	Yes	No	Culture or Xpert	GA	73 (39–94)	97 (89–100)
Dubale et al.[21]; 2022	Ethiopia	152	XSR	No	No	No	Culture, Xpert or smear microscopy	Sputum or GA	100 (69–100)	100 (97–100)
de Haas et al.[17]; 2021 (SOS)	Ethiopia	123	XSR	No	No	No	Culture or Xpert	Sputum and GA	78	–
Ainan et al.[22]; 2021	Tanzania	225	DW and XSR	Yes	No	No	Xpert and/or Culture	Sputum or GA	62.5 (25–92)	100 (98–100
Song et al.[23]; 2021	Kenya	294	PBS, NALC-NaOH, XSR	No	Yes	No	Culture or Xpert	Sputum	60 (36–81)	89 (52–100)
Ngadaya et al.[24]; 2020	Tanzania	590	DW and XSR	Yes	Yes	No	Culture	Sputum	84 (81–87)	93.4 (98.5–99.9)
Andriyoko et al.[15]; 2019	Indonesia	36	PBS and XSR	No	No	No	Xpert	GA or IS	100	87.5
LaCourse et al.[25]; 2018	Kenya	165	PBS, NALC-NaOH, XSR	No	Yes	No	Culture or Xpert	Sputum or GA	70 (35–93)	100 (97–100)
Walters et al.[14]; 2018	South Africa	280	PBS and XSR	Yes	No	Yes	Xpert	Sputum, IS or GA	44.4 (13.7–78.8)	99.1 (96.8–99.9)
Memon et al.[26]; 2018	India	100	PBS, NALC-NaOH, XSR	No	No	No	Culture	GA or IS	11.5 (2.4–30.1)	98.6 (92.7–99.9)
Hasan et al.[27]; 2017	Pakistan	50	PBS and XSR	Yes	Yes	No	Culture or Xpert	Sputum or GA	82 (48–98)	95 (82–99)
Chipinduro et al.[28]; 2017	Zimbabwe	218	PBS and XSR	Yes	Yes	No	Culture or Xpert	IS	68 (43–87)	98 (95–99)
Walters et al.[19]; 2017	South Africa	351	PBS, NALC-NaOH, XSR	No	Yes	No	Culture	GA, IS, string sample	32 (21–44)	100 (98–100)
Banada et al.[12]; 2016	South Africa	38	Commercial buffer	Yes	No	Yes	Xpert	GA or IS	85 (62–97)	100 (98–100)
Moussa et al.[29]; 2016	Egypt	115	DW, PBS, NALC-NaOH, XSR	No	Yes	No	Culture	Sputum or IS	83 (67–94)	99 (93–100)
Nicol et al.[10]; 2013	South Africa	115	PBS and XSR	No	Yes	No	Culture	IS	47 (23–72)	99 (94–100)

DW: Distilled water; GA: Gastric Aspirate; IS: Induced Sputum; NALC-NaOH: N-acetyl-cysteine-sodium citrate-sodium hydroxide; PBS: Phosphate buffered saline; XSR: Xpert sample reagent.

suspected of having TB, especially in settings where obtaining respiratory samples is challenging. The algorithm may involve a combination of clinical evaluation, medical history, and risk assessment, followed by Stool Xpert MTB/RIF testing as a first-line diagnostic tool.
2. Sample Collection and Processing: Develop standardized protocols for collecting and processing stool samples for Stool Xpert MTB/RIF testing in pediatric patients. Ensure proper training and adherence to quality control measures for sample collection and transportation to maintain the integrity of the samples.
3. Diagnostic Algorithm Flow: Stool Xpert MTB/RIF can be incorporated as an initial diagnostic test alongside other conventional methods such as smear microscopy and culture. Positive Stool Xpert MTB/RIF results can be considered definitive evidence for the presence of TB, especially in settings where resources for culture and drug susceptibility testing are limited. Negative Stool Xpert MTB/RIF results may require further investigation with additional tests, such as culture, especially in cases with high clinical suspicion of TB.
4. Drug Resistance Detection: Stool Xpert MTB/RIF can simultaneously detect rifampicin resistance-associated mutations in the rpoB gene, allowing for early identification of drug-resistant TB cases. Positive rifampicin resistance results should trigger further testing for additional drug susceptibility testing, considering the need for appropriate treatment regimens.
5. Extrapulmonary TB Diagnosis: To include Stool Xpert MTB/RIF in the diagnostic algorithm for extrapulmonary TB, evidence will need to be generated as some EPTB patients have pulmonary involvement too.
6. Data Management and Reporting: Establish systems for proper documentation and reporting of Stool Xpert MTB/RIF results to facilitate monitoring and evaluation of diagnostic performance and patient outcomes. Ensure integration with existing surveillance systems to contribute to epidemiological data and monitoring of TB trends in pediatric populations.
7. Cost-effectiveness Considerations: Conduct cost-effectiveness analyses to assess the financial implications of integrating Stool Xpert MTB/RIF into pediatric TB diagnostic algorithms. Consider the potential reduction in costs associated with early diagnosis, reduced hospitalizations, ease of sample collection and prevention of disease transmission.

It is crucial to adapt the integration of Stool Xpert MTB/RIF into diagnostic algorithms based on local resources, epidemiological context, and healthcare settings. Regular evaluation and refinement of the algorithm are necessary to optimize diagnostic accuracy, patient outcomes, and resource allocation for pediatric TB diagnosis.

5. Conclusion

Stool CBNAAT holds promise for microbiological confirmation of pediatric pulmonary TB. In the peripheral resource limited settings, where there is a severe dearth of trained medical professionals, obtaining representative specimens of the respiratory tract is difficult and attempts at microbiological confirmation of disease are therefore limited. We propose that stool is a potential sample which can be used to achieve microbiological confirmation of diagnosis. Although utilizing centrifugation technique shows higher sensitivity, there is further need to compare all stool processing methods and standardize one method most suitable as per the local situations.

Author's respective contributions

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The authors have no competing interests to declare that are vrelevant to the content of this article.

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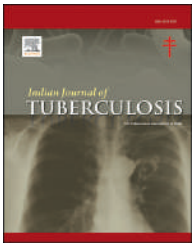

Review article

Latent TB infection in children and adolescents: Scientific rationale and programmatic management

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ABSTRACT

As we march towards the goals of TB elimination, one area of focus is on TB preventive therapy which deals with treatment of latent TB infection, the pool from which future TB cases are generated.

Children are particularly vulnerable to disseminated TB and seriously ill TB like TB meningitis, which highlights the need for addressing latent TB infection in the age group of 0–18 years.

The national TB elimination program has extended it's strategy to include TB preventive therapy from treating children <5 years and PLHIV to treating children ≥5 years, adolescents and adult household contacts of TB cases and at risk immunosuppressed groups.

Newer regimens including weekly INH and Rifapentine for three months (3HP) has been recommended in the program.

Concerns and opportunities for operational research in this area include surveillance and monitoring for drug toxicity and resistance, strategies to ensure adherence and improve treatment completion and outcomes.

1. Introduction

Ever since Robert Koch's historical announcement of the discovery of Tuberculous bacilli, various attempts have been made to control the deadly disease of Tuberculosis right from Koch's lymph, to sanatorium treatment, collapse, and gold therapy and various chemotherapy regimens, with varying degrees of success.

Latent TB infection (LTBI) is a state where the bacilli have entered the body but there is no clinically manifest disease. The term Latent TB infection is being discarded and replaced by TB infection since no infection can be considered latent. The current End TB strategy[1,2] that is followed has much focus on the treatment of latent TB infection, this being the reservoir from which future TB cases are generated.

The lifetime risk of an individual breaking down into Tuberculous disease from latent TB infection is 10%, this risk increasing 16 to 21 times in patients with HIV and 3 to 4 times in patients with Diabetes and other immunosuppressive conditions highlighting the importance of treating at the latent TB infection stage itself. This risk appears to be higher in contacts of bacteriologically confirmed cases and within the first two years of contact.[3]

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Treatment of latent TB infection has been shown to reduce this risk by almost 90% which means that the lifetime risk of breakdown to TB disease reduces from 11.1% in those untreated to 1.2% for those treated for latent TB infection.[4]

Preventive strategies in the national strategic plan include three strategies namely active case finding, contact tracing, and investigating for TB among household contacts of TB patients and TB preventive therapy. The expectation is that these preventive measures will bring down the current decline in incidence from 2.5% to 10% towards meeting the End TB targets.[5]

India has a relatively younger population with 41% of its population of 140 crores, comprising children in the age group 0–18 years according to UNICEF. Among the cases notified to the National TB elimination program about 6–7% are children in this age group which indicates a high burden in terms of absolute numbers.[6]

As we march towards our goals of TB elimination, it is important that latent TB infection is identified and treated early in children who are the architects of the future of India. Latent TB infection in children indicates a recent infection, unlike adults who could have been infected a long time back. Also, children are more at risk for developing more disseminated TB and seriously ill forms of TB like TB meningitis which is a reason why LTBI treatment becomes a priority in the pediatric age group.

2. Scientific rationale and review of historical trials of TB preventive therapy in children

Historically, clinical trials on latent TB infection to study the effect of TB preventive therapy were done on the general population in high-burden areas and on high-risk groups like household contacts of recent active TB. These trials were done in the 1950s and published in the 1960s and 1970s.[7] These studies showed that TB preventive therapy was effective in preventing cases of TB among populations at high risk of TB and also that it conferred a longer-lasting post-treatment effect on the TB burden.

The first trials initiated in 1956 by Horowitz et al involving more than 8000 villagers in Greenland showed an overall reduction of 29% in the annual rate of smear-positive TB.[7] In modern times, a trial initiated by the international union against TB in 1982, involving 27,830 individuals with positive TST and negative sputum culture showed a marked reduction in TB activation when treated with INH.

A study done by WHO (Nyobe et al, 1963) in Tunisia showed a marked reduction in TB infection in children treated with INH for TPT despite low adherence, with only 20% completing the treatment.[8]

Hanson et al in 1967 documented a marked reduction in latent TB infection among children treated with INH in a previous study done in Alaska in 1962, indicating an enormous reduction in transmission of mycobacterium tuberculosis.

Among people living with HIV, preventive treatment reduced the overall risk for TB by 33%. Among TST positive the reduction was 64% and among TST negative the reduction was 14%.[9]

The combination of INH and Rifapentine as once-weekly therapy for TB preventive therapy has been found to be safe and effective and ensures higher treatment completion rates.[10]

Based on the increased risk of progression of TB infection to active TB disease in high-risk groups, on the burden of TB in the community, the availability of resources and infrastructure, and the feasibility of programmatic implementation, WHO has shifted its policy from the treatment of latent TB infection in children less than 5 years and PLHIV to including children more than 5 years of age, adolescents, and adult household contacts of TB cases. Other risk groups like patients on immunosuppressive therapy, silicosis, anti-TNF treatment, dialysis, and preparing for organ or hematological transplant have been included.[8]

3. A brief review of India's programmatic approach to TB preventive therapy in children[4]

India has an estimated 35 to 40 crore latent TB infection cases with 18–26 lakh Indians developing TB annually. The baseline TB infection (TST ≥5 mm or QGIT ≥35 IU/ml) in household contacts has been estimated to be 71% and household contacts developing incidental Tb 2%. The highest risk of developing TB among household contacts has been found to be in the first two years of contracting the infection. Hence, WHO recommends TB preventive therapy to all household contacts of PTB patients residing in high-burden countries like India. However, it is found that the number of patients actually completing the entire cascade of care is <20%.[4]

The cascade of care recommended in the program includes identifying the high-risk population and ruling out active TB disease, testing for TB infection, offering appropriate treatment regimens, ensuring adherence and completion of treatment, monitoring for adverse drug reactions, and evaluating the program.

For the programmatic purpose, a child in India is defined as 0–18 years of age, which includes adolescents of 10–18 years.

Regarding diagnosis, the currently recommended tests for detection of TB infection include Tuberculin skin tests (TST) and interferon Gamma release assay (IGRA), which are based on the principle of type IV or delayed-type hypersensitivity to mycobacterial proteins. In programmatic conditions, testing is not required for PLHIV and children <5 years and can be offered to children >5 years and adult household contacts where available. *Non-availability of testing should not be a reason for not offering TB preventive therapy to the children and adult household contacts of the index TB case.*

The contraindications to TB preventive therapy include active TB disease, active or chronic hepatitis, concurrent use of other hepatotoxic drugs, alcoholism, peripheral neuropathy, and known allergy or hypersensitivity to the drugs used in the regimen.

Currently, two regimens have been approved by the national program,[4] viz 6 months of isoniazid (6H) in all the risk groups including PLHIV, adult and paediatric household contacts, and 3 months of weekly isoniazid with Rifapentine (3HP) in PLHIV, adult household contacts and in children >2 years of age. The dose of INH is 5 mg/kg/day in children ≥10 years of age and 10

mg/kg/day in children <10 years. The 3 HP regimen is given as FDC and loose drugs based on the weight band of the patient.

Recommendations for the contacts of INH monoresistant TB include 4 months of rifampicin (4R) in the dose of 10 mg/kg/day for ≥10 years of age and 15 mg/kg/day in children <10 years.

For Rifampicin-resistant and fluoroquinolone-sensitive patient contacts, a 6-month regimen of levofloxacin (6 LFX) based on the weight band has been recommended.

The patients are said to have completed treatment if they complete 80% of the recommended doses (90% for the 3 HP regimen) within an extended time for treatment completion which is defined as the treatment duration plus 33% of additional time.

4. Recent advances in the diagnosis and treatment of latent TB infection[11]

Given the low sensitivity of Tuberculin skin tests in BCG-vaccinated individuals and the high cost and expertise needed for IGRA, various other tests are in the pipeline for the diagnosis of latent TB infection. Noteworthy of these are the C-tb, C- TST, and DPPD tests which are based on the ESAT -6 and CF10 antigens of Mtb which are not shared in the BCG strain. QIAreach QFT is an advance in IGRA which is being evaluated at the PHC level.

For people exposed to DRTB, PHOENIx, and TB CHAMP trial results are expected in 2023. TB CHAMP compares six months of Levofloxacin to placebo in household contacts of people with exposure to DRTB. TB CHAMP is mainly for infants and children <5 years including children with HIV. PHOENIx study is based on a six-month regimen of Delaminaid for adult and child contacts of people with drug-resistant TB.

5. Concerns in program implementation and opportunities for operational research

There are four main concerns in the implementation of TB preventive therapy.

Firstly there is a *fear of generating drug resistance*. Analysis done of various randomized controlled trials of INH preventive therapy in both non-HIV and HIV patients,[12] showed that though the relative risk for isoniazid-resistant TB after preventive therapy is not statistically significant, the point estimate and upper boundary of the 95% Confidence interval are consistent with an increased risk. In PLHIV, Isoniazid preventive therapy has been found to be a safe, low-cost intervention with the potential to reduce illness and death caused by TB. The main cause of antituberculous drug resistance is inadequate treatment and failure to identify active TB which needs to be ruled out carefully before starting Isoniazid preventive therapy. Therefore, the program needs to carefully weigh the risk of an increase in drug resistance while starting preventive therapy with the benefits of TB reduction achieved with such therapy. Also, cross-resistance between Rifapentine and Rifampicin has been documented earlier[13–15] which raises concern about the emergence of drug-resistant Tuberculosis in a country with a high burden of drug-resistant TB like India. There is a felt need for operational research for surveillance and monitoring of the emergence of drug resistance while implementing TB preventive therapy in programmatic conditions.

The second concern is *the risk of drug toxicity*. Historical studies[7] have documented drug-induced side effects like hepatotoxicity, headache, nausea, vomiting, and peripheral neuropathy among patients receiving INH preventive therapy but the toxicity has been at low rates and has not been documented in children and adolescents. This is another area where operational research should focus on while rolling out the program.

Thirdly the *issue of adherence* has always been a concern while implementing preventive therapy, especially in an asymptomatic patient. Currently, only 20% of patients complete the entire cascade of care and the program needs to focus on ensuring adherence and follow-up of these patients so as to avert the development of further drug resistance in the future due to inadequate and incomplete treatment.

Lastly, the *ethical issue of treating an asymptomatic patient* with potentially toxic drugs has to be addressed.

6. Conclusion

As we march towards the goals of TB elimination, one of the areas of focus is the treatment of TB infection or TB preventive treatment which aims to address the reservoir of latent TB infection from where future TB cases will be generated. The program needs to carefully weigh the benefits accrued by treating latent TB infection with the risks like drug toxicity and future development of resistance which has been at a low level in studies so far. Operational research and surveillance to ensure adherence and monitor the development of drug resistance may be prudent. The health of children, who account for 41% of our population and who are the architects of tomorrow's India is vital and all necessary steps should be taken to prevent Tuberculosis in this cohort. As the oft quoted saying goes, *"Healthy children build healthy nations*".

Conflicts of interest

The author has none to declare.

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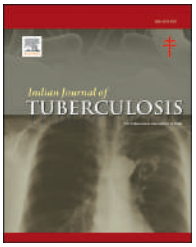

Review article

Tuberculosis and childhood cancer – A review of literature

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ABSTRACT

Tuberculosis and malignancy are major public health problems in developing countries like India and causes significant morbidity and mortality. *Mycobacterium tuberculosis* is an aerobic acid-fast bacilli which is an important pathogen especially complicating clinical status of paediatric oncology patients and treatment of infection with this bacilli is challenging in this subpopulation of patients because of ongoing immunosuppression and relative lack of published guidelines. Atypical presentations of tuberculosis in children also complicate the diagnosis and management. All the more, in tuberculosis endemic area lung cancer may be mistakenly diagnosed as tuberculosis or vice versa and this wrong diagnosis increases the burden on country's health status. It is noted that tuberculosis prevalence is high in children with haematological malignancy and head and neck tumours compared to other solid organ tumours. Moreover, it is found that morbidity and mortality from tuberculosis is more in children from WHO listed high TB burden countries who undergo hematopoietic stem cell and solid organ transplantation. Use of immune checkpoint inhibitors as novel therapy in treatment of childhood malignancies has led to modification of the body's immunological response and has resulted in increased latent tuberculosis infection reactivation as one immune-related infectious consequence.

Latent TB infection screening is important concept in management of paediatric oncology patients. Currently, the tests employed as screening diagnostics for LTBI are interferon-gamma release assay (IGRA) blood test and the tuberculin skin test (TST). Various regimens have been suggested for the treatment of LTBI. But, after a positive IGRA or TST and prior to latent TB treatment, active tuberculosis should be ruled out by detailed history taking, examination and appropriate investigations so as to minimize the risk of drug resistance with anti-tuberculosis monotherapy used in LTBI treatment. To add on to literature, Non tuberculous mycobacteria are universally present environmental organisms. However, in immunocompromised children especially in subpopulation of malignancy, NTM is known to cause infections which needs protocol based management. Also importance has to given to implementation of adequate preventive and corrective measures to prevent such opportunistic infection in paediatric oncology subpopulation.

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In this review, we provide an overview of tuberculosis in paediatric oncology patients and summarize the expansive body of literature on the tuberculosis mimicking carcinoma, tuberculosis burden in transplantation patients and those receiving immune check point inhibitors, latent TB infection screening and management, and NTM infection in children with malignancy.

1. Introduction

Tuberculosis (TB) may be present as a serious, life-threatening disease for patients with both hematological as well as solid organ cancers. Only a few case reports of *Mycobacterium tuberculosis* in children with oncologic or hematologic diseases are reported in literature.[1] It is becoming a big global public health issue because tuberculosis and cancer, two primary causes of increasing morbidity and death rates in humans, are on the rise. Immune deficiency in malignancy occurs, due to underlying disease per se or drug-induced due to chemotherapeutic agents or corticosteroids or immune checkpoint inhibitors and immunosuppressant, favor the development of tuberculosis in these groups.[2]

Mycobacterium tuberculosis is a prime pathogen in patients with malignant diseases and should be suspected early and investigated promptly in high clinical suspicion cases. Management of mycobacterial infection in pediatric oncology is challenging because of immunosuppression and lack of adequate published guidelines. Empirical anti-tuberculosis medication is required, when clinical radiological findings strongly imply tuberculosis, especially in patients from high endemic areas. Chemotherapy for cancer is, however, not a barrier to treatment of pediatric tuberculosis.

Childhood tuberculosis classically presents fever, cough, lymphadenopathy, loss of appetite and weight loss. Atypical presentations are commonly reported in children.[3] Manifestation varies depending upon organ affected (Fig. 1). Latent Tuberculosis should be diagnosed and treated in children with hematological or solid tumors, especially if the child is from an endemic area. Impaired cellular immunity is one of the fundamental aspects for TB in children with hematological malignancies.[4] According to statistics, people with hematologic illnesses have a 2 to 40 times higher frequency of TB than the broader population.[5,6] In underweight, immunocompromised children, signs and symptoms of the disease may be nonspecific. Also, they have a difficult time getting a tuberculosis diagnosis because they are less likely to develop a positive Mantoux test and sputum staining for the disease.

Childhood cancers and tuberculosis (TB) have superimposed appearances and are frequently misdiagnosed. Depending on their exposure to TB, level of infection, and degree of illness, children who have been exposed to mycobacterium tuberculosis are divided into three primary diagnostic groups. Either a tuberculin skin test (TST) or an interferon-gamma release assay (IGRA) indicates that a child has TB infection. As a result of hematogenous and lymphatic spread, TB typically exhibits symptoms, physical indicators, or radiographic abnormalities, and children might develop TB illness in lymph nodes, bones, joints, the central nervous system, military TB, and intra-abdominal organs are examples of extrapulmonary locations.

Children with tuberculosis are thought to be less contagious than adults since the illness has a paucibacillary nature, and as a result, it has received less attention from the public. Mycobacteria are challenging to be found in clinical specimens, which frequently results in missed or delayed illness diagnosis.[7] Young children are more prone to get TB from an infection and are more prone to developing extra-pulmonary manifestations.

Paediatric population who have high risk of developing tuberculosis disease and life threatening manifestation of disease than adults are those children with following comorbidities.

- Human immunodeficiency virus (HIV),
- Hematologic malignancies,
- Solid organ transplant (SOT) or
- Hematopoietic stem cell transplant (HSCT).[8]

2. Tuberculosis mimicking carcinoma in children

It may be difficult to distinguish PTB and lung cancer clinically and radiologically.[9] In both these conditions respiratory examination can be unremarkable but symptoms such as productive cough, chest pain, hemoptysis, anorexia, and weight loss can be observed.[10]

PTB on imaging rarely presents as a lung mass in immunocompetent children, and it can be difficult to distinguish PTB from primary lung cancer, especially when GeneXpert, MTB, and repeated negative sputum tests for acid-fast bacilli are present.[11,12] Contrarily, lung malignancy can sometimes be mistakenly identified as PTB, especially in areas of the world where tuberculosis is endemic and carries a heavy toll.[2,13] In India, high percentage of primary lung cancer cases receive prior TB therapy, delaying the diagnosis and leading to appalling results. In addition, when both tuberculosis and lung cancer occur together in exceedingly rare conditions where sputum examination for the presence of mycobacterium is performed, the diagnosis of lung cancer may go unreported.[2,14]

Retrospective research by Van Heerden et al. assessed the diagnosis of TB in paediatric population with cancer included

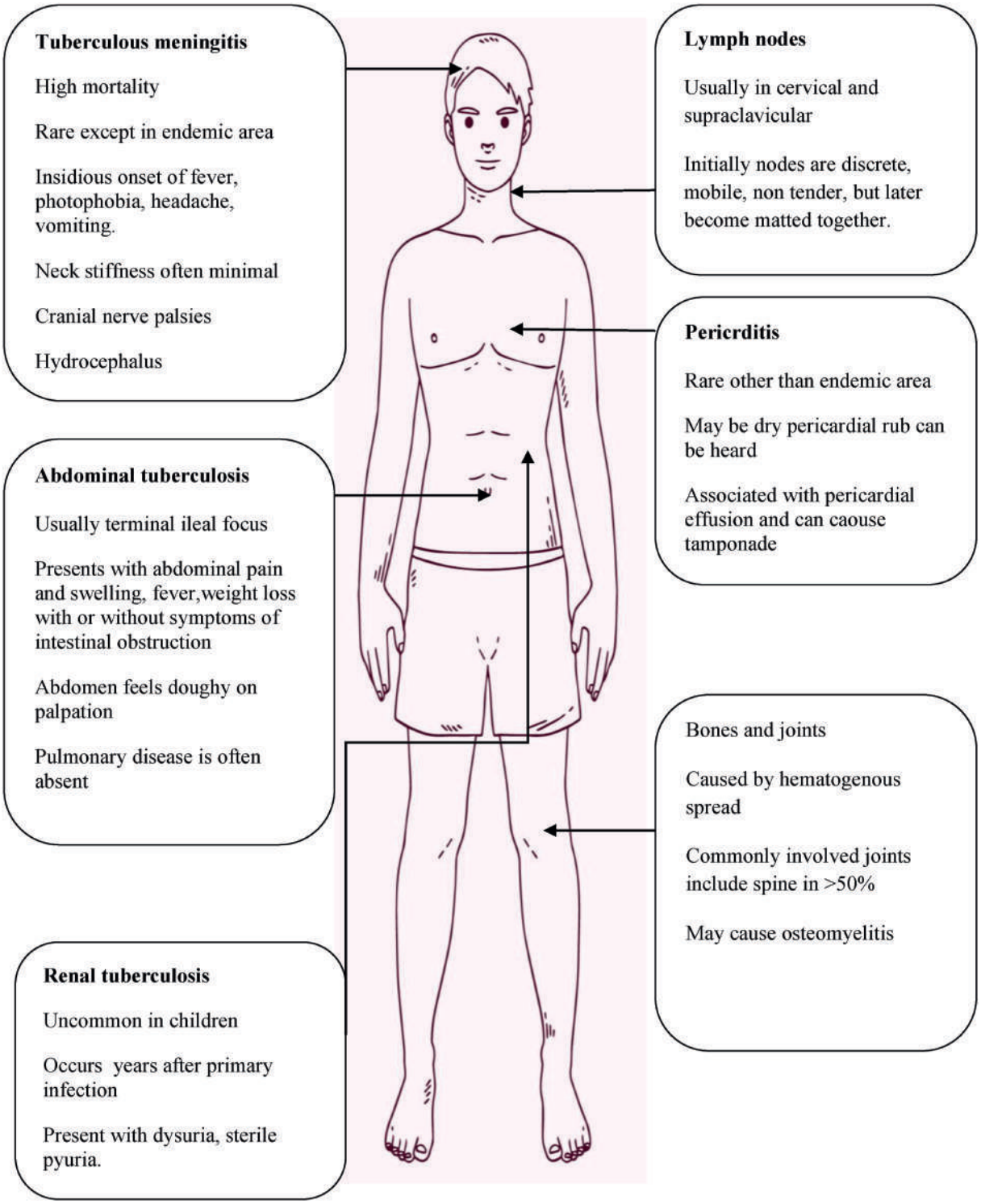

Fig. 1 – Extra pulmonary manifestations in childhood tuberculosis.

in the Tygerberg Hospital Childhood Tumor Registry (2008–2018). The study involved 539 children who were getting anti-tuberculosis medication (ATT) and those who had just been diagnosed with cancer and TB. The findings revealed that extrapulmonary and pulmonary TB hindered ATT in 27 (5%) patients who had started therapy prior to a cancer diagnosis. Six of the twelve children who were on ATT at the time of their cancer diagnosis had a positive tuberculin skin test, and 22 of the 27 patients encountered a TB patient. 11/27 of the children who were confirmed to have cancer had chest radiographs that were deemed to be TB-indicative. The average amount of time between TB and cancer diagnoses was 25 days, with an interquartile range of 3.5–58. 204 of the 539 children with cancer diagnoses died of the disease, and 18/30

of them were either receiving ATT at the time of their diagnosis or had received a tuberculosis diagnosis within one month of their cancer diagnosis, with an odds ratio of 2.6.[15]

Mohakud et al., presented a case report of a 1 year 7 months old toddler referred with complaints of wet cough, intermittent low-grade fever, and inadequate weight gain for about three months. In the chest radiograph, a significant radio-opacity was seen on right upper and middle zones. History revealed that the child's grandfather had TB. Mantoux test, Nucleic acid amplification testing, and gastric lavage (AFB) were all negative. A USG guided biopsy revealed numerous Langhans giant cells and well-delineated epithelioid cell granulomas with large areas of caseous necrosis. For the first 10 months, Antitubercular chemotherapy (ATT) was started, and 3 months follow-up showed clinical improvement along with a resolution of the lung lesion in the radiograph.[16] The collection of lymph nodes in primary tuberculosis is known as lymph nodal pseudotumor. A granulomatous parenchymal mass develops due to a unique immune reaction to the mycobacterial antigen released from endobronchial discharge of cavity contents, the parenchymal mass development takes place. In the absence of endobronchial decompression, parenchymal mass is created mostly in progressive primary illness by expanding necrotic regions.[17]

Both in infants and adults, the parenchymal pseudotumoral form of tuberculosis is extremely uncommon and resembles malignant tumours strikingly from a clinical, bronchoscopic, and radiological standpoint.[18,19,20] It could be misinterpreted for pleuropulmonaryblastoma or intrathoracic sarcoma.[21]

The approximate time delay it takes to diagnose pseudotumoral TB is between 4 and 10 weeks with no clear-cut clinical signs. It could appear as an endobronchial tumour or an ulceration during bronchoscopy.[20] Tuberculosis may cause a large radio-opaque lesions on a chest X-ray, mostly in the upper and middle zones, which are well-ventilated and have a high propensity for tuberculosis; also a mediastinal or parenchymal mass may be found on a CT scan. Lymph nodes with peripheral augmentation and core hypodensity from necrosis may be caused by tuberculosis, however this is not a pathognomonic sign.[14] Tuberculosis can also result in calcified nodes.[14]

Methods like sputum AFB and bronchoalveolar lavage (BAL) fluid AFB are originally performed for reliable microbiological identification, although these may produce unfavourable results in situations of paucibacillary TB. In that situation, FNAC, bronchoscopic biopsy, USG or CT-guided FNAC or biopsy, or surgical biopsy, are performed.[17]

Regarding the management of pseudotumoral tuberculosis, scant information is available. Patients who get short-course ATT typically exhibit full resolution.[17] In cases of paucibacillary and extrapulmonary illness, the course of treatment may be extended based on clinical justification.

3. Pediatric bone marrow transplantation patients and tuberculosis

Prolonged fever is commonly seen in bone marrow transplant (BMT) recipients and pediatric oncology cases. In a multicenter study by Andrea T Cruz et al, six children were identified with malignancy and bone marrow transplantation, and all had fever whereas a child had chronic cervical adenopathy. In four out of five children with cancer, tuberculin skin tests (TSTs) were found to be positive. Five out of six children had cultures that were positive, but one of the children also had a positive TST and a pleural punch biopsy that showed granulomas.[22]

Only one child out of the six was tested for latent TB infection (LTBI) before beginning chemotherapy, and when the results were positive, he was started on isoniazid as chemoprophylaxis. Massive intrathoracicadenopathy and a tiny apical cavitary lesion indicative of cavitating lymphoma were both visible on his pre-treatment radiograph. While the adenopathy improved with treatment of his lymphoma, he had doubling of a pulmonary cavitary lesion by computed tomography. As he could not produce sputum, an aspirate of this lesion was performed and revealed acid-fast organisms; the culture grew *M. tuberculosis*.

There are three potential risk factors for TB in pediatric population. The first is the risk of acquisition of Latent TB infection LTBI, which is an epidemiologic phenomenon. The second is the risk of progression to disease, which is associated with immune status and age. The third is severity of disease; this is associated with age and immune status and symptom duration prior to diagnosis of TB. The most recent edition of the Red Book states that "an initial TST or interferon gamma release assay should be performed before initiation of immunosuppressive therapy, including prolonged corticosteroid administration, use of TNF-alpha antagonists, or other immunosuppressive therapy in any child requiring these treatments.[23] Despite this recommendation, many oncology patients are not routinely tested for LTBI prior to starting chemotherapy.

Kaplan M et al., in their study found that there is increased TB illness rates in paediatric population with head and neck cancers and hematologic malignancies.[24] In a study by Andrea T Cruz et al., six oncology or BMT patient were assessed, and almost all children had potentially preventable tuberculosis disease which would have been prevented if they had been checked for latent TB infection (LTBI) before starting chemotherapy, and the benefit of doing so outweighs the danger of LTBI treatment.

Two months after receiving an allogeneic stem cell transplant, a child from Ukraine developed *M. tuberculosis* pneumonia, according to Biral et al.[25] The authors noted that compared to children from countries with low TB prevalence, children from high TB-incidence countries have a greater risk of developing post-transplant TB. It's unclear if it occurred because of post-BMT exposure or untreated LTB1 in the donor. In an Indian study it was found that Mycobacterium TB contributes to almost 2% of infections after BMT.[26]

4. The incidence of tuberculosis and pediatric hematopoietic stem cell recipients and solid organs

Tuberculosis (TB) is expected to cause a significant potential for disease and death in children who receive hematopoietic

stem cell transplants and solid organ transplants. Immunosuppression and medication interactions complicate the management of tuberculosis in the post-transplant environment.

5. Tb epidemic in transplant recipients

A total of 30 countries across the world have been declared with high TB burdens, high TB/HIV burdens, and high multidrug resistant TB burdens (MDR-TB) by the World Health Organization (WHO). Compared to individuals who have received solid organ transplants (SOT), pediatric transplant patients from specific countries are more likely to get TB after the transplant. Patients who have received a hematopoietic stem cell transplant (HSCT) have a lower risk of developing TB. The restoration of T-cell function may be possible because HSCT patients don't receive lifelong immunosuppression.[27]

Several factors contribute to the risk of post-transplant tuberculosis in children undergoing HSCT, including social factors; allogeneic transplantation, indications for stem cell transplantation, conditioning therapies prior to transplantation, namely T-cell depleting agents and total body irradiation, and complications associated with transplants, including chronic graft versus host disease (Table 1).[28]

In the post-transplant setting, there are three mechanisms through which tuberculosis may develop.

- Endogenous reactivation
- Donor-derived TB or
- A de novo infection.

Table 1 – Risk factors for Solid organ transplant and Hematopoietic stem-cell Transplant.

Solid organ transplant	Hematopoietic stem-cell transplant
Social factors	Social factors
Birth or residence in high-endemic area	Birth or residence in high-endemic area
Infectious Disease History and Co-infections	Underlying indication for transplantation
Patient or donor history of TB infection or disease	Hematologic malignancy
HIV infection	Conditioning Regimen
Other coinfections: Mycoses, CMV, PJP or Nocardia	Busulfan
Non-infectious co-morbidities	Total body irradiation
Diabetes mellitus	T-cell depleting agents
Chronic liver disease	Type of stem-cell transplant
Chronic renal insufficiency or hemodialysis	Allogeneic transplant
Organ Transplanted	Mismatched allograft
Lung transplant	Transplant complications
Non-renal transplant	BOOP
Immunosuppressive therapies	Chronic GVHD
Cyclosporine	Graft failure
Intensification of immunosuppression for graft rejection	Other risk factors
Mycophenolate mofetil	Corticosteroid use
OKT33 or anti-T lymphocyte antibodies	
Tacrolimus	
Transplant Complications	
Allograft rejection	

The existence of or prior travel to an endemic area is a major risk factor for endogenous reactivation. Donor-derived TB, which often develops early after transplantation, is a significant mechanism of transmission. It is crucial to take precautions to prevent new TB infection in recipients of transplants since endogenous reactivation accounts for around 25% of MTB infections in HSCT recipient children.

SOT patients may present with typical or atypical TB manifestations. Two children (19 months and 9 years old) in a case study following juvenile liver transplantation developed widespread mycobacterial infections over the course of a year.[29] A case of hepatic graft TB was reported in a 10-month-old female child with history of biliary atresia, likely transmitted from the living related donor; the transplant recipient died of pneumonia on day 273 after transplantation.[30] Anti-T-cell antibodies, disease spread, organ rejection in the past, and delayed diagnosis can all increase mortality in SOT patients.

The development of pulmonary TB in children with acute lymphoblastic leukemia (ALL) who had HSCT has been documented.[31] Atypical manifestations including fever of unknown origin and non-specific clinical manifestations may also occur. Multidrug-resistant strains, widespread illness, and a delayed start to therapy are all factors that increase the risk of mortality in HSCT patients.

Most instances of TB occur in SOT and HSCT patients within the first year following transplantation, with a midpoint of the clinical presentation duration of 6–11 months.[32] TB cases have been observed more than a year after transplantation, which may be attributed to community MTB exposure in TB-endemic countries. The median time to diagnosis in a study of 6 pediatric liver transplant recipients in the United Kingdom (UK) with tuberculosis (TB) was 8 months.[33]

Pre-engraftment, post-engraftment (before day 100), and late phases are the three categories used to classify post-HSCT problems. The late period is when post-transplant TB cases predominate.

Children usually have a complete pre-transplant examination prior to SOT and HSCT in order to detect risk factors for the emergence of TB infection. The patient's recent medical background, current medications, and previous TB infection or illness history, prior TST/IGRA findings, prior BCG vaccination history, and exposure history are all thoroughly reviewed as part of this examination. As children may have recently come into touch with an adult who has infectious pulmonary TB, it is crucial to ask in regard to incidences of contagious TB in children. Additionally, a thorough physical examination that pays special attention to the respiratory system and screens for hepatosplenomegaly and lymphadenopathy is required (Fig. 2).[28]

Then, prior to transplantation, all transplant candidates should have a TST or an IGRA test to check for TB infection.[33] IGRAs can be either T-SPOT or QuantiFERON-TB Gold In-Tube TB.[34] In those who have a history of receiving the BCG vaccine, IGRAs are often preferred over TST because the antigens utilized in them are not included in the BCG vaccine, which reduces the likelihood of false positive results. As patients

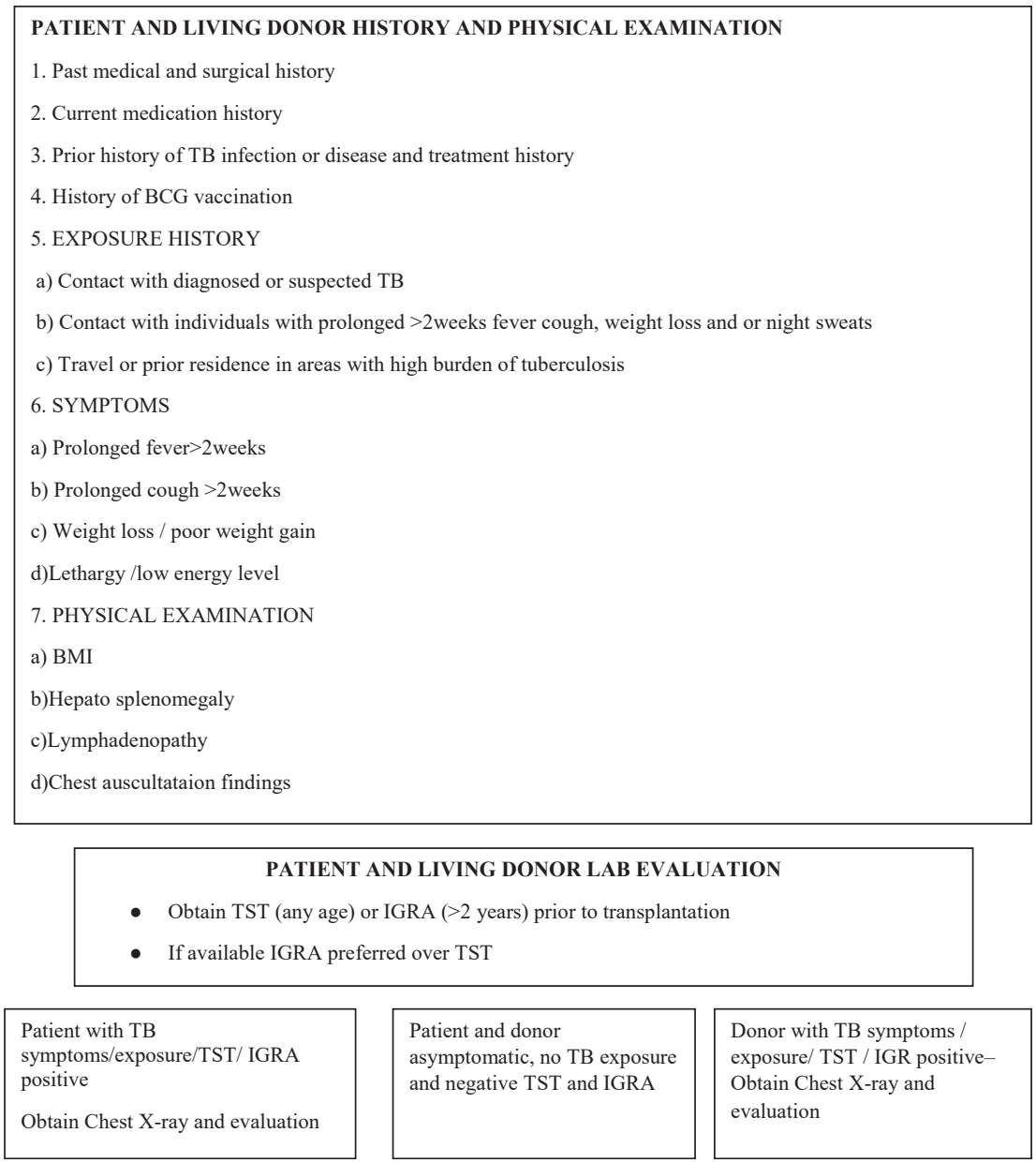

Fig. 2 – Pre-transplant evaluation for TB for pediatric solid organ and hematopoietic stem-cell transplant candidates and living donors. (World Health Organization 2019).

undergoing HSCT or SOT are often on immunosuppressive drugs, there can be falsely negative or indeterminate results. T-SPOT. TB may be preferred in immunocompromised patients as it appears to have slightly higher sensitivity for detecting MTB infection compared to QFT.[35]

Prior to beginning treatment, it is essential to thoroughly examine individuals who have had TB exposure or infection for TB illness. Because the start of TB infection therapy can result in undertreatment and the development of drug resistance in patients that are actively being treated. A chest radiograph should be performed on children if they exhibit symptoms suggestive of tuberculosis and their TST or IGRA results are favorable (Fig. 2).[28] The best course of action is to treat for TB infection before transplantation if screening for TB infection is positive but there is no evidence of active TB illness. However, this might not always be possible.

To minimize additional liver damage from antituberculosis therapy (ATT), it may be prudent to wait to start treatment in liver transplant recipients until liver function has stabilized following the procedure. Alternatively, a less hepatotoxic medication may be used in place of ATT.[36] HSCT needs to be postponed till clinical judgment deems TB illness managed.

Living donors should undergo thorough pre-transplant screening for TB infection and illness together with transplant candidates. This includes collecting accurate information about their exposure and symptoms, as well as doing TST or IGRA testing. For the benefit of the recipient, it is advised

not to use an organ from a live or deceased donor who has TB illness.

The live Bacille Calmette-Guérin (BCG) vaccination, which protects against meningitis and widely spread TB illness, is given to infants in most nations at birth. In rare cases, children with compromised immunity who get the BCG vaccine may develop disseminated BCG illness. Children with a history of BCG vaccination and underlying immunodeficiency are at risk for developing disseminated BCG illness after transplant, according to a case study of a 23-month-old girl with Bare Lymphocyte Syndrome type II who received allogeneic HSCT and developed the infection.[37]

6. Immune checkpoint inhibitors and pediatric tuberculosis

Immune checkpoint inhibitors (ICPIs) have revolutionized cancer management and greatly improved outcomes for a range of pediatric malignancies. But one immune-related consequence that might arise from further ICPI modulation of the body's immune response is the reactivation of latent tuberculosis infection (LTBI). Since the initial approval of Ipilimumab (a monoclonal antibody blocking CTLA-4) for the treatment of metastatic cancer, five additional drugs that target the PD-1/PD-L1 pathways, including nivolumab and pembrolizumab (against PD-1) and atezolizumab, avelumab, and durvalumab (against PD-L1), have been introduced. But because these substances stimulate the immune system, they may also trigger immune-related adverse events (irAEs), which can have a negative impact on almost all organs and even be deadly.[38] The administration of high doses of corticosteroids is advised for the treatment of irAEs, and if damage persists, additional immunosuppression may be required with steroid-sparing regimens (e.g., anti-tumor necrosis factor-alpha (TNF-a) agents or mycophenolatemofetil), which will again increase the risk of contracting opportunistic infections or unmask chronic underlying or opportunistic infections.[39]

In a recent study by Del Castillo M et al, the overall prevalence of these severe immune-related infections was calculated to be 7.3% in 740 pediatric patients with metastatic melanoma who were receiving ICPIs.[40] The majority of MTB patients continue to have clinically asymptomatic or dormant illness. Over the course of their lifespan, 5–10% of them will experience reactivation. Studies from indian pediatric population on immune check point inhibitors with TB reactivation is very rarely reported in literatures and an area yet to explore.

7. Recommendations for latent Tb infection (Ltbi) screening in pediatric cancer

Reduced likelihood of developing active MTB infection or reactivation is induced by the short-term immunosuppression brought on by adjuvant cancer therapy in earlier stages of the disease and the poor prognosis in metastatic stage in these people. LTBI testing is recommended when the estimated 5-year survival rate is more than 25% in patients at low risk for hepatotoxicity (mainly because of isoniazid). But it is not recommended when it is greater than 50% in patients at high risk for hepatotoxicity.[41] The type of malignancy, mycobacterium exposure, disease prognosis, host comorbidities, and predicted treatment adverse effects should all be taken into account when thinking about a latent TB screening.

8. Recommended Ltbi screening tests

The interferon-gamma release assay (IGRA) and the tuberculin skin test (TST) are utilized for latent TB screening. Two currently approved IGRAs that just require blood samples to obtain results within 8–30 hours are T Spot TB and QuantiFERON-TB Gold In-Tube tests. For the diagnosis of LTBI in those at low-to moderate-risk of developing an active illness, IGRA is favored over TST.

9. Screening for targeted Ltbi prior to immunotherapy

Before beginning an Immune Check Point Inhibitor (ICPI), it is recommended to screen for Latent TB, especially in cancer patients who have independent risk factors for tuberculosis (such as host comorbidities, exposure to MTB endemic regions, and immunosuppression). ICPIs are temporarily withheld in cases of proved active TB. Any further immunosuppression must be discontinued, and prompt antituberculosistherapy should be started. Furthermore, it is unknown how soon after the anti-TB therapy, in patients with latent or active tuberculosis, ICPIs may be resumed safely. Most frequently, a time frame of 2–4 weeks is suggested.

According to the Gustave Roussey Cancer Center's French prospective registry, the prevalence of TB among cancer patients on anti-PD1/PD-L1 drugs was estimated to be around 1/1000 patients.[41] Because anti-PD1 medications may promote the reactivation of tuberculosis, Picchi et al. in their suggested that all cancer patients should undergo an IGRA screening for LTBC before receiving immunotherapy.[42] Prior to starting any anti-TNF treatment, it is advised to do LTBC screening. If the results are positive, proper anti-tuberculosis medicine should then be used.

The treatment includes 3 months of once-weekly isoniazid andrifapentin, 4 months of rifampicin, or 9 months of isoniazid are all included in the therapy of LTBI.[43] Throughout the course of LTBC treatment, patients should undergo routine checkups for any clinical hepatitis signs and drug toxicity. It is more acceptable to start treatment (for example, ensuring patient acceptance of anti-tuberculosis prophylaxis before initiating anti-PD-1/anti-PD-L1 antibodies, 2 weeks prior.

10. Management and exclusion of active tuberculosis in patients with pediatric oncology

Before starting latent TB treatment, following a positive IGRA or TST, an evaluation to rule out active tuberculosis must be conducted. This will lower the risk of drug resistance with anti-tuberculosis therapy. A detailed physical examination, chest radiographare all included. The active incidence of MTB

remains high for the first year following a cancer diagnosis and the start of treatment. For example, in hematological malignancies, the active incidence falls from 12.01% (95% CI: 10.81—13.30) in the first six months to 2.70 (95 CI: 2.13.39) after 2 years.[44]

There isn't yet a proven method for treating reactivated MTB during immunotherapy. It is widely acknowledged that ICBs should be avoided when an infection is active due to the possibility of an excessive inflammatory response. Before restarting immunotherapy, it is typically advised to wait two weeks while receiving anti-tuberculosis medication. Close monitoring is necessary to identify any overlap in toxicities of either anti-tuberculosis therapy or anti-PD-1/PD-L1 maintenance, notably liver toxicity.

11. Non-tuberculous mycobacteria (Ntm) Infection in pediatric oncology

Nontuberculous mycobacteria (NTM) are common environmental organisms, but infrequently attacks hosts with impaired immune system. A cluster of NTM bacteremia in a haemato-oncology unit was investigated clinically, microbiologically, and epidemiologically, and the treatment of this infection was described by Baird SF and his colleagues.[45] Five patients receiving treatment for haematological malignancies had blood cultures that revealed *Mycobacterium mucogenicum* in four individuals and *Mycobacterium neoaurum* in one patient. NTM was also removed from the hospital water system after an investigation of the environment, particularly the water system.

To stop additional occurrences of NTM bacteremia in those individuals, central venous catheters (CVCs) were removed, and the patients were effectively treated with antibiotics, environmental modifications, and improvements in CVC management. *Mycobacterium mucogenicum*, *Mycobacterium phocaicum*, and *Mycobacterium chelonae* are a few of the species that have been identified.[46] Diagnosis may be delayed if the early microbiological results are misinterpreted. Among the clinical effects of infection include bacteremia, septic emboli, lung colonization, and infections of the soft tissue and bones. In order to avoid and control instances with the proper corrective actions, it is crucial to quickly identify and investigate groups of cases.[47]

12. Hodgkin's lymphoma and tuberculosis in children

Hodgkins Lymphoma is one of the most common malignancies of pediatric age group involving lymph nodes and extranodal sites. In India, Tuberculosis, being one of the most common diseases with an annual risk of infection between 2% and 5% in children[45] Cell-mediated immunodeficiency in Lymphoma may predispose them to many infections including[45] Due to the similarities in their clinical, radiological, and pathological characteristics, TB and Hodgkins Lymphoma can arise before, concurrently, during, or after therapy for HL, and this can make diagnosis difficult. It may be challenging to distinguish between relapse or resistant disease, if tuberculosis develops during or after the treatment of lymphoma.

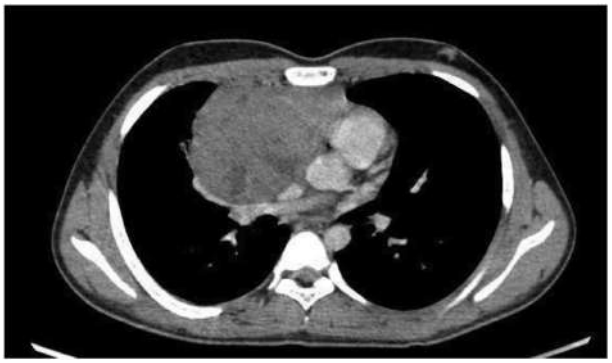

Fig. 3 – A 14-year-old boy with fever, weight loss anorexia and urticarial rashes. CT Thorax- Large necrotic mediastinal lymph node came out as Hodgkin's lymphoma on biopsy.

Because both TB and HL may present with similar symptoms and sometimes radiology, a site specific biopsy and histopathology is most accurate and sensitive diagnostic tool.

Both HL and NHL exhibit the caseating or necrotizing granulomatous lesions characteristic of TB. For the diagnosis of classical HL, Reed Steinberg (RD) cells must express the CD15 and CD30 antigens. Similar to this, AFB must be present in the biopsy or culture to confirm the existence of TB.

172 patients with HL were included in major Indian study from Chennai by Radhakrishnan et al, despite the fact that 32 of them had received empirical ATT prior to the diagnosis of HL and none of them had evidence of active TB at the time of diagnosis.

Malignancy-related TB might present with unusual symptoms, making it difficult to distinguish between the two, which can delay diagnosis and further care. A definitive diagnosis can be made by histopathological examination using biopsy, immunohistochemistry, and the presence of Acid-fast Bacilli (AFB). In our center a 14-year-old boy was presented with urticarial rashes, anorexia, fever and cough of 1 month duration. He was started on ATT from outside centre and there was noclinico radiological response. CT thorax showed a large necrotic mediastinal lymph node - Endo bronchial ultrasound (EBUS TBNA) and biopsy results confirmed it as Hodgkin'slymphoma with histopathology showing Reed Sternberg cells, and chemotherapy was initiated (Fig. 3). A high index of suspicion of concomitant TB and HD is needed, especially in a country like India where TB is very prevalent. The difficulty in differentiating the two can cause delay in the diagnosis and further management.

13. Conclusion

There is a need for Anti tuberculous medications with less toxicities, child-friendly formulations, and shortened duration regimens. Given the hepatotoxicity of ATT, many liver transplant recipient children are at risk for interruptions in therapy and graft rejection. Shorter duration regimens for

tuberculosis are key to prevent these complications and improving patient compliance. MDR-TB treatment remains a major threat worldwide, with a need for clinical trials in children and treatment availability in low-resource settings. Young children remain vulnerable to TB complications, as it is difficult to obtain a microbiological diagnosis in this age group. Since immunosuppression may alter TST and IGRA responses, there is a high need for diagnostic tests for TB infection that do not rely on an intact T-cell response. Further, a test that distinguishes TB infection and disease also is needed. Children with malignancy should have a comprehensive pre chemo or pre-transplant evaluation plan to screen for TB infection and active disease in order to reduce the risk of post-transplant TB. Children undergoing SOT and HSCT have their immune response impaired, due to decreased T-cell function from conditioning regimens and immunosuppressive regimens. Pediatric transplant recipients are at greatest risk for pulmonary TB, but can additionally have extra pulmonary and disseminated disease with atypical presentations which hamper the diagnosis and early treatment. It is important to maintain a high degree of suspicion for TB in these children. Patient characteristics and drug susceptibility patterns should be considered when choosing a treatment regimen. Literatures regarding tuberculosis in childhood cancer from high TB burden areas are limited. Hence we recommend more epidemiological studies to be conducted on this subject.

Conflicts of interest

The authors have none to declare.

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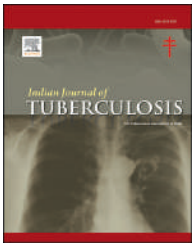

Review article

The 'pulmonary diseases spectrum' in HIV infected children

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ABSTRACT

Despite advances in diagnostic, therapeutic and preventive strategies for HIV, pulmonary diseases continue to be the major cause of morbidity and mortality in infants and children infected with HIV. With effective programs to prevent perinatal HIV-1 transmission to early diagnosis in infants, we have seen a substantial decline in paediatric HIV incidence. Early initiation of Highly Active Anti-Retroviral Therapy (HAART) in all HIV infected children coupled with consistent use of *Pneumocystis* prophylaxis in all HIV exposed/infected children under 5 years of age has considerably reduced associated infections overall and respiratory infections in particular. In developing countries already burdened with poverty, malnutrition, suboptimal immunization coverage and limited access to health care and treatment, acute and chronic HIV-associated respiratory disease remain a major cause for concern. Prevention of severe respiratory infections in advanced HIV disease among children consists mostly of rapid and optimal HAART initiation & continuation, preventing severe TB disease with BCG and TB preventive treatment, preventing *Pneumocystis jirovecii* pneumonia with cotrimoxazole prophylaxis and administering age-appropriate vaccinations and catch-up vaccines as per National Immunization schedule.

1. Introduction

As of 2021, globally 2.73 million (2.06–3.47 million) Children and adolescents <19 years are living with HIV, of whom 52% (42–65%) have had access to treatment; an estimated 110,000 (80,000–160,000) deaths have been due to HIV; mostly because of inadequate access to HIV prevention, care and treatment services, each day in 2021 approximately 850 children are said to have contracted HIV and 301 children have died from AIDS related causes.[1]

Pulmonary infections remain a leading cause of morbidity and mortality and one of the most frequent causes of hospital admission in HIV infected patients worldwide.[2] Most HIV-related pulmonary disease presents in infancy or early childhood. Preventable and treatable respiratory infections are common and include upper respiratory infections. HIV-infected children have a higher risk of increased frequency as well as increased severity of respiratory infections. In advanced disease, there are usually more than one pathogen. In the absence of HAART, up to 90% of children will develop a severe respiratory illness sometime in the course of their

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disease.[3] Furthermore there is increasing evidence suggesting that HIV-exposed but uninfected children are also at greater risk of respiratory infections compared to those who are born to an HIV-uninfected mother.[4] Clinical, epidemiological and postmortem studies have established that Lower Respiratory Tract Infections (LRTIs) caused by common childhood respiratory pathogens (viral and bacterial), as well as opportunistic pathogens, such as *Pneumocystis jirovecii* (PCP), cytomegalovirus (CMV) and *Mycobacterium tuberculosis* (TB) are the major cause of morbidity and mortality in HIV-infected children.[5,6] Lung function impairment with predominantly irreversible lower airway obstruction and reduced aerobic function are often found in HIV-infected children and adolescents.[7] Increase in immune activation within the lung and direct infection of pulmonary macrophages and lymphocytes play an important role in the pathogenesis of pulmonary disease.[8] Defects in humoral (B cell) immunity leading to impaired ability to generate antigen specific response in addition to T cell destruction and impaired cell-mediated immune response contribute to infections.[9]

There are clear associations between immunosuppression and the risk of developing specific pulmonary infections. Common respiratory problems such as sinusitis and bronchitis, bacterial pneumonia and TB may occur at any CD4 count. Declining immune function increases the risk for all HIV-associated respiratory diseases including AIDS-defining opportunistic infections and neoplasms - PCP, MAC, fungal and viral pneumonias, Kaposi's sarcoma, cancers.

Pulmonary diseases seen in HIV infected children are listed in Table 1[10]

Important changes in the epidemiology of HIV-related pulmonary infections have occurred in the era of HAART; currently, bacterial pneumonia, especially pneumococcal pneumonia, seems to be the most frequent diagnosis in developed countries, with Pneumocystis Pneumonia being the second, the third being tuberculosis.[11] In contrast, in countries where Tuberculosis (TB) is endemic, pulmonary TB far exceeds PCP or Lymphoid Interstitial Pneumonitis (LIP). The development of chronic lung disease is common in HIV-infected children and may result from recurrent or persistent pneumonia of varied etiology. Many abnormalities of large and small airways and pulmonary parenchyma can produce chronic cough. Nevertheless, underlying cardiac disease should always be considered in the differential diagnosis of respiratory problems and rarely neoplastic pulmonary diseases.

Diagnosis and management is often difficult in resource limited settings, especially as most HIV-related pulmonary disease presents in infancy or early childhood. In the context of HIV, children are at increased risk of diagnostic errors because of overlapping clinical and radiographic features of most pulmonary diseases.

In line with the WHO Guidelines, all Children living with HIV (CLHIV) less than 5 years of age are categorized as having advanced disease regardless of CD4 cell counts or percent and clinical disease and are offered advanced disease package - evaluation for pneumonia, TB, bloodstream bacterial infections, diarrhoeal disease and malnutrition.[12] Moreover, most infections require multiple drugs for prolonged periods for effective therapy and prevention of development of drug resistance making drug to drug interactions and drug toxicities more common and treatment adherence more complicated. This poses as an enormous challenge to both clinicians and public health workers. Systematic approaches coupled with improvements in diagnosis and available treatment options/preventive and prophylactic strategies have led to earlier diagnosis and improved survival.

2. Pulmonary Tuberculosis (PTB)

In 2019 approximately 11% (1.1 million) of total TB cases occurred among children <15 years; 13.8% of all TB deaths were in children, of which 6.5% deaths were in children with HIV infection.[13] Coexisting HIV infection has been the most important factor for the resurgence of TB worldwide. TB and HIV facilitate each other in multiplying rapidly, leading to early clinical progression and death. The diagnosis of childhood TB that has always been difficult, remains even more challenging in the face of the dual epidemic of HIV and TB, especially in resource limited settings.[14] TB in HIV infected children can be more severe and progression to death, more rapid. As in India, in many countries, BCG vaccination at birth is a norm for all babies including babies born to HIV-positive mothers as it gives protection against disseminated and severe forms TB.

TB in children is a direct consequence of adult TB and is a marker of current transmission of TB in the community. Infection with HIV is a strong risk factor for progression from latent to active tuberculosis. Risk of recurrent TB infection is relatively high even among cured TB cases in HIV infected children. Childhood TB with concurrent severe malnutrition and HIV infection, contribute to increased mortality. HIV and TB co-infection occurs in up to 50% of children living with HIV. Clinical manifestations at the time of initial infection vary according to the age of the child and immune response. The common presenting symptoms are cough, fever, wheeze, decreased appetite and fatigue. Children may be devoid of symptoms despite abnormal radiographic findings and vice versa. Respiratory distress is infrequent unless there is extensive disease or co-infections. Atypical features, extrapulmonary & miliary TB are common among younger children. Older children and adolescents may present with cavitary tuberculosis. As HIV infection progresses and immunity declines, TB rapidly progresses to disseminated disease.

In the presence of co-infection, children are at increased risk of diagnostic errors because of overlapping clinical and radiographic features. In general, the clinical features and radiological manifestations in children with relatively preserved immunocompetence may be indistinguishable from that in non-HIV infected children, although the disease is usually more severe. TB can occur in HIV with any level of CD4 count.

To prevent and ensure seamless management of HIV-TB coinfection, emphasis is laid on strengthening of **'3Is' strategy**, i.e. **I**ntensive **C**ase **F**inding (ICF), **A**irborne **I**nfection **C**ontrol (AIC) and **I**soniazid **P**reventive **T**herapy (IPT), along with the provision of daily anti-TB treatment (ATT) at all Anti-Retroviral Treatment centres (ARTC) as part of 'single-

Table 1 – Causes of Lung diseases in HIV positive Children.[10]

Opportunistic infections			
Bacteria	Fungi	Viruses	Protozoa
-*Mycobacterium tuberculosis*	- Pneumocystis jerovicii (PCP)	- Cytomegalovirus (CMV)	- Toxoplasma gondii
-*Streptococcus pneumoniae*	- *Candida albicans*	- Epstein–Barr Virus (EBV)	- *Strongyloides stercoralis*
-*Haemophilus influenzae*	- *Cryptococcus neoformans*	- Herpes Simplex Virus (HSV)	
-*Staphylococcus aureus*	- *Histoplasma capsulatum*	- Varicella Zoster Virus (VZV)	
- *Pseudomonas aeruginosa*	- *Coccidioides immitis*		
- *Klebsiella pneumoniae*	- Penicillium marneffei		
- Atypical mycobacteria			
- *Moraxella catarrhalis*			
- Anaerobes			
Neoplasms			
- Lymphoma			
- Tumors of smooth muscle origin			
- Mucosa associated lymphoid tissue lesions (MALT)			
- Pulmonary Kaposi sarcoma			
Others			
- Upper Respiratory Tract illness- URTI, sinusitis, otitis media, bronchitis, pertussis			
- LIP (Lymphoid Interstitial Pneumonia)			
- Asthma			
- Bronchiectasis			
- Immune Reconstitution Inflammatory Syndrome (IRIS)			
- Spontaneous pneumothorax			
- Foreign body aspiration			
- Severe gastroesophageal reflux			
- Metabolic derangement - systemic acidosis			
- Pulmonary Lymphoid Hyperplasia (PLH)			
- Lymphoproliferative thymic cysts			
- Pulmonary symptoms associated with cardiac disease - Congestive cardiac failure, Cardiomyopathy, Cardiomyopathy secondary to Zidovudine exposure			
- Cystic fibrosis			
- Alveolar hemorrhage			
- Sarcoid			

window services' by National AIDS Control Organisation (NACO) along with Central TB Division (CTD).[15]

All children should be screened for TB using **4S screening tool** which includes current cough, fever, reported weight loss or confirmed weight loss >5% or no weight gain since last 3 months and history of contact with a person with any form of active TB within last 2 years. A child with any one of these four symptoms should be thoroughly investigated for active TB with chest x-ray, CBNAAT on (induced) sputum, gastric aspirate, or other extra-pulmonary samples as relevant. In the presence of HIV, the diagnosis can be obscured by the frequency of atypical clinical and radiographic findings and overlap with other AIDS-associated infections; anergy on tuberculin testing and increased likelihood of a negative acid-fast smear of sputum add to the diagnostic dilemma making over-diagnosis as well as under-diagnosis a common occurrence. Basic components of history - signs and symptoms including previous TB or contact with infectious case, 'Tuberculin skin testing (induration of > 5 mm), radiographic findings, with or without microbiologic confirmation with smear/culture are all essential for diagnosis. WHO strongly recommends using lipoarabinomannan (TB-LAM) to assist in diagnosing active TB among CLHIV less than 5 years of age with signs and symptoms of TB or those that are seriously ill regardless. Urinary LAM assays have shown greater sensitivity in people living with advanced HIV disease & lower CD4 cell counts.

The IAP algorithm for pediatric intra-thoracic TB among children with no risk factors for drug resistance is provided in Flow Chart 1.[16]

Both HIV and TB require multiple drugs for prolonged periods for effective treatment and prevention of development of drug resistance. Pill burden, serious drug interactions between some Anti-retroviral drugs and Anti-TB drugs, adverse effects & risk of developing Immune Reconstitution Inflammatory Syndrome (IRIS) lead to challenges in treating co-infection, warranting vigilant monitoring. Nevertheless, prompt and early treatment of tuberculosis in the HIV infected child is critically important. In newly diagnosed cases, initiation of anti-TB treatment takes priority regardless of CD4 cell count, followed 2 weeks later by initiation of ART (except when signs and symptoms of meningitis are present when ART initiation should be delayed up to 8 weeks). ART is lifelong and optimal adherence is key to the successful management. Undue delay in starting ART could result in significant risk of HIV related deaths especially in drug resistant patients.

The ART regimen needs to be modified in all CLHIV receiving rifampicin-based ATT. The dose might also need modification as these children start gaining weight with successful management. However, drugs remain unchanged unless the child develops intolerance or toxicity warranting a change.

Rifampicin (RIF) stimulates the activity of the cytochrome P450 liver enzyme which is involved in the metabolism of many Anti-retroviral drugs, like Protease-inhibitors (PI) and Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI), leading to their sub-optimal blood levels. In turn, PIs and NNRTIs can enhance or inhibit the same enzyme system leading to altered blood levels of RIF. Isoniazid (INH) used for TB treatment may aggravate peripheral neuropathy with concomitant use of certain NRTIs like Didinosine, Zalcitabine, Stavudine. Furthermore, Anti-TB drugs may have adverse interactions with other medications prescribed for HIV patients, like fluconozole, ketoconozole, clarithromycin, etc. Bedaquiline, metabolized by the CYP3A4 has multiple drug interactions with PI and NNRTI. Suboptimal HIV treatment and/or TB treatment, occurrence of drug toxicities (especially hepatotoxicity) can be caused by drug-to-drug interactions and should be vigilantly monitored. Cotrimoxazole Prophylaxis (CPT) has to be continued in all HIV-TB co-infected children as it reduces morbidity and mortality.

ART regimens in CLHIV with TB are summarized in Table 2.[17]

Efavirenz based ART (no longer used as 1st line ART), however does not require any modification with RIF-based ATT. For babies older than 4 weeks and weighing 3 kg, the use of Dolutegravir (DTG) 5 mg has been approved by FDA (not available for use in our country yet).[18]

For treatment of RIF sensitive pulmonary TB in children, the recommendation is 2HRZE + 4HRE in appropriate doses; ethambutol is added in both the intensive and continuous phases of therapy, as 12–14% of background resistance to INH is prevalent in our country.[19] At the end of treatment, six months of INH Preventive therapy (IPT) is also advised to reduce the risk of relapse (secondary prophylaxis). Pyridoxine supplementation (10 mg/day) should be given along with INH.

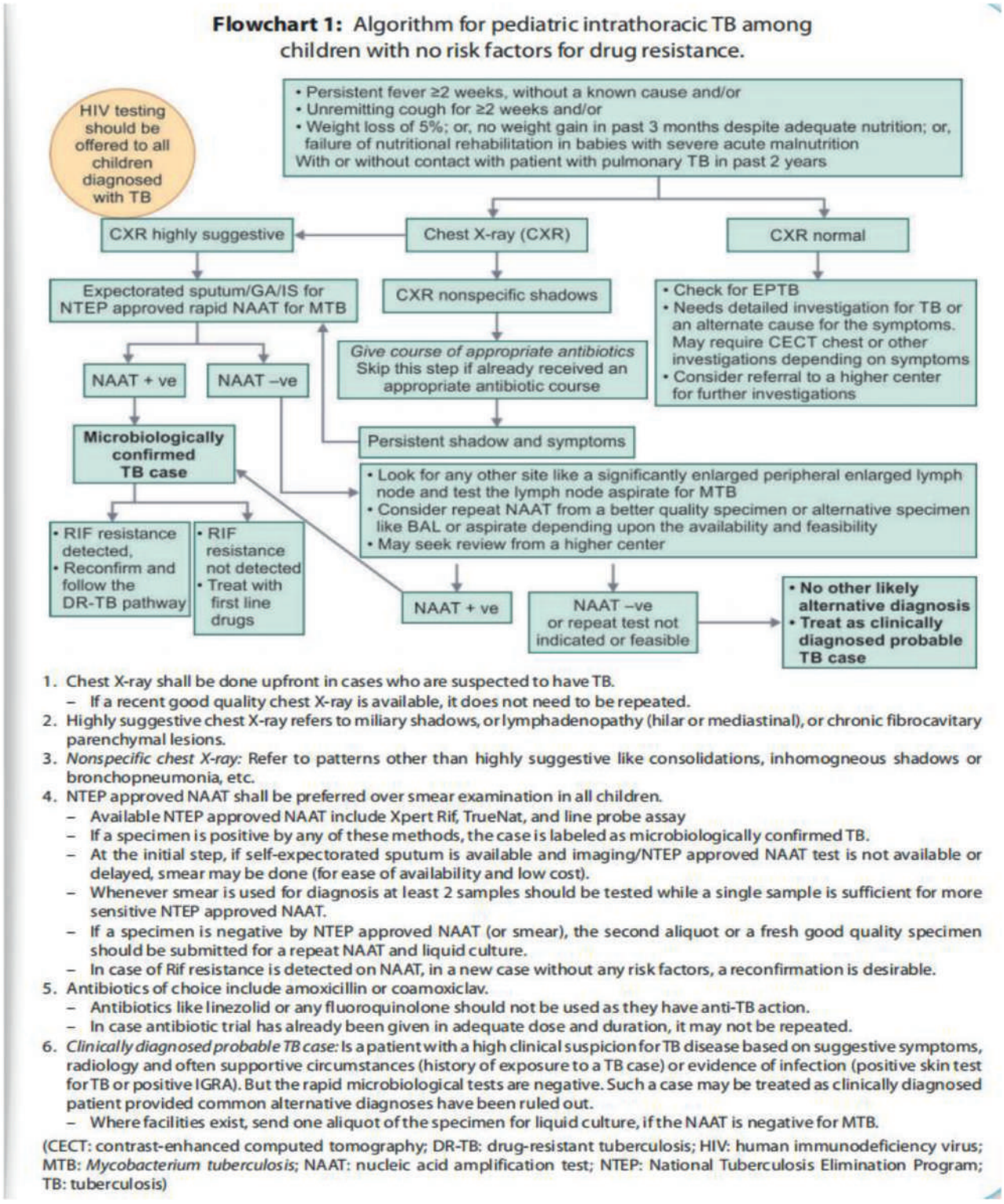
WHO recommends a shortened 4-month regimen (2HRZ(E)/2HR) regimen in children and adolescents under 16 years of age with non-severe, presumed drug-susceptible TB, rather than the standard 6-month regimen (2HRZ(E)/4HR).[20]

When a child already on ART presents with active TB, ART modification may need to be done depending on the ongoing ART regimen, to maintain optimal efficacy of ATT as well as ART. ART treatment failure should be suspected especially if clinical and immunological evidence of disease progression is present.

2.1. *Drug-Resistant (DR) TB & HIV*

In children who show poor/no response clinically or radiological deterioration despite adherence to ATT by the end of 4 weeks, a probable DR should be suspected, especially if there are one or more of the following risk factors: exposure to a DR TB case and/or history of recent death in family due to TB, children with recurrent TB, who were earlier lost to follow up (LFU) and whose microbiological tests are negative/or no access to specimen. It is important to exclude other Opportunistic Infections (OIs) and diseases before suspecting a probable DR case. The treatment is the same as in HIV negative individuals. However, in the context of HIV, DR TB treatment is much more challenging with adverse events being more common. Rigorous monitoring is required in order to reduce default, ensure adherence to both, identify and treat adverse events and make necessary drug modifications.

Dolutegravir can be used safely with the new TB drugs - Bedaquiline and Delamanid. Bedaqualine with Efavirenz and Lopinavir/ritonavir based ART regimens should be used with caution ensuring frequent clinical and cardiac evaluation. Co-administration of Delamanid with Tenofovir, Lopinavir/



1. Chest X-ray shall be done upfront in cases who are suspected to have TB.
   - If a recent good quality chest X-ray is available, it does not need to be repeated.
2. Highly suggestive chest X-ray refers to miliary shadows, or lymphadenopathy (hilar or mediastinal), or chronic fibrocavitary parenchymal lesions.
3. *Nonspecific chest X-ray:* Refer to patterns other than highly suggestive like consolidations, inhomogeneous shadows or bronchopneumonia, etc.
4. NTEP approved NAAT shall be preferred over smear examination in all children.
   - Available NTEP approved NAAT include Xpert Rif, TrueNat, and line probe assay
   - If a specimen is positive by any of these methods, the case is labeled as microbiologically confirmed TB.
   - At the initial step, if self-expectorated sputum is available and imaging/NTEP approved NAAT test is not available or delayed, smear may be done (for ease of availability and low cost).
   - Whenever smear is used for diagnosis at least 2 samples should be tested while a single sample is sufficient for more sensitive NTEP approved NAAT.
   - If a specimen is negative by NTEP approved NAAT (or smear), the second aliquot or a fresh good quality specimen should be submitted for a repeat NAAT and liquid culture.
   - In case of Rif resistance is detected on NAAT, in a new case without any risk factors, a reconfirmation is desirable.
5. Antibiotics of choice include amoxicillin or coamoxiclav.
   - Antibiotics like linezolid or any fluoroquinolone should not be used as they have anti-TB action.
   - In case antibiotic trial has already been given in adequate dose and duration, it may not be repeated.
6. *Clinically diagnosed probable TB case:* Is a patient with a high clinical suspicion for TB disease based on suggestive symptoms, radiology and often supportive circumstances (history of exposure to a TB case) or evidence of infection (positive skin test for TB or positive IGRA). But the rapid microbiological tests are negative. Such a case may be treated as clinically diagnosed patient provided common alternative diagnoses have been ruled out.
   - Where facilities exist, send one aliquot of the specimen for liquid culture, if the NAAT is negative for MTB.

(CECT: contrast-enhanced computed tomography; DR-TB: drug-resistant tuberculosis; HIV: human immunodeficiency virus; MTB: *Mycobacterium tuberculosis*; NAAT: nucleic acid amplification test; NTEP: National Tuberculosis Elimination Program; TB: tuberculosis)

Flow Chart 1 – Algorithm for Pediatric intra-thoracic TB in children with no risk factors for drug resistance.[16]

ritonavir, or Efavirenz based ART regimens can be safely done, as demonstrated by lack of significant CYP-related drug–drug interactions in clinical studies and *in-vitro* studies.[21]

Tuberculosis Preventive Treatment (TPT) with six months of isoniazid (6H) is recommended after pro-actively ruling out active TB disease in all HIV-infected infants who are in contact with a patient of pulmonary TB. Adolescents and children >12 months living with HIV should be screened for TB using four-symptom complex, and TPT provided to those without symptoms or after ruling out active TB in those with

Table 2 – ART drug regimens for CLHIV during co-treatment of Drug-Sensitive TB (2HRZE+ 4HRE regimen).[17]

Weight and Age	Recommended ART regimen
Weight: Less than 20 kg or Age below 6 years	FDC of Abacavir + Lamivudine twice daily as per weight band + Lopinavir/ritonavir + super-boosting with additional ritonavir (ratio of 1:1), twice daily as per weight band
Weight: Between 20–30 kg and Age between 6–10 years	FDC of Abacavir + Lamivudine twice daily as per weight band + Dolutegravir (DTG) 50 mg twice daily
Weight: Above 30 kg and Age above 10 years	FDC of Tenofovir + Lamivudine + Dolutegravir (DTG) once daily + Tab Dolutegravir 50 mg after 12 hours

* DTG dose to remain twice daily for 2 weeks AFTER stopping RIF-containing ATT.
*Super-boosting of Lopinavir with ritonavir to continue for 2 weeks AFTER stopping RIF-containing ATT.
FDC: Fixed Drug Combination; RIF -containing ATT: Rifampicin -containing Anti-TB-Treatment.

symptoms. TPT should be given to all irrespective of the degree of immunosuppression, whether on ART or had previous TB treatment.[15]

3. Mycobacteria Other Than TB (MOTT)

Commonly found in the environment and referred to as Mycobacterium Avium Complex (MAC), they rarely cause respiratory symptoms and isolated pulmonary disease. MAC disease typically occurs in patients with severe immunesuppression and presents with fever associated with night sweats/chills, weight loss, muscle wasting, abdominal pain, tiredness, pallor and diarrhoea with enlarged liver, spleen and lymph nodes. CXR may show pulmonary infiltrates. A confirmed diagnosis is based on compatible clinical signs and symptoms coupled with the isolation of MAC from cultures of blood, lymph node, bone marrow. Treatment is a combination therapy using clarithromycin/azithromycin/ethambutol/RIF/ciprofloxacin. Most effective way to prevent disseminated MAC infection is to preserve immune function with effective HAART. Primary prophylaxis with Azithromycin (20 mg/kg per week PO) is indicated in children with advanced immunosuppression. Secondary prophylaxis is indicated for life in all confirmed cases to prevent recurrence.

4. Bacterial pneumonias

Bacterial pneumonias are very common in HIV infected children and recurrent bacterial pneumonias are a severe manifestation of HIV disease. It can occur at any time during the course of HIV infection and at any CD4 lymphocyte count. The frequency and severity however, increases with increasing immunosuppression.

The commonest cause is Sreptococcus pneumoniae and response to treatment is usually satisfactory. Other causes include *Haemophilus influenzae*, Salmonella, *Staphylococcus aureus*, Klebsiella pnemoniae and *Escherichia coli*. These pathogens are sensitive to commonly available recommended antibacterials. The severity of illness and need for hospitalization should be classified according to the WHO Integrated Management of Neonatal & Childhood Illness algorithms.[22] PTB should be considered when there is a poor clinical response to standard antibiotics and mother has TB. Pneumonia due to Staphylococcus or Klebsiella may be a problem in HIV infected children with chronic lung disease. Recurrent pneumonias can result in acute-on-chronic alterations in lung architecture and can cause cystic changes and cavitation including bullous lung disease and bronchiectasis. Because of difficulties in obtaining appropriate specimens (e.g. sputum), the diagnosis of pneumonia is often made on the basis of clinical features: high fever, cough, respiratory distress with or without hypoxemia, wheeze and radiographic findings. In patients with underlying chronic lung disease/Lymphoid Interstitial Pneumonitis (LIP), superimposed bacterial pneumonia may significantly worsen the respiratory status. Focal infiltrates or consolidation are seen on Chest radiographs. The local prevalence of resistance to common infectious agents and the recent use of prophylactic or therapeutic antibiotics should be considered when initiating empiric therapy. HIV infected children whose immune systems are not seriously compromised and who are not neutropenic can be expected to respond well and should be treated with the usual antimicrobial agents recommended for the most likely bacterial organisms. When the organism is identified, therapy should be based on antibiotic susceptibility results.

Prompt treatment with antibiotics, physiotherapy and nutritional support will reduce or prevent bacterial infections in general; the incidence of invasive disease can be reduced in particular with *Hemophilus influenzae* & Polysaccharide pneumococcal vaccines.

5. Pneumocystis jerovecci Pneumonia (PCP)

PCP is the most common AIDS defining condition and most life-threatening OI in the developed countries. Primary asymptomatic infection occurs in immunocompetent hosts as evidenced by presence of serum antibodies against the organism in most children by 2–4 years of age.[23] Patients most at risk remain those with advanced HIV infection with impaired cell mediated immunity. HAART and routine use of prophylaxis when needed, has considerably brought down the incidence of PCP. The highest incidence is in the 1st year of life, with cases peaking at age 3–6 months and at a much higher CD4 cell count than older children or adults. Hence, it is mandatory for all HIV-exposed infants to receive primary prophylaxis for PCP from 6 weeks of age till HIV infection as been ruled out. In all confirmed HIV-infected children, CPT should be continued till 5 years of age in the dose of 5 mg/kg/day of Cotrimoxazole (Sulfamethoxazole and Trimethoprim

combination) once daily; also recommended in older children with WHO Stage 3 and 4 regardless of CD4 cell count, or with CD4 <350 cells/mm^3 regardless of WHO staging.[17]

Because clinical presentation, blood tests and chest radiographs are not pathognomonic for PCP and because the organism cannot be demonstrated routinely, a presumptive diagnosis should be made in all infants & young children, with clinical symptoms, low CD4 count, presence of oral candidiasis, inadequate response to bacterial pneumonia treatment, suboptimal PCP prophylaxis/HAART in the past. Demonstration of organisms in tissue (by Wright–Giemsa staining/ Gomori's methenamine silver stain/Immunofluorescence staining), Broncho-alveolar-lavage (BAL) or induced sputum sample are required for a definitive diagnosis of PCP. Suspect PCP if tachypnoea, cyanosis and relatively few lung signs compared to the degree of respiratory difficulty. The chest x-ray commonly shows bilateral diffuse parenchymal infiltrates with 'ground-glass' or reticulogranular appearance. In mild cases, perihilar parenchymal infiltrates progressing peripherally to the apical regions are seen. Rarely lobar, cavitary or miliary lesions, pneumothorax or pneumomediastinum may be seen.

As the definitive diagnostic test for PCP is not readily available, it is advisable to empirically treat all infants with HIV infection & severe pneumonia with oral cotrimoxazole (20 mg of trimethoprim and 100 mg of sulfamethoxazole per kg per day), every six hourly for 21 days. Treatment should not be withheld for want of confirmation. The early use of steroids in infants with hypoxaemia and respiratory failure in addition to co-trimoxazole therapy significantly reduces mortality. If there is no clinical improvement after a few days of PCP treatment, other causes should be pursued including treatment failure, co-infection with other bacteria, CMV. Lifelong suppression as secondary prophylaxis is indicated following treatment for PCP to prevent recurrence. Between 1 and 5 years old, cotrimoxazole is not empirically recommended in CLHIV with severe pneumonia as prevalence of PCP in this age group is low.[17]

6. Viral and fungal pneumonias

The viruses which cause lower respiratory tract infections in immunocompetent children also infect children with HIV infection - respiratory syncytial virus (RSV), para influenza viruses, influenza viruses, adenoviruses, etc. They may occur in isolation or worsen a pre-existing/concurrent opportunistic infection. Potential systemic involvement and prolonged viral excretion should be kept in mind. CMV pneumonia is generally interstitial in nature with gradual onset of shortness of breath and a dry non-productive cough with minimal auscultatory findings. Other end organ CMV disease should be pro-actively looked for with profound immunosuppression; prognosis is very poor with high early mortality.

Severe Acute Respiratory Illness (SARI) is an important cause of mortality in young children, especially with HIV infection. A study done in South Africa analyzing SARI programme data which included 5297 children aged <5 years with SARI-associated hospital admission, showed increased mortality in HIV-infected children than in HIV-uninfected children[24]

6.1. *COVID-19 & HIV infection*

The respiratory system is usually affected with clinical manifestations being upper respiratory tract symptoms, cough, dyspnoea, fever and sore throat. Symptoms appear to be same as reported in the general population, with good outcomes mostly; in severe cases, pneumonia with ARDS, acute hypoxic respiratory failure and death may occur. The interaction with regards to severity and outcomes remains little understood. None of the HIV and antiretroviral treatment-related variables have been found to be associated with greater infection risk or protection; a systematic review done recently reveals there is no association established between COVID-19 and HIV.[25,26]

Fungal pneumonias although rare, are being increasingly encountered in HIV infected children as a consequence of severe immunodeficiency. Aspergillosis, histoplasmosis, candidiasis, penicilliosis, cryptococcosis, coccidiodomycosis and nocardiosis may present with pulmonary involvement and progressive pneumonia/disseminated infection. They cause high morbidity and mortality despite the recent advances in anti-fungal therapy due to limited availability, safety and efficacy. Aspergillosis may occur as locally invasive pulmonary and sinus disease requiring surgical removal of the affected area along with high dose Amphotericin B or Voriconazole and long term suppressive therapy.

7. Lymphoid Interstitial Pneumonitis (LIP)

A distinctive marker of pediatric HIV infection, it may be the first clinical manifestation of HIV. It occurs in 30–50% of children with perinatally acquired HIV if they are not treated with HAART and usually presents in the 2nd-3rd year of life; LIP as the first AIDS indicator disease shows improved survival compared with other AIDS indicator diseases.[27] A WHO Clinical Stage 3 criterion, it represents an abnormal lymphoproliferative response, either to HIV alone, or due to superinfection with another virus, probably Epstein–Barr virus.

LIP can present with a varied spectrum - asymptomatic disease to severe pulmonary insufficiency. The disease is often insidious in onset and slowly progressive, with mild cough, dyspnea, chronic painless parotid enlargement, generalized lymphadenopathy, hepatosplenomegaly and clubbing. The natural history of the disease varies, as it may spontaneously resolve or worsen, but it is generally benign with a favourable prognosis. LIP should be suspected in a child who despite being given appropriate antibiotics or ATT, does not respond clinically or radiologically. The development of LIP is well correlated with serum IgG levels greater than 2500 mg/dL.Typically bilateral diffuse reticulonodular infiltrates in the lower lobes with enlarged mediastinal or hilar lymph nodes may be seen on CXR. Chest Computed Tomography (CT) is used to establish the interstitial pattern and also for monitoring the disease, but lung biopsy is the only definitive diagnosis. Many children having repeated hospital admissions for LRTIs may have underlying LIP.

The important features that help to differentiate between LIP, miliary TB and PCP are listed in Table 3.

There is no specific treatment for LIP. Systemic corticosteroids (Prednisolone 1–2 mg/kg daily may be given for 2–4 weeks and then tapered gradually over 2 weeks) for severely symptomatic patients with significant hypoxemia and pulmonary insufficiency. Some may be refractory to therapy requiring prolonged low dose treatment. Oxygen, bronchodilators and other supportive measures may be required for severe hypoxemic episodes.

8. Chronic respiratory diseases

Chronic HIV associated lung disease include chronic infections, LIP, IRIS, bronchiectasis, malignancies, and interstitial pneumonitis. Underlying cardiac causes should be considered and ruled out. Chronic lung disease may present with chronic symptoms and persistent chest X-ray changes resulting from recurrent or persistent pneumonia due to polymicrobia. TB is an important cause to be ruled out. Definitive diagnosis can be challenging. Treatment includes early initiation and continuation of HAART, specific therapy, steroids for children with IRIS or LIP who are hypoxic or who have airway compression from tuberculous nodes and pulmonary clearance techniques as and when required. Timely immunization, HAART with good adherence, chemoprophylaxis and micronutrient supplementation all play a major role in prevention.

Bronchiectasis is a permanent abnormal dilatation of the bronchi and usually results from damage to the pulmonary mucosa and the bronchial wall by previous infectious processes causing irreversible alteration in shape and function, predisposing to recurrent infections. In HIV infected children it is usually secondary to LIP or TB. Finger clubbing, halitosis and cough productive of copious purulent/blood-stained sputum, with Chest Xray/CT often revealing evidence of bronchial wall thickening/honey-combing give clue to the diagnosis. Prompt anti-microbial therapy, aggressive chest physiotherapy and anti-inflammatory therapy are all important in management.

9. Immune Reconstitution Inflammatory Syndrome [IRIS]

It is defined as a collection of signs and symptoms resulting from the ability to mount an immune response to antigens/organisms associated with immune recovery on ART; it results in paradoxical clinical deterioration after starting ART from an improving immune system as evidenced by increase in CD4 counts from baseline. Frequency is roughly 10% of all patients initiating ART and up to 25% among patients initiating ART with a CD4 cell count <50 cells/mm^3 or severe clinical disease.[28] It usually presents within 6 weeks of starting ART, but may take weeks to months. It is a diagnosis of exclusion and care must be taken to differentiate from an opportunistic infection or drug toxicity. IRIS is usually due to an active or sub clinical infection by an opportunistic organism (TB, PCP, MAC, Candida, Aspergillosis, Hep B/C, CMV,

Table 3 – Important features to differentiate between LIP, Miliary TB and PCP.

	LIP	MILIARY TB	PCP
Age	Older	Any	Younger
Immuno-suppression	Mild to severe	Mild to severe	Severe
Clinical features	Chronic cough, no fever	Fever, cough, weight loss	Acute onset of cough + tachypnoea
X ray findings	Persistent reticulo-nodular opacities ± Hilar lymphadenopathy	Miliary mottling	Perihilar diffuse infiltrates
Response to treatment	No response to antibiotics or ATT	Responds to ATT	Responds to Cotrimoxazole ± Prednisone

Herpes, etc) but may be due to noninfectious causes like sarcoidosis and auto-immune disorders. Clinical presentations vary and depend on the causative agent and the organ system involved. A child on HAART with improving immunity, presenting with high fever, lymphadenopathy, worsening of the original TB lesion and/or deteriorating radiographic manifestations, including the development of miliary pattern or pleural effusion should be suspected of TB-IRIS. Specific treatment should be initiated for the offending organism with continuation of HAART under close monitoring. In severe cases steroids for 2–4 weeks with gradual tapering is usually effective and rarely temporary discontinuing of ART may be warranted.

10. Pulmonary Malignancies

Although there is increased incidence of malignancies in HIV, it is generally rare in children. Presentation may be that of hilar adenopathy, mediastinal mass, isolated parenchymal nodules, diffuse interstitial disease incidentally found on chest radiographs or large pleural effusion which is bloody on aspiration: Non Hodgkin's Lymphoma, tumors of smooth muscle origin, Mucosa Associated Lymphoid Tissue lesions, or pulmonary Kaposi's Sarcoma. Presence of typical lesions on the skin, palate or conjunctiva may give a clue to the diagnosis. Lung biopsy is required for definitive diagnosis.

Conflicts of interest

The author has none to declare.

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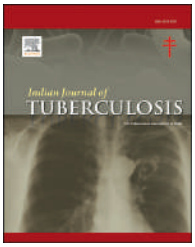

Original article

Missed phenotypic drug resistance in pediatric tuberculosis: A cause of concern in a resource-limited setting

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ABSTRACT

Background: Multi-drug resistance (MDR) in pediatric tuberculosis (TB) is a growing global threat. Unavailability of conventional or molecular drug susceptibility test (DST) in resource-limited settings often impede the determination of the extent of first line anti-tubercular drugs deployed in national programs.

Materials and method: Pulmonary and extra pulmonary specimens were collected from clinically suspected pediatric TB cases, who were microbiologically confirmed. Resistance to first-line anti-TB was detected by 1% proportion method. *KatG315* and *inhA-15* genes were amplified by PCR and detection of mutations were done by sequencing. Genotypic resistance for rifampicin was detected by Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, California).

Results: Fifty-one cases of pediatric tuberculosis were confirmed microbiologically. Resistance to isoniazid, streptomycin, rifampicin and ethambutol were 5 (14%), 4 (11%), 2 (5.5%) and 2 (5.5%) respectively by 1% proportion method. Genotypic Rifampicin and isoniazid resistance was found in 2 (5.5%) and 7 (14%) samples respectively.

Conclusion: Existing genotypic methods, detect targeted mutations conferring rifampicin resistance, however isoniazid (INH) resistance often go undetected. Since the resistance to pivotal anti-TB drugs are often encoded by multiple genes which may not be targeted by widely available molecular tests, discrepancies in molecular and culture-based DST reports should be interpreted with caution.

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1. Introduction

Since the advent of the first anti-tubercular drug, streptomycin, the biological phenomenon of drug resistance has emerged hand in hand. The earliest therapy involving the injection of intra venous streptomycin, continued to show treatment failure with the emergence of streptomycin resistant *Mycobacterium tuberculosis*.[1] Discovery of drugs like thioacetazone and para-aminosalicylic acid in 1948 and isoniazid in 1952 paved the way for combination drug therapy, which in time formed the crux of anti-tubercular chemotherapy. The discovery of the mycobactericidal agent rifampicin, which favorably possessed strong sterilizing potential, abetted the formulation of short and more effective isoniazid- and rifampicin-containing short-course chemotherapy protocol, DOTS (directly observed treatment short course).

Chaotic treatment protocols and repeated use of short course chemotherapy for polydrug resistant *M. tuberculosis* strains, paradoxically amplified the emergence of first line anti-tubercular drug resistance.[2] Community and facility based nosocomial transmission in areas of high tuberculosis (TB) prevalence substantially contributed to the spread and emergence of more resistant strains of TB.[3,4] The estimated 1 million children developing TB annually, merely underscores the enormous health concern.[5] Lack of diagnostic and treatment amenities accounted for a staggering global death toll of 208,000 children in 2019 as per World Health Organization (WHO); among which more than 70% of the deaths occurred in southeast Asia and Africa.[6] Drug resistance to first line anti-tubercular drugs like isoniazid and rifampicin continue to add on to an accruing burden of MDR-TB (multi-drug resistant tuberculosis) in children.[7]

The prevalence of MDR-TB in pediatric population, has been steadily increasing from 5.6% pre-2010 to 7% post-2010.[8] Few studies have been done to determine the prevalence and assess the modalities for diagnosing MDR-TB in pediatric population. The complexities of performance of tests along with infrastructure often impede determination of anti-TB drug susceptibility test (DST) in remote health care settings. The study was thus undertaken to ascertain susceptibility to all first line anti-TB drugs by conventional culture-based 1% proportion method and genotypic resistance to isoniazid and rifampicin by molecular techniques like cartridge-based nucleic acid amplification (CB-NAAT), PCR and sequencing.

2. Materials and method

A prospective cross sectional study was conducted at a tertiary care center to ascertain the burden of pulmonary and extra-pulmonary tuberculosis in pediatric population in India, over a period of one year. The study was approved by the institutional ethics committee (approval number IEC-HR/2017/32/62). All cases of clinically suspected TB referred from the department of Pediatrics to the department of Microbiology and DOTS (Directly Observed Treatment Short course) center, who were confirmed upon smear microscopy and/or culture and/or CBNAAT and who were ≤14 years of age, were included in the study. Pulmonary and extra-pulmonary specimens were collected as per Revised National Tuberculosis Control Program (RNTCP) guidelines. Approximately 5–6 mL of appropriate sample, where ever applicable, was collected from each study subject under aseptic conditions. Study subjects were traced back to obtain the relevant clinical details of the study subject after obtaining consent. All clinical samples were subjected to Zeihl Neelsen staining for direct demonstration of acid fast bacilli and culture on Lowenstein Jensen medium as per laboratory standards. Drug susceptibility tests (DST) for all first line anti-tubercular drugs was performed upon all culture confirmed study isolates of *M. tuberculosis* by 1% proportion method and interpreted as per RNTCP guidelines (Quality control: H37RV).

CB-NAAT was performed on all study specimens with the help of Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, California) using standard operating procedures. Sputum specimens were tested as per manufacturer's instructions, by adding buffer in 1:2 proportion. While for all other specimens standard operating procedures designed by Revised National Tuberculosis Program (RNTCP) were adopted. A cycle threshold of 38 was considered positive for *M. tuberculosis*.

Conventional polymerase chain reaction (PCR) was performed on all isolates for detection of *M. tuberculosis* by amplification of 130 bp fragment of insertion sequence *IS6110*. The primers used for amplification of katG315 and inhA-15 were (katG315F: GGCCCCGAACCCGAGGCT; katG315R: AACGGGTCCGGGATGGTGCCG; inhA-15F: CCGCCGATGAGAGCGGTGAGC; inhA-15R: CCACTGCTTTGCCGCCACCGC). The PCR was carried out with a pre-denaturation of 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, extension at 72 °C for 30 seconds and a final extension at 72 °C for 5 min. The H37Rv strain of *Mycobacterium* was used as positive control and sterile nuclease free water was used as negative control. PCR products of katG315 and inhA-15 were further sequenced after purification with the help of QIAquick PCR purification Kit. Purified bands visualized on agarose gel with the help of 100 bp DNA ladder. Forward and reverse gene specific primers were used for sequencing of PCR amplicons, using the BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic analyzer. Obtained sequencing files were analyzed with the help of freely available software: NCBI Basic Local Alignment Search Tool (BLAST). (Control: H37Rv strain of *M. tuberculosis*).

3. Results

During the study period, fifty one cases of pediatric TB confirmed by either smear microscopy or culture or CB-NAAT

Table 1 – Pulmonary and extra pulmonary pediatric tuberculosis diagnosed by various modalities.

	Pulmonary TB n = 40 (%)	Extra pulmonary TB n = 11 (%)
Smear microscopy	14 (35)	4 (36)
Culture	28 (70)	8 (73)
CB-NAAT	38 (95)	11 (100)

were included in the study. Table 1 depicts the number of pulmonary and extra pulmonary cases of pediatric tuberculosis, diagnosed by various modalities. The age of the study subjects ranged from 8 months to 14 years (mean 8.5 ± 0.48 years). Majority of the study population were female (55%, n = 28), whereas 45% were male subjects (n = 23). Among the entire study population, 78% (40) were diagnosed with pulmonary tuberculosis, while 22% (11) were suffering from extra-pulmonary tuberculosis as defined by Revised National tuberculosis Control Programme (RNTCP). The extra pulmonary TB cases comprised of tubercular meningitis (36.4%, n = 4), skeletal tuberculosis (36.4%, n = 4), tubercular lymphadenopathy (18.2%, n = 2) followed by tubercular psoas abscess (9%, n = 1).

3.1. *Drug susceptibility by 1% proportion method*

DST for the first line anti-tubercular drugs was done on all culture positive isolates as per 1% proportion method. Out of the total 36 culture positive isolates on which DST was done, 28 (78%) were sensitive to all the first line anti-tubercular drugs and eight (22%) samples showed anti-tubercular drug resistance in various forms. Table 2 illustrates the specimen wise frequency distribution of resistance to first line anti tubercular drugs by 1% proportion method (n = 36).

Among all culture positive isolates, 5 (14%) isolates were mono resistant, 2 (5.5%) were poly resistant and 1 (2.7%) isolate was an MDR. Isoniazid mono resistance and isoniazid resistance along with any other drug was present in 5 (14%) isolates. Mono-isoniazid resistance was found in 2 (5.5%) culture positive isolates and isoniazid resistance along with streptomycin or ethambutol was seen in 2 (5.5%) isolates. Resistance to streptomycin, rifampicin and ethambutol were 4(11%), 2(5.5%) and 2 (5.5%) respectively. Rifampicin mono-resistance was found in 1 (2.7%) isolates and rifampicin resistance along with other drugs was found in 1 (2.7%). Table 3 illustrates the profiles of eight isolates showing phenotypic drug resistance to first line anti tubercular drugs by 1% proportion method.

3.2. *Genotypic detection of isonizad resistance by PCR and sequencing*

PCR confirmed 50 isolates of *M. tuberculosis* by amplification of a 130 bp fragment of the insertion sequence *IS6110*. Furthermore PCR for *kat*G315 and *inh*A-15 genes confirmed the presence of *kat*G315 and *inh*A-15 genes in all fifty isolates. Upon sequencing, mutations in either *kat*G315 or *inh*A-15 genes were found in 7 (14%) samples out of fifty PCR products subjected to sequencing. Six (85.7%) had mutation in *kat*G315

Table 2 – Specimen wise frequency distribution of resistance to first line anti tubercular drugs by 1% proportion method (n = 36).

Culture positive Specimens	Positive by direct smear examination	Positive by CBNAAT	Rifampicin resistance by CBNAAT	Resistance to the first line Anti tubercular drugs by DST [Streptomycin (S), Isoniazid (I), Rifampicin (R), Ethambutol (E)] No. (%)			
				S	I	R	E
Gastric aspirate (n = 21)	10	21	1	2 (9.5)	4 (19)	1 (4.7)	2 (9.5)
Sputum (n = 4)	2	4	0	0	0	0	0
Pus (n = 2)	2	2	0	0	0	0	0
CSF (n = 5)	0	5	0	1 (20)	1 (20)	0	0
Pleural fluid (n = 2)	1	1	0	0	0	1 (50)	0
LN aspirate (n = 1)	1	1	0	1 (100)	0	0	0
Axillary tissue (n = 1)	0	1	0	0	0	0	0
Total no. (%)	16	35	1	4 (11)	5 (14)	2 (5.5)	2 (5.5)

Table 3 – Microbiological profile and resistance pattern of eight isolates showing phenotypic drug resistance to first line anti tubercular drugs by 1% proportion method.

S. No.	Type of TB (Clinical sample)	Smear microscopy finding	CBNAAT result (Rifampicin resistance)	Pattern of drug resistance	First line anti tubercular drugs [Streptomycin (S), Isoniazid (I), Rifampicin (R), Ethambutol (E)]
1	Pulmonary TB (Gastric Aspirate)	Positive	Positive (sensitive)	Mono resistance	I
2	Extra pulmonary TB (LN aspirate)	Positive	Positive (sensitive)	Mono resistance	S
3	Pulmonary TB (Gastric Aspirate)	Positive	Positive (sensitive)	Mono resistance	I
4	Extra pulmonary TB (CSF)	Negative	Positive (sensitive)	Poly-drug resistance	I,S
5	Pulmonary TB (Pleural fluid)	Positive	Not recommended	Mono resistance	R
6	Pulmonary TB (Gastric Aspirate)	Negative	Positive (sensitive)	Poly-drug resistance	I,S,E
7	Pulmonary TB (Gastric Aspirate)	Positive	Positive (Resistant)	Multidrug resistance	I,R,S
8	Pulmonary TB (Gastric Aspirate)	Negative	Positive (sensitive)	Mono resistance	E

I: Isoniazid; S: Streptomycin; R: Rifampicin; E: Ethambutol.

Table 4 – Phenotypic and genotypic profile of first line anti-tubercular drug resistant study isolates.

Clinical specimen	Smear microscopy finding	CBNAAT result (Rifampicin resistance)	Culture finding	Pattern of phenotypic drug resistance	Mutation on sequencing of *kat*G315 and *inh*A-15 genes
1 Pulmonary TB (Gastric Aspirate)	Positive	Positive (Sensitive)	Positive	Mono isoniazid resistance	*kat*G315
2 Pulmonary TB (Gastric Aspirate)	Positive	Positive (Sensitive)	Positive	Mono isoniazid resistance	*kat*G315
3 Pulmonary TB (Sputum)	Negative	Positive (Resistant)	Negative	___	*kat*G315
4 Extra pulmonary TB (CSF)	Negative	Positive (Sensitive)	Positive	Poly-drug resistance	*kat*G315
5 Pulmonary TB (Gastric Aspirate)	Negative	Positive (Sensitive)	Positive	Poly-drug resistance	*kat*G315
6 Pulmonary TB (Gastric Aspirate)	Positive	Positive (Resistant)	Positive	Multidrug resistance	*kat*G315
7 Extra pulmonary TB (Pus)	Negative	Positive (Sensitive)	Negative	___	*inh*A-15

gene, causing AGC to ACC substitution and 1 (14.2%) had mutation in *inh*A-15 gene with C to T substitution. Out of these seven samples, 5 (71.4%) were culture positive and demonstrated isoniazid resistance phenotypically (Table-4). These five samples were found to have only *kat*G 315ACC mutation. No mutations for *kat*G315 and *inh*A-15 genes were detected in thirty one isoniazid drug susceptible isolates which in turn were detected phenotypically.

Resistance to any drug in some form either by phenotypic method, CBNAAT or sequencing, was seen in 10 (19.6%, n = 51) specimens. Phenotypic isoniazid resistance along with genetic mutations conferring the same, were associated more frequently with higher age group (Fig. 1). Among the total cases with either phenotypic or genotypic isoniazid resistance, 5 (71.4%, n = 7) were females.

3.3. *Genotypic detection of rifampicin resistance by CBNAAT*

Genotypic Rifampicin was found in 2 (5.5%) samples. One of them was a culture positive gastric aspirate isolate which was resistant to Isoniazid, Rifampicin and Streptomycin by 1% proportion method and simultaneously harbored the *kat*G315 mutation conferring isoniazid resistance. The other isolate showing genotypic rifampicin resistance by CBNAAT, was isolated from a culture negative sputum sample, carrying the *kat*G315 mutation.

4. Discussion

The development of resistance to anti-tubercular drugs is a major public health problem that threatens the progress made in the management and control of TB worldwide. Among the new TB cases, an estimated 3.3% have multidrug-resistant TB and this proportion is much higher (up to 20%) among the previously treated cases of TB.[9] Very little is known about the magnitude of pediatric TB and anti-tubercular drug resistance among children; which in turn may be partially attributed to the paucibacillary nature as well as commoner extra-pulmonary presentation of the disease in children in comparison to adult patients. Additionally the unavailability of representative lower respiratory tract sample due to the inability of most children to expectorate optimally, further lay hindrances in establishing microbiological diagnosis.[10] The diagnosis of merely 40% of pulmonary pediatric TB are successfully established microbiologically and the percentage dwindles further for extra-pulmonary TB.[10,11] Since DST is often performed by genotypic methods directly on specimens or on culture isolates, difficulties in optimal specimen

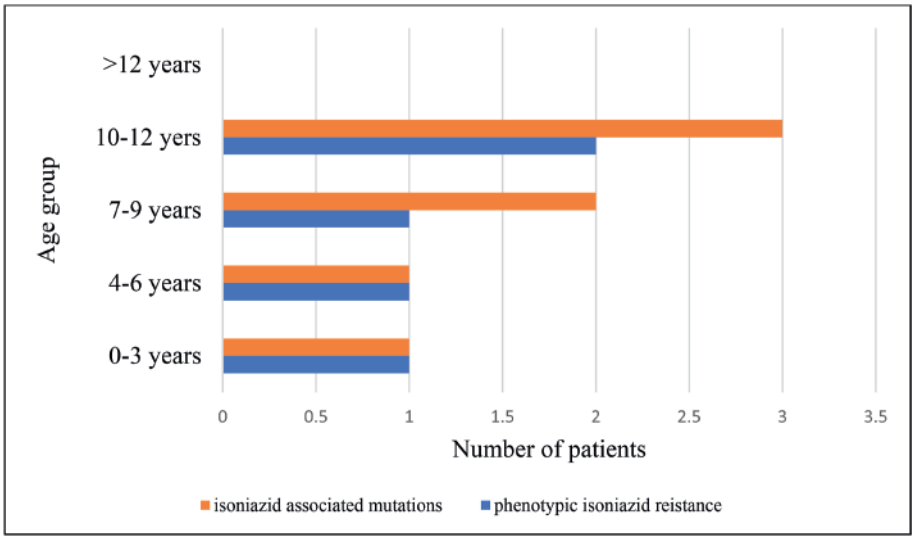

Fig. 1 – Age distribution of patients harboring phenotypic and genotypic isoniazid resistance.

collection often deter the establishment of DST profiles of *M tuberculosis* isolates causing TB in children.[11,12]

DST forms the mainstay for detection of MDR-TB and extensive-drug resistant tuberculosis (XDR-TB). When conventionally performed by culture-based phenotypic 1% proportion or resistance ratio method, DST requires weeks to months for generating reports. However, the relative steep learning curves along with expensive infrastructure add to roadblocks to the performance of phenotypic DST by these conventional methods in resource limited settings. On the other hand, the development of molecular based genotypic methods have revolutionized the detection of MDR-TB worldwide. Ranging from polymerase chain reaction (PCR) for detection of anti-tubercular drug resistance-inducing mutations to commercially available systems like GeneXpert System (Xpert MTB/RIF, Cepheid, USA), GenoType MTBDRplus and MTBDRsl assays (Hain Lifescience GmbH, Germany), and INNO-LiPA Rif.TB line probe assay (Innogenetics Inc., Belgium), there is a wide array of molecular tests available for the detection of drug resistance in TB. In addition to being almost fully automated, faster results, their ease of performance as well as widespread availability make them an attractive choice for anti-TB DST. Since there are no available "gold standard" detecting anti-TB drug resistance in pediatric population, both phenotypic and genotypic methods are conveniently deployed.

Prevalence of MDR pediatric TB in this study was lower (2.7%) than other parts of the country.[13,14] However, systematic review and meta-analysis have documented similar prevalence of MDR-TB in children from India (2.9%); while the higher income countries like the USA and UK reported lower rates of MDR-TB isolation. Countries like South Africa (6%) and China (9.8%) have reported higher rates of isolation of MDR-TB from children as per the same systematic review.[15] Population and case density and the extent of coverage and implementation of preventive strategies as per the national guidelines collectively might contribute to these variations in prevalence of MDR-pediatric TB in these varied geographical locations. The first line drug isoniazid was found to be resistant in 5 (14%) isolates, which not only emphasizes the futility of first line treatment regimens, in such patients, but also points to the brewing threat of emergence of MDR-TB and XDR-TB by selecting resistant strains. Similar to other studies conducted among pediatric population, some form of isoniazid resistance was observed in 5 (14%) isolates. Approximately 12% of children suffering from tuberculosis are estimated to harbor isoniazid resistance, contributing 120,000 cases annual cases.[16] As per reports, 166,000 new isoniazid mono-resistant infections in children are added to the global pool annually.[17] Concurrent to the findings of other studies from varied geographical locations, isoniazid mono-resistance was found in 5.5% culture positive isolates.[13,18] Considering the role of isoniazid specifically in the treatment of latent and isoniazid preventive therapy (IPT) in pediatric age groups, an estimate of the isoniazid susceptibility pattern, as determined by various testing modalities, is crucial.[19]

Rifampicin resistance among the study population was relatively lower than other studies conducted from different parts of the same country.[13] Systematic reviews and meta-analysis have established slightly higher (7.53%) prevalence of rifampicin resistance among pediatric population.[15] Mono-resistance to rifampicin is relatively uncommon and most rifampicin-resistant strains are concurrently known to harbor resistance to other anti-tubercular drugs, especially isoniazid-resistance. Considering that ≥90% of rifampicin-resistant strains are also resistant to isoniazid, detection of rifampicin resistance by molecular tests, may be considered diagnostic of MDR-TB.[20,21] Similarly, all genotypic rifampicin -resistance in this study, were detected in conjunction with *katG*315 mutation for isoniazid resistance. However, the molecular techniques like GeneXpert determines rifampicin resistance by detecting mutations in *rpoB* gene. Mutations in several other genes like the *rpoA* or *rpoC* genes have also been implicated in conferring rifampicin resistance in *M. tuberculosis*.[22] In fact, about one-third of rifampicin -resistant TB has been known to be associated with *rpoB* mutant strains with companion *rpoC* or *rpoA* mutations, which are not targeted by conventional molecular tests.[23]

The current study depicted discrepancies in detecting isoniazid resistance by 1% proportion method and molecular method (PCR and sequencing) in 2 pediatric TB patients, which was lower in comparison to other studies comparing the performances of conventional DST to genotypic MTBDRplus test.[24] The two discrepant isolates comprised of one culture negative sputum isolate which was resistant to rifampicin by CBNAAT and was harboring *katG*315 mutation conferring isoniazid resistance. While the other discrepant isolate was an extra-pulmonary pus isolate that was susceptible to rifampicin and had *inhA*-15 mutation conferring isoniazid resistance. Molecular tests may detect clinically significant mutations conferring low levels of resistance in culture negative as well as culture positive isolates, thus all molecular tests need not routinely be confirmed by culture-based DST techniques. The discrepancies in molecular and phenotypic resistance, though low in this study, the possible dilemma arising in clinical practice may pose several therapeutic challenges. False-resistant genotypic DST would ensue treatment with erroneous ATD therapy instituted for prolonged duration, which in turn would lower efficacy of treatment, increase side effects and hamper treatment compliance by patient. On the contrary, a false-susceptible DST report would render therapeutic failure along with the potential of selecting and spread of resistant strains. Nevertheless, the relationship between genotype and phenotype is complex, and discrepancies in phenotypic and genotypic DST results should be interpreted with caution.

5. Conclusion

The commonly used molecular tests like GeneXpert System (Xpert MTB/RIF, Cepheid, USA), readily detect rifampicin resistance, but due to the dearth of infrastructure conventional culture based DST are deployed in most resource-poor settings. Though molecular based tests like MTBDR*plus* are capable of detecting katG and inhA genes, 20% of isoniazid resistance has been established to be mediated by mutations

in other genes like ahpC. However, due to the lack of molecular or conventional DST tests in resource-poor settings, isoniazid mono-resistance often remain undetected.[24] Resistance to isoniazid has been increasingly linked to poor treatment outcomes, higher relapse rates and death. Multiple genetic loci have been implicated in the orchestration of phenotypic isoniazid resistance, which impedes the use of rapid sequencing techniques for routine diagnostics for determining appropriate treatment regimens.[16] On the other hand, though resistance to rifampicin has relatively better outcomes, extended treatment regimens up to 18 months, often severe and long term adverse effects limited availability of child-friendly formulations often impede therapy.[25] Thus, establishing an overview of existing resistance patterns to first line anti-TB drugs like isoniazid and rifampicin in varied geographical settings, is imperative to institute initial therapy and preventive regimens in pediatric population.

Conflicts of interest

The authors have none to declare.

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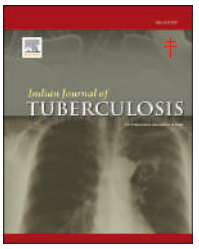

Original article

Comparative analysis of cartridge-based nucleic acid amplification test (CBNAAT) with conventional methods in the diagnosis of pediatric tuberculosis at a tertiary care center

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ABSTRACT

Background: Tuberculosis is an important cause of morbidity and mortality among children. Early diagnosis and treatment in children are challenging, more so in resource-limited, tuberculosis-endemic countries. In 2017, the WHO endorsed the use of CBNAAT for tuberculosis diagnosis. We have undertaken this study to evaluate the diagnostic value of CBNAAT in pediatric tuberculosis in comparison to other methods like microscopic detection of acid-fast bacilli and detection of mycobacteria-by-mycobacteria growth indicator tube (MGIT).

Material and methods: This hospital-based, cross-sectional, observational prospective study was conducted in the department of pediatrics, at a tertiary care center. A detailed history, general physical examination, and relevant physical examination were performed systematically and the findings were noted in the proforma. All necessary basic investigations

Abbreviations: WHO, World Health Organisation; DOTS, Directly Observed Treatment Shortcourse; B.C, Before Christ; TB, Tuberculosis; HIV, Human Immunodeficiency Virus; AIDS, acquired immunodeficiency syndrome; TST, Tuberculin Skin Test; M.Tb, Mycobacterium Tuberculosis; Rif, Rifampicin; PCR, Polymerase Chain Reaction; DNA, Deoxyribonucleic Acid; CBNAAT, Cartridge Based Nucleic Acid Amplification Test; RNTCP, Revised National Tuberculosis Control Programme; MGIT, Mycobacteria Growth Indicator Tube; ZN, Ziehl Neelsen; HAART, Highly Active Retroviral Therapy; BCG, Bacille Calmette Guerin; GC, Guanine Cytosine; CFU, Colony Forming Unit; BAL, Bronchioalveolar Lavage; PTB, Pulmonary Tuberculosis; EPTB, Extra Pulmonary Tuberculosis; AFB, Acid Fast Bacilli; IL, Interleukin; IFN, Interferon; Th1, T Helper 1; NCCT, Non-Contrast Computed Tomography; CT, Computed Tomography; MRI, Magnetic Resonance Imaging; LJ, Lowenstein Jensen; TBM, Tubercular Meningitis; TU, Tuberculin Unit; IRIS, Immune Reconstitution Inflammatory Syndrome; USA, United State of America; CD4, Clusters of Differentiation 4; CR, Complement Receptor; NTM, Non-Tuberculous Mycobacteria; CNS, Central Nervous System; SIADH, Syndrome of Inappropriate Anti Diuretic Hormone; IGRA, Interferon Gamma Release Assay; CSF, Cerebrospinal Fluid; WBC, White Blood Cell; PMN, Polymorphonuclear; ADA, Adenosine Deaminase; PPD, Purified Protein Derivative; PPC, Progressive Primary Complex; FAST, Finding TB cases Actively, Separating safely and Treating effectively; FNAC, Fine Needle Aspiration Cytology; FIND, Foundation for Innovative New Diagnostics; BACTEC, Becton Dickinson and Company; RRDR, Rifampicin Resistance Determining Region; rpoB, RNA Polymerase Subunit Beta; TLR, Toll Like Receptor; LiPA, Line Probe Assay; ESAT 6, Early Secretory Antigenic Target-6; CFP 10, Culture Filtrate Protein 10; NTEP, National Tuberculosis Elimination Programme.

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Extrapulmonary tuberculosis
ZN staining
MGIT culture

like CBC, ESR, X-Ray, etc., and advanced investigations like MRI, CT, and FNAC were done as per the requirement of the subjects and the results were mentioned in the study proforma. Sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy were calculated for various methods. A comparison between the two methods was done using the Mc Nemar test. p-value $\leq$0.05 was taken as statistically significant. All statistical analyses were done using Epi info version 7.2.1.0 statistical software.

Results: Among 102 children suspected to be suffering from tuberculosis, the maximum number of TB cases were found in the age group of 11–16 years (43.2%), there were 58.2% of females, 58.8% belonged to the rural population, fever (78.4%) was the most common presenting symptom and 35.3% had a history of contact. In the present study, CBNAAT and ZN staining had equal sensitivity (60.8%) and specificity (100%) while the yield for MGIT culture was quite low (sensitivity 37.3%, specificity 100%).

Conclusions: CBNAAT as a test was found to be useful, especially for early diagnosis and detection of rifampicin resistance in pediatric tuberculosis against MGIT culture. Since MGIT results become available only after 42 days and have a relatively lower yield so they can be utilized only in a selected clinical situation or in patients with high suspicion of tuberculosis where another test is not able to detect the organisms.

1. Introduction

Tuberculosis is an important cause of morbidity and mortality among children, mainly in developing countries.[1] According to WHO, since 2015 TB has surpassed HIV/AIDS as the leading cause of death from an infectious disease worldwide.[2] In absolute numbers, about 10 million people fell ill with TB, out of which 12% were children aged less than 15 years according to the global TB report 2020.[3] In India 2.69 million cases, out of which 342,000 are children affected by tuberculosis according to Central TB Division, Ministry of Health and Family Welfare, Government of India 2020.[4]

Early diagnosis and treatment in children are challenging, more so in resource-limited, tuberculosis-endemic countries, and are largely based on history, clinical examination, tuberculin skin test (TST), and radiological evidence.[5–7] Diagnosis of pediatric tuberculosis with microbiological tests is further complex due to its paucibacillary nature as well as the difficulty to obtain a good quality specimen. Mycobacterial culture takes 2–6 weeks for final results and requires expensive and sophisticated laboratory facilities which cannot be afforded in most resource-limiting settings.[8]

The cartridge-based nucleic acid amplification test, (CBNAAT, the Xpert MTB/RIF assay) is a promising test for TB and RIF resistance providing results within 2 h. It can detect mycobacterium TB complex DNA in any body fluid sample except blood, urine, and stool.[9–11] A series of meta-analyses have shown CBNAAT to have a high specificity with variable sensitivity in different types of specimens for TB diagnosis.[12–14] In 2017, the WHO endorsed the use of CBNAAT for tuberculosis diagnosis in pediatric presumptive pulmonary and extra pulmonary tuberculosis cases to achieve the global objective of improved TB case and control and early TB case detection.[15,16]

We have undertaken this study to evaluate the diagnostic value of CBNAAT in pediatric tuberculosis in comparison to other methods like microscopic detection of acid-fast bacilli and detection of mycobacteria-by-mycobacteria growth indicator tube (MGIT).

2. Material and methods

This hospital-based, cross-sectional, observational prospective study was conducted in the department of pediatrics, SMS Medical College, Jaipur from September 2018 to November 2020 after obtaining requisite clearance from the institutional ethics committee (No.: 1119/MC/EC/2021-dated 01/12/21) and informed written consent from parents, family members, or available close relatives of all enrolled children. Children between 6 months and 18 years of age with clinical features suggestive of pulmonary and extrapulmonary tuberculosis like-persistent fever, persistent cough for more than 2 weeks, loss of weight or no weight gain, patient in contact with TB case, progressive enlargement of lymph node more than 2 weeks, size more than 2 cm or sinus formation, patients having HIV infection, headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness or lethargy, patients having ascites with hepatosplenomegaly, the baby having mother suffering from active tuberculosis were included in the study. Cases already diagnosed as TB by CBNAAT and those with negative consent were excluded. A detailed history including history of contact and duration of illness, general physical examination, and relevant physical examination of the affected system was performed systematically and the findings were noted in the proforma. All necessary basic investigations like CBC, ESR, X-Ray, etc., and advanced investigations like MRI, CT, and FNAC were done as per the requirement of the subjects and the

results were mentioned in the study proforma. Fasting gastric lavage, spontaneous sputum, and induced sputum samples were collected and sent for AFB, GeneXpert (CBNAAT) and culture. These selected patients were subjected to investigations to find out an appropriate diagnosis and severity of the disease.

2.1. *Statistical analysis*

Data collected was compiled in an MS Excel spreadsheet as a master chart. Data were presented as tables, figures, and charts. Nominal/categorical variables were summarized as frequency and percentage and were analyzed using the Chi-square test. Continuous variables were summarized as mean and standard deviation and were analyzed using the independent sample *t*-test. Sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy were calculated for various methods. A comparison between the two methods was done using the Mc Nemar test. p-value $\leq$0.05 was taken as statistically significant. All statistical analyses were done using Epi info version 7.2.1.0 statistical software.

3. Results

Present study, a Cross-sectional observational prospective study was conducted in the department of pediatrics at a tertiary care center to study the role of CBNAAT in the diagnosis of tuberculosis in children and the diagnostic value of CBNAAT in pediatric tuberculosis in comparison to other diagnostic tests like microscopic detection of acid-fast bacilli and detection of mycobacteria-by-Mycobacteria growth indicator tube (MGIT) test. The total number of study subjects was 102, out of which 51 were TB cases and 51 were non-TB cases.

Present study subjects had the following characteristics and all the observations were done in microbiologically confirmed TB cases:

Out of 102 cases, the maximum number of TB cases were found in the age group of 11–16 years (43.2%) followed by 6–11 years (27.4%). It was found that the number of cases of tuberculosis was higher with increasing age (Table 1). In the present study, there were 58.2% of females outnumbered males (42.2%) among children suffering from tuberculosis, and based on religion 2/3rd cases were hindus (72.5%) and 27.5% were muslims in microbiologically confirmed TB cases and 58.8% belongs to rural population and 41.1% belongs to urban population (Table 1). Among 102 children suspected to be suffering from tuberculosis, fever (78.4%) was the most common presenting symptom followed by cough (36.3%) (Fig. 1).

Based on the contact history, 64.7% had no history of contact and 35.3% had a history of contact in the present study. According to the type of tuberculosis. 54.9% of the cases were pulmonary tuberculosis and 45.1% were extrapulmonary tuberculosis and the respiratory system (47.1%) was the most commonly affected system (Table 1).

In the patients suffering from pulmonary tuberculosis hilar lymphadenopathy (35.4%) was the most common finding followed by pleural effusion (25%) and most of the chest X-rays were normal in patients suffering from extrapulmonary tuberculosis (66.6%) (Table 2).

Table 1 – Microbiologically confirmed TB in relation to demographic profile.

Age group (years)	TB		Non-TB		Total
	N	%	N	%	
<1 years	7	13.7	3	5.9	10
1–6 years	8	15.7	14	27.4	22
6–11 years	14	27.4	18	35.3	32
11–16 years	22	43.2	16	31.4	38
Total	51	100	51	100	102
Chi-square = 4.684 with 3 degrees of freedom; P = 0.262 LS = Not Significant					
Gender	**TB**		**Non-TB**		**Total**
	N	%	N	%	
Male	19	42.2	27	57.8	46
Female	32	58.2	24	41.8	56
Total	51	100	51	100	102
Chi-square = 1.940 with 1 degree of freedom; P = 0.164 LS = Not Significant					
H/o Contact	**TB**		**Non-TB**		**Total**
	N	%	N	%	
Absent	33		64.7	31	60.8
Present	18		35.3	20	39.2
Total	51		100	51	100
Chi-square = 1.940 with 1 degree of freedom; P = 0.164 LS = Not Significant					
Religion	**TB**		**Non-TB**		**Total**
	N	%	N	%	
Hindu	37	72.5	39	76.5	76
Muslim	14	27.5	12	23.5	26
Total	51	100	51	100	102
Chi-square = 1.940 with 1 degree of freedom; P = 0.164 LS = Not Significant					
Residence	**TB**		**Non-TB**		**Total**
	N	%	N	%	
Rural	30	58.8	27	57.8	62
Urban	21	41.1	24	41.8	40
Total	51	100	51	100	102
Chi-square = 1.940 with 1 degree of freedom; P = 0.164 LS = Not Significant					

Among 51 TB cases, 28 cases (54.9%) have pulmonary TB and 23 subjects (45.1%) have extrapulmonary TB (Table 3). In our observations, CBNAAT was found to be positive in 30.4% of cases out of 102 suspected enrolled patients while the sensitivity was found to be 60.8% and specificity was 100% in a patient diagnosed with tuberculosis. In the present study, out of 102 study subjects result of ZN staining among the suspected children suffering from tuberculosis AFB was positive in 30.4%, and out of 51 diagnosed cases of tuberculosis, the sensitivity of AFB was 60.8% specificity of 100%. In 102 suspected patients suffering from pulmonary tuberculosis, 18.6% had M. Tb detected by MGIT culture and in the diagnosed patients of pulmonary tuberculosis, sensitivity was 37.3% with 100% specificity (Table 4). Observations were made that the sensitivity of CBNAAT and ZN staining was found to be similar to 60.8% and the sensitivity of MGIT was 37.3% in the

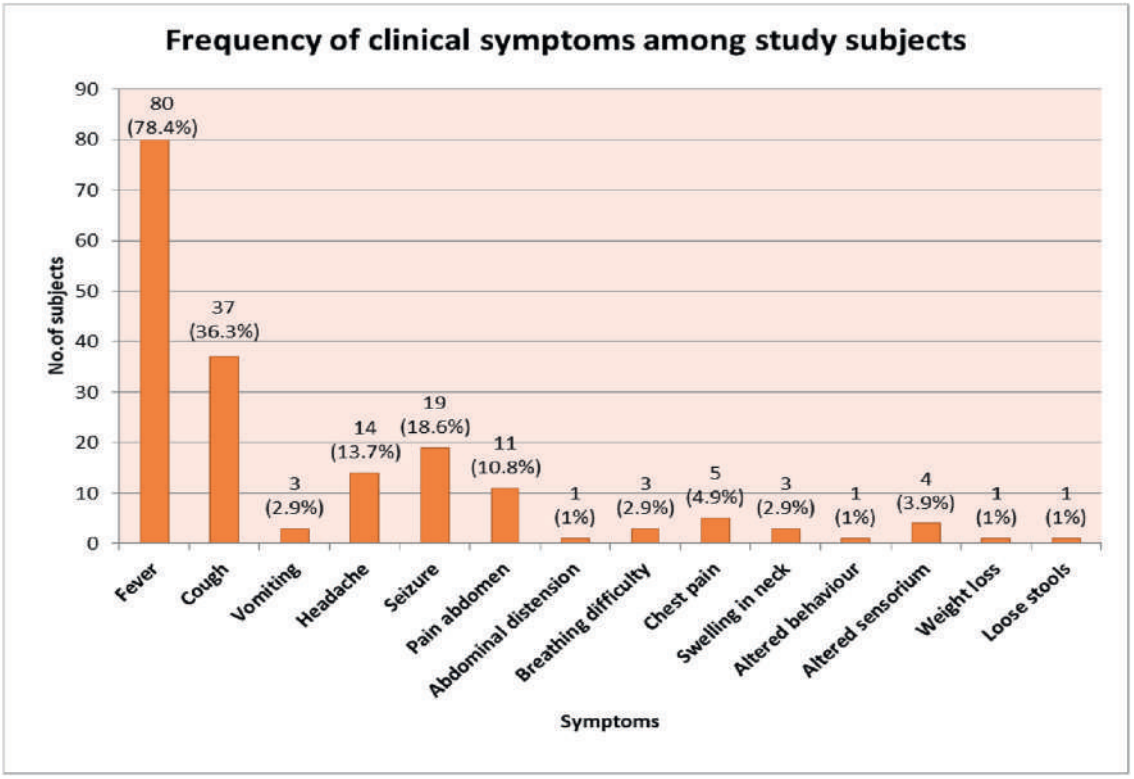

Fig. 1 – Frequency of symptoms.

Table 2 – Chest X-Ray findings in suspected cases of TB.

Chest X Ray finding	Suspected cases of pulmonary TB		Suspected extra pulmonary TB cases	
	Number	Percentage (out of 48)	Number	Percentage (out of 54)
Hilar lymphadenopathy	17	35.4	10	18.5
Pleural effusion	12	25	2	3.7
Infiltrate	8	16.7	7	12.9
Consolidation	8	16.7	2	3.7
Miliary Lesion	2	4.1	1	1.8
Normal	8	16.7	36	66.6

*Figures are overlapping.

patients suffering from pulmonary tuberculosis. And also, the diagnostic accuracy of CBNAAT and ZN staining was found to be similar 80.4% and that of MGIT was found to be 68.6% in the patients of pulmonary tuberculosis. Among 102 study subjects suspected to be suffering from tuberculosis, the sensitivity of MT was 68.6% and out of 51 diagnosed cases of tuberculosis, sensitivity was 56.9% with 19.6% specificity (Table 5).

4. Discussion

Present study aimed to study the role of CBNAAT in the diagnosis of Tuberculosis in children and the diagnostic value of CBNAAT in pediatric tuberculosis in comparison to other diagnostic tests like microscopic detection of acid-fast bacilli and detection of mycobacteria-by-mycobacteria growth indicator tube (MGIT) test.

In our study, we found the maximum number of suspected TB cases in the population with the age group of 11–16 years (37.2%) followed by 6–11 years (31.4%). And out of 51 microbiologically confirmed TB cases 43.2% belong to the age group of 11–16 years followed by 27% of cases belonging to 6–11 years. The results of the present study differ from other studies depicting the majority of children in the <5 years age group,[17–20] and other studies revealing 42% cases between the

Table 3 – Distribution of microbiologically confirmed cases according to type of TB.

Type of TB	Number	Percentage
Pulmonary TB	28	54.9
Extra-Pulmonary TB	23	45.1
Total	51	100

Table 4 – CBNAAT, ZN Staining, MGIT and Mantoux test results among study subjects.

CBNAAT	TB		Non-TB		Total	
	N	%	N	%	N	%
MTb Detected	31	60.8	0	0	31	30.4
MTb Not detected	20	39.2	51	100	71	69.6
Total	51	100	51	100	102	100
ZN staining	TB		Non-TB		Total	
	N	%	N	%	N	%
Positive	31	60.8	0	0	31	30.4
Negative	20	39.2	51	100	71	69.6
Total	51	100	51	100	102	100
MGIT Culture	TB		Non-TB		Total	
	N	%	N	%	N	%
MTb Detected	19	37.3	0	0	19	18.6
MTb Not detected	32	62.7	51	100	83	81.4
Total	51	100	51	100	102	100
Mantoux test	TB		Non-TB		Total	
	N	%	N	%	N	%
Positive	29	56.9	41	80.4	70	68.6
Negative	22	43.1	10	19.6	32	31.4
Total	51	100	51	100	102	100

5–10 years of age group.[21] This may due to the difference in the age group of the enrolled subjects.

In the present study, female subjects outnumbered males, in which 45.1% were males & 54.9% were females in suspected cases of tuberculosis. And out of 102 study subjects, 58.2% were females 42.2% were males in microbiologically confirmed tuberculosis. Contradictory to our study, other studies report more male subjects.[17,19,20,22,23] This may be attributed to increased rates of immunization against tuberculosis among males in comparison to female children & NTEP providing free investigation, treatment, and transport to all children suffering from Tuberculosis and their parents in India.

In the present study, 2/3rd cases were hindus in suspected cases of tuberculosis, 72.5% were hindus and 27.5% were muslims in microbiologically confirmed TB cases out of 102 study subjects. The composition of the population of Rajasthan according to the census 2011, consists of 88.49% of hindus & 9.07% of muslims. The proportion of tuberculosis is higher among muslim patients i.e., 1/3rd of the total which is over and above the census percentage of 2011 (9.07%). Poverty, illiteracy, large family, overcrowding, and poor rates of immunization may be the factors responsible.

Table 5 – Diagnostic parameters of various tests for diagnosis of TB.

Parameter	CBNAAT	ZN stain	MGIT culture	MT
Sensitivity	60.8	60.8	37.3	56.9%
Specificity	100	100	100	19.6%
PPV	100	100	100	42.0%
NPV	28.2	28.2	61.5	31.3%
Diagnostic accuracy	80.4	80.4	68.6	38.2%

In the present study majority of the cases were from the rural population (60.8%) followed by 39.2% from the urban population. Out of 51 microbiologically confirmed TB cases, 58.8% belong to the rural population and 41.1% belong to the urban population. According to the 2011 census, Rajasthan consists of 75.1% rural population & 24.8% urban population.

The frequency of clinical symptoms among subjects of different studies was similar to the present study.[21,24] Most of the studies have reported fever as the most common presenting symptom. On the contrary, one study reported cough as the most common presenting symptom.[25] In the present study, the seizure was the next common symptom, while in other studies the patient also presented with respiratory difficulty & weight loss as one of the common symptoms.

In the present study, 64.7% had no history of contact with microbiologically confirmed cases of tuberculosis and 35.3% had a history of contact. Almost similar findings were seen in most studies regarding the presence of contact history in children suffering from Tuberculosis. A relatively higher percentage of positive contact history has been observed in some studies[19,20] from Kolkata. In a study done in China, contact history was traceable in nearly 10% of the study subjects.[22] The reporting of positive contact history will always be under the real status, all over the world and more so from the developing and underdeveloped countries. Children are more likely to develop tuberculosis after infection compared to adults, it is estimated to be between 5 and 10% in adults, 15% in adolescents, 24% in children below 5 years, and high as 43% in children under 1 year of age.[26]

On comparing different studies[19,23,27,28] based on the type of tuberculosis in children; the studies[19,23,28] reported that pulmonary tuberculosis was higher than extrapulmonary tuberculosis in our study. One study found that most of the cases were extrapulmonary tuberculosis, almost 70%. The higher number of extrapulmonary tuberculosis might be due to the selection of patients based on clinical findings. Out of 107 children, 69 children were clinically diagnosed with neuro tuberculosis.[27]

In the present study, hilar lymphadenopathy was the most common finding in the patients suffering from pulmonary tuberculosis followed by pleural effusion and most of the chest X-rays were normal in the patients suffering from extrapulmonary tuberculosis. Whereas in one study, 44% of the patient showed consolidation and 32% had hilar lymphadenopathy in the chest x-ray in the patient suffering from pulmonary tuberculosis.[21] This difference might be due to the lesser number of children suffering from pulmonary tuberculosis (27%) as compared to extra pulmonary tuberculosis (30%) out of 254 children. Hilar lymphadenopathy is the radiological hallmark of the primary pulmonary complex seen in 96% of children suffering from tuberculosis.[29]

In our observations, out of 102 suspected enrolled patients, CBNAAT was found to be positive in 30.4% of cases while the sensitivity was found to be 60.8% and specificity was 100% in a patient diagnosed with tuberculosis. The sensitivity of CBNAAT is highly variable in different studies ranging from 50% to 90%.[17,18,22,30,31] Variations in the sample collection method might affect sensitivity and specificity. Pediatric studies reveal that compared to culture, the sensitivity and specificity of Xpert are 62% and 98% on induced or expectorated

sputum and 66% and 98% on gastric aspirates.[32] Transportation of the specimen is ideally done between the temperature range of 4–80 Celsius to prevent the activation of nucleases and DNAses. An inappropriate specimen dilution during the decontamination procedure can also affect the test sensitivity.

In the present study, out of 102 suspected cases, AFB was positive in 30.4%, and out of 51 diagnosed cases of Tuberculosis, the sensitivity of AFB was 60.8% specificity of 100%. Considerably lower status for ZN staining in the diagnosis of pulmonary tuberculosis has been reported by one study reporting 31.6% sensitivity and 100% specificity of ZN staining among childhood pulmonary tuberculosis.[18] One study reported 39.09% sensitivity and 88.85% specificity of ZN staining among children suffering from pulmonary tuberculosis.[19] Another study found a much lower sensitivity of 8.4%, and 100% specificity of ZN staining among patients of pulmonary tuberculosis.[22] This might be due to the suboptimal quality of sample collection, and the lesser capability to diagnose by this particular method. With the renewed approach and enhanced emphasis by the national government, under the NTEP program lot of emphasis has been given to improving technical facilities and training of the persons involved.

Based on the MGIT Culture, out of 102 suspected patients, 18.6% had MTb detected by MGIT culture and in the diagnosed patients of pulmonary tuberculosis, sensitivity was 37.3% with 100% specificity. Similarly, one study reported that the sensitivity of the culture was 28.9% and specificity of 100% in the children suffering from pulmonary tuberculosis.[22] Another study found 29% sensitivity and 94.7% specificity of MGIT in patients with tuberculosis.[18] The lower sensitivity of MGIT can be partly explained by a few factors like higher contamination rates, inappropriate transport of the sputum, and inadequate training in handling liquid culture.

In the present study sensitivity of CBNAAT and ZN stain was found to be similar to 60.8% and the sensitivity of MGIT was 37.3% in the patients suffering from pulmonary tuberculosis. The diagnostic accuracy of CBNAAT and ZN Stain was also found to be similar 80.4% and that of MGIT was found to be 68.6% in the patients of pulmonary tuberculosis.

In the present study, out of 102 study suspected cases, the sensitivity of MT was 68.6% and out of 51 diagnosed cases of tuberculosis, the sensitivity was 56.9% with 19.6% specificity. For patients in whom there is the highest risk of progression of tuberculosis, the sensitivity of the tuberculin test is most important while the specificity of this test is more important for the patient who has a low risk of progression to tuberculosis.

5. Conclusions

In the diagnosis of pediatric tuberculosis – Chest X-Ray, ESR, MT, ZN staining, CBNAAT, and MGIT are the frequently done investigations. Each one of them has its merits and limitations. No single test can be considered the gold standard test. In the present study, CBNAAT and ZN staining had equal sensitivity (60.8%) and specificity (100%) while the yield for MGIT culture was quite low (sensitivity 37.3%, specificity 100%). CBNAAT as a test was found to be useful, especially for early diagnosis and detection of rifampicin resistance in pediatric tuberculosis against MGIT culture. Since MGIT results become available only after 42 days and have a relatively lower yield so they can be utilized only in a selected clinical situation or in patients with high suspicion of tuberculosis where another test is not able to detect the organisms. Despite the availability and good performance as a diagnostic test of CBNAAT and MGIT, the basic workup should start from the radiological test, sonography, FNAC, MT test, and ZN staining of different body fluids. However, it may be less or more, the utility of this test is time-tested and continue to remain the same.

5.1. *Recommendations*

However with the ongoing NTEP program at the national and state level, most of the early detection, diagnosis, and treatment part of clinical TB and MDR TB have been looked after well by the government. The present study reemphasizes the importance of history taking, general physical examination, relevant systemic examination, and the basic tubercular workup for all the children clinically suspected to be suffering from tuberculosis. Looking at a relatively lesser yield of tests like CBNAAT and MGIT more research is needed for making them more sensitive and specific. To further confirm our results, a study with a larger number of subjects with a sufficient number of patients belonging to TB of different organ systems should be undertaken. Better and meticulous training of the persons involved in the collection, handling of samples and putting the samples in the machine, inoculating cultures, microscopy, and other technical skills. Also, clinicians must be trained to improve their capabilities for the diagnosis and management of tuberculosis in children.

Conflicts of interest

The authors have none to declare.

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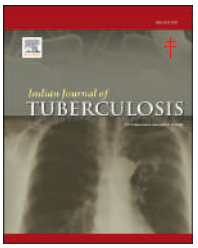

Original article

Contact investigation in healthcare personnel exposed to pediatric patients with pulmonary tuberculosis at the pediatric ward, Vajira hospital, Thailand

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ABSTRACT

Background: Tuberculosis (TB) is an important public health problem, and airborne transmission is easily transmitted. Latent TB infection (LTBI) detection and treatment is the key strategy to prevent TB.

Objective: This study aimed to perform contact investigation of LTBI in healthcare personnel (HCPS) working with pediatric patients with active pulmonary TB and report the side effects of 12-week-isoniazid and rifapentine (3HP).

Methods: This contact investigation reviewed HCPS who were in close contact with pediatric pulmonary TB. Close contact with pulmonary TB is defined as contact for 8 h per day and 120 h per month, or contact in poor air ventilation spaces. HCPS who participated in this study were tested for the active TB and LTBI by history using physical examination, chest X-ray, and tested with interferon-gamma releasing assays (IGRAs). LTBI is defined as a positive IGRA without any evidence of active TB.

Results: A total of 82 HCPS, including 39 nurses (47%), 13 nursing assistance (16%), 12 pediatrician doctors (15%), 12 pediatric residents (15%), and 6 medical students (7%) had close contact with pediatric pulmonary TB. Of the total, 75 HCPS were women, and the mean age and standard deviation was 33 ± 10.4 years. IGRAs revealed positive results in 14 (17%) HCPS who were diagnosed with LTBI and treated with 3HP, of whom, 12 (86%) completed the LTBI treatment, but 2 discontinued the treatment due to the side effects of 3HP. No serious adverse events from 3HP were reported. The most reported side effect of 3HP was nausea and vomiting, occurring in 50% of the HCPS. No diagnosis of TB was seen after 1-year follow-up.

Conclusion: This contact investigation in HCPS of the pediatric ward revealed a large number of LTBI. The LTBI treatment with 3HP showed favorable outcomes and showed minor side effects. Therefore, 3HP was preferred to treat LTBI.

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1. Background

Tuberculosis (TB) is an important global public health. The World Health Organization (WHO) reported TB as one of the top ten causes of death and >10 million people were diagnosed with TB in 2019.[1] The incidence of TB in Thailand is approximately 105,000 people per year which presents a considerable problem for TB in Thailand.[2] Approximately 25% of people worldwide have latent TB infection (LTBI).[1]

TB is an airborne transmission, and approximately 30% of contacts with TB will develop LTBI. Additionally, 10% of LTBIs will progress to TB disease.[3] The WHO determines the strategy to end TB in 2035; one of strategy is LTBI treatment.[4] The WHO recommends testing and treating LTBI, especially in human immunodeficiency virus infection, household contacts of pulmonary TB, immunocompromised hosts, healthcare personnel (HCPS), immigrants from countries with high TB incidence, the homeless, drug users, and prisoners.[5] LTBI treatment can reduce the incidence of TB, especially in healthcare workers, as treating LTBI in healthcare professionals offers several advantages. It effectively prevents the development of active TB, which is highly contagious and poses a risk to healthcare professionals and their patients. It helps protecting patients and provides personal health benefits and broader public health implications, thereby reducing the risk of TB transmission, containing outbreaks, and contributing to the overall goal of TB elimination.[6] Nowadays, many drug regimens are used to treat LTBI, and 12 weekly isoniazid with rifapentine (3HP) is the new feasible regimen to complete treatment approved by the WHO.[5,7] HCPS has a higher incidence of LTBI than non-healthcare workers.[8,9] Therefore, HCPS should detect and treat LTBI, especially in pediatric and immunocompromised wards which are at high risk for developing severe TB.

2. Methods

2.1. Study site, design, and population

The hospital is a tertiary care hospital serving more than 700,000 outpatient visits and 30,000 inpatient admissions annually.

A 13-year-old Thai patient in the pediatric ward was diagnosed with active pulmonary TB in August 2021. However, she was already admitted to the general pediatric ward since April 2021 for acute lymphoblastic leukemia. The estimated beginning of the infectious period in people with contagious TB is 3 months before the first positive finding consistent with TB.[10] Therefore, we could manage to perform the contact investigation with HCPS who had close contact with this patient from May 2021 to August 2021. Data from HCPS who had close contact with this patient from May 2021 to August 2021 were reviewed. All HCPS's close contact with active TB patient was taken as the history and physical examinations which were compatible with active TB such as prolonged fever, chronic cough, significant weight loss, abnormal lungs sound, abnormal lymphadenopathy, etc. were conducted. HCPS were tested with IGRAs by the QuantiFERON-TB Gold test method and chest X-ray (CXR). Additionally, HCPS with positive IGRA, normal CXR, and no clinical compatibility with active TB were diagnosed with LTBI and treated with 3HP, including 900 mg of isoniazid and 900 mg of rifapentine per week. HCPS received 3 tablets per week for 12 weeks. The side effects from 3HP and the 1-year follow-up for TB were collected. This prospective cohort study was conducted 1 year after contact investigation.

2.2. Data collection and data analysis

We assemble clinical data, including demographic data, clinical manifestations, laboratory findings, treatment, side effects, and 1-year follow-up outcomes, on an individual notebook with a secretory password.

Descriptive statistics were performed with mean, standard deviation, and percentage.

Definition[10,11]

1. Close contact TB refers to contact with contagious TB for 8 h per day in the same place, 120 h per month, or in poor air ventilation.
2. LTBI is defined as persons who have positive IGRA without clinical TB.
3. Serious adverse events are defined as death, life-threatening, hospitalization, disability, and required intervention to prevent permanent impairment.

2.3. Ethical consideration

This study was approved by the institutional review board Faculty of Medicine, Navamindradhiraj University (Approval No. COA 161/2565).

3. Results

A total of 82 HCPS had close contact with patients with pulmonary TB in the pediatric ward from May 2021 to August 2021. Of them, 92% were women, and the mean age was 33 ± 10.4 years. Additionally, 47% and 16% were nurses and nurse assistants, respectively. Moreover, 50%, 37%, and 13% were in close contact for 8 h per day in the same place, in poor ventilation, and 120 h per month, respectively. Underlying diseases were found in 37% of HCPS, and none were taking immunosuppressive drugs. The most common underlying disease is allergic rhinitis and dyslipidemia. All close contact with TB neither have a TB history nor symptoms. CXR was normal. IGRA by QuantiFERON-TB Gold test method revealed that 17% were positive (Table 1).

Contact investigation revealed 14 HCPS diagnosed with LTBI and all were treated with a 3HP regimen. The LTBI treatment was completed by 12 HCPS. However, 2 HCPS decided to stop the LTBI treatment after 3 weeks of therapy due to the side effects of 3 HP. The side effects such as headache with nausea-vomit made both HCPS to discontinue. All the HCPS treated with 3 HP did not report serious adverse events; however, 7 HCPS showed minor side effects, including 50%, 29%, and 21% of nausea and vomiting, fatigue, and headache, respectively (Table 2).

Table 1 – The demographic data of healthcare personnel who had close contact with TB patient.

Characteristic	Healthcare personnel (N = 82)
Age (years old); mean ± SD	33 ± 10.4
Gender; n (%)	
- Female	75 (92)
- Male	7 (8)
Occupational; n (%)	
- Nurses	39 (47)
- Nursing assistance	13 (16)
- Pediatrician doctors	12 (15)
- Pediatric residents	12 (15)
- Medical students	6 (7)
Time exposure of TB patient; n (%)	
- 8 hours per day in the same place	41 (50)
- Poor air ventilation	30 (37)
- 120 hours per month	11 (13)
History of TB; n (%)	0 (0)
Symptoms of TB; n (%)	0 (0)
Underling disease; n (%)	
- No	52 (63)
- Yes	30 (37)
- Dyslipidemia	8 (10)
- Allergic rhinitis	8 (10)
- Hypertension	6 (7)
- Diabetes mellitus	4 (5)
- Asthma	3 (4)
- Hypertension	3 (4)
- Hypothyroidism	3 (4)
- Gynecological problems	3 (4)
- Gastroesophageal reflux disease	3 (4)
- Major depressive disorder	1 (1)
Chest X-RAY results; n (%)	
- Normal	82 (100)
- Abnormal	0 (0)
IGRA test; n (%)	
- Negative	65 (79)
- Positive	14 (17)
- Deny testing	3 (4)

The 1-year follow-up revealed normal CXR and no TB symptoms in 75 HCPS, and 7 HCPS have no CXR follow-up data due to their resignation. No diagnosis of TB was seen after the 1-year follow-up (Table 3).

Table 2 – The latent TB infection treatment and side effects.

Outcome	LTBI (n = 14)
Treatment with 3HP regimen; n (%)	14 (100)
Complete treatment; n (%)	
- Yes	12 (86)
- No	2 (14)
Side effects; n (%)	
- Nausea-vomit	7 (50)
- Fatigue	4 (29)
- Headache	3 (21)
- Dizziness	2 (14)
- Fever	1 (7)

Table 3 – The outcome of 1-year followed-up of close contact TB patient.

Outcome	Healthcare personnel (N = 82)
Complete follow-up; n (%)	75 (92)
Loss to follow-up; n (%)	7 (8)
Chest X-RAY results; n (%)	
- Normal	75 (92)
- Abnormal	0 (0)
- Not done	7 (8)
TB disease; n (%)	0 (0)

4. Discussion

A previous study revealed 7.2% LTBI in HCPS in high TB incidence countries[12] and approximately 6.3%–9.5% in Thailand.[13,14] The annual incidence of TB among HCPS in countries with high TB incidence was 1180 per 100,000 persons.[12] The airborne transmission of TB is a concerning issue. The occurrence of TB among HCPS is a huge problem due to TB transmission not only among HCPS but also among patients. Receiving MTB can develop severe TB, especially in pediatric patients and immunocompromised hosts. Additionally, this study revealed that LTBI prevalence in HCPS of the pediatric ward was 17% higher than in the previous study. This study investigated LTBI prevalence in one population, and not all HCPS in the pediatric ward were included which may have a higher prevalence.

HCPS of the Vajira Hospital must take CXR annually. However, asymptomatic TB with abnormal CXR appropriate with TB can transmit MTB to others 3 months earlier. Therefore, annual CXR is not the best strategy to prevent TB. Nowadays, no clear consensus about the frequency of CXR in HCPS is reached. Therefore, each hospital should have a policy about the frequency of CXR and tests of LTBI, especially in the pediatric and immunocompromised wards where the patients may have severe symptoms of TB. However, treating LTBI is the only strategy where HCPS may come into contact with patients with TB again after the treatment. Therefore, other measurements should be performed in HCPS such as infection control practices which include isolation rooms for patients with TB, use of personal protective equipment against airborne transmission, receiving training on TB infection control practices, recognizing TB symptoms, and implementing appropriate precautions while dealing with patients with TB. Moreover, This study revealed the completion rate in 3HP was acceptable. The completion rate of 3HP in this study was 86% compared to 62% of 9H in the previous study.[15] The 3HP regimen is an excellent LTBI treatment for HCPS. The acceptable 100% to receive 3HP showed healthcare personnel realized and needed to treat LTBI.

Study limitations include the limited number of HCPS who were in close contact with patients with TB. We could not test LTBI in everyone due to its high cost. In the future, we plan to test and treat LTBI all HCPS to prevent TB, especially in pediatric and immunocompromised wards.

5. Conclusion

This contact investigation in HCPS of the pediatric ward revealed a large number of LTBIs. The LTBI treatment with 3HP gave favorable outcomes and showed minor side effects. Hence, 3HP was preferred to treat LTBI.

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Conflict of interest

The author has none to declare.

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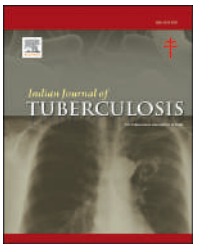

Original article

Adverse drug reactions in children and adolescents on daily antitubercular regimen: An observational longitudinal study

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ABSTRACT

Objectives: Subsequent to introduction of daily fixed dose combination (FDC) regimen with increased dosages and inclusion of ethambutol in continuation phase of antitubercular therapy (ATT) in India, this study was done to evaluate adverse drug reactions (ADRs) in children and adolescents.

Methods: Longitudinal observational study conducted in tertiary teaching hospital. Children (1 month–18 year), with newly diagnosed drug sensitive tuberculosis, started on daily FDC regimen of ATT, were included. Participants were followed up at 2 weeks, 8 weeks and 6 months. Division of AIDS (DAIDS) severity grading and World Health Organization-Uppsala Monitoring Centre (WHO-UMC) causality assessment was done.

Results: In 99 participants, 29 experienced ADRs. Most commonly ADRs involved hepatobiliary (11.1%) and gastrointestinal (8.1%) systems. Grade 3 severity noted in 35.5% ADRs. Certain causality classified in 19.3%. Presence of ADRs was significantly higher in participants with vs without malnutrition [40.5% vs 21.1% ($p = 0.036$)]. Tendency for more severe ADRs noted in participants with vs without malnutrition [Grade 3 ADRs out of all ADRs: 64.7% vs 0% ($p < 0.001$)].

Conclusion: Incidence and severity of ADRs has increased after introduction of daily FDC of ATT. Most common ADR observed were hepatobiliary. Malnutrition and less weight for age were risk factors for occurrence and severity of ADRs.

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1. Introduction

In India, intermittent (3 times weekly) regimen of antitubercular therapy (ATT) for the treatment of tuberculosis (TB) was adopted in 1997 when directly observed treatment (DOT) was introduced. The advantages of this were less frequent treatment visits, better adherence to treatment and low incidence of drug toxicity as the doses of the drugs used were low.[1] The set objectives of cure rate (85%) and case detection rate (70%) were achieved in 2007 with intermittent ATT.[2]

An updated systematic review and meta-analysis concluded that thrice weekly dosing throughout therapy appeared to have worse microbiological treatment outcomes when compared with daily therapy.[3] The World Health Organization (WHO), in 2010, strongly recommended daily administration of ATT for the treatment of TB.[4] The reason for this was stated to be better adherence and preventing acquired drug resistance.

With the aim of elimination of TB by 2025, the National Tuberculosis Elimination Program (NTEP) of India adopted some changes in 2017. Daily drug regimen of fixed dose combination (FDC) with weight appropriate bands was introduced for pediatric TB patients.[5] The dose of Rifampicin, was 15 mg/kg/day (range 10–20 mg/kg) (maximum of 600 mg) and Isoniazid, was 10 mg/kg/day (range 7–15 mg/kg) (maximum of 300 mg), in the newly introduced FDCs. The new regimens also included Ethambutol at 20 mg/kg/day (range 15–25 mg/kg) as the third drug in the continuation phase. The regimen was started in India in March–April 2019.

Undoubtedly, ATT has increased the life expectancy and improved the quality of life in the patients, but it is associated with adverse drug reactions (ADRs). This is one of the reasons for reduced compliance, treatment failure and acquisition of drug resistance. ADRs range from mild rashes to life threatening events. The incidence of ADRs varies from 8% to 85% in patients receiving ATT.[6]

There is paucity of literature on ADRs of ATT in children and adolescent population, subsequent to the introduction of daily FDC regimen with increased dosages and ethambutol inclusion in the continuation phase, in India. This study was therefore planned for analysis and evaluation of ADRs occurring due to daily regimen and increased dosages of FDC regimen of ATT in a tertiary care teaching hospital in New Delhi, India.

2. Methods

This longitudinal observational study was conducted in a tertiary care teaching hospital in India from March 2020 to October 2021 after approval from the Institutional ethics committee. Assuming the confidence limits as 10%, anticipated frequency of 57% (according to the study by Tutu, et al) and confidence level of 95%, the sample size was calculated to be 95 using openepi.com.[7]

Children and adolescents (1mo–18y old) with newly diagnosed drug sensitive TB and started on daily FDC regimen of ATT as per weight appropriate bands according to the NTEP guidelines were included in the study.[8] Patients on medication for any other condition, those with known chronic illnesses like chronic kidney disease or chronic liver disease or with known psychiatric illness were excluded from the study. Informed consent and/or assent from all parents and children, as applicable were obtained. A detailed history was taken and physical examination was performed at the time of enrollment. These findings were entered separately for each participant in pre-designed proformas. The WHO classification of nutritional status of infants and children and the revised Indian Academy of Pediatrics growth charts were used to classify the participants.[9,10] Participants were asked to maintain a diary to record any adverse reactions during the therapy period.

The participants were followed up in the out-patient department (OPD) at 2wk, 8wk and 6mo after initiation of the ATT. On all these visits, a focused examination, weight measurement and history and monitoring for ADRs experienced by the participants were noted in pre-designed proformas. They were enquired for any adverse reaction encountered between the past and the present visit by method of recall and review of the diary. The Central Drugs Standard Control Organisation (CDSCO) suspected ADR Reporting Form was used to report the ADRs.[11] The Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events, corrected version 2.1 was used to categorize the reported ADRs according to severity.[12] The World Health Organization-Uppsala Monitoring Centre (WHO-UMC) causality assessment system was used to assess causality of the ADRs.[13] The WHO-UMC causality categories are certain, probable/likely, possible, unlikely, conditional/unclassified and unassessable/unclassifiable.

The data collected were entered and analysed using IBM SPSS Statistics version 25.0. Categorical variables such as age group, gender, nutritional status, type of TB, presence of ADRs, organ system involved in the ADRs, severity of ADRs and causality category of ADRs were presented in the form of counts and percentages. Histograms were plotted and Kolmogorov–Smirnov test was applied to assess the distribution of continuous variables.[14] Normal distributions such as weight and dose of individual drugs were described as mean and standard deviation. Skewed distributions such as age, duration of fever and weight for age z-score were described as median and interquartile ranges. Associations of presence of ADRs with categorical variables like age group, gender, nutritional status, etc. were assessed using Chi-squared test and Fisher's exact test. Association of the presence of ADRs with normally distributed continuous variables like dose of individual drugs was assessed using the T test. Association of the presence of ADRs with non-normally distributed continuous variables like age was assessed using the Wilcoxon-Mann-Whitney U test. Association of severity of ADRs with non-normally distributed continuous variables like weight for age z-scores was assessed using Kruskal Wallis test. A p-value <0.05 was considered as statistically significant.

3. Results

A total of 99 children and adolescents were enrolled in the study. Demographic and clinical details of the enrolled

participants at time of enrolment is summarized in Table 1. Participants up to 5y of age were 7 (7.1%), 6–12y were 36 (36.4%) and 13–18y of age were 56 (56.6%).

In the 99 participants who were followed up for a period of 180d (6mo) a total of 31 ADRs were experienced by 29 (29.3%) participants. Two participants experienced both drug induced hepatitis and optic neuritis. Amongst all the participants, distribution of the ADRs experienced according to the organ systems involved were hepatobiliary (hepatitis): 11 (11.1%), gastrointestinal (nausea, vomiting, epigastric pain, anorexia and bloating sensation): 8 (8.1%), allergic reactions (erythematous cutaneous reactions): 4 (4.0%), ophthalmological (blurring of vision and painless loss of vision): 4 (4.0%), musculoskeletal (joint pain, back pain and myalgia): 3 (3.0%) and neurological (peripheral neuropathy): 1 (1.0%). No hematological, psychiatric or renal system related ADRs were reported in our study.

Majority of the ADRs occurred in intensive phase of treatment. Median duration between initiation of ATT and occurrence of ADR was 8d (IQR 3–31). Seventeen (17.2%), 6 (6.1%) and 6 (6.1%) participants, reported ADRs on the first, second and third follow up visits, respectively. Most of the allergic skin reactions (100%), musculoskeletal (100%), gastrointestinal (87.5%) and hepatobiliary (81.8%) ADRs were observed in the intensive phase. All Ophthalmological and neurological ADRs were reported in continuation phase of ATT.

According to DAIDS grading of severity, out of the 31 ADRs, 11 (35.5%) were of Grade 1, 9 (29.0%) were of Grade 2 and 11 (35.5%) were of Grade 3 severity. Distribution of ADRs in the DAIDS severity grading according to the follow-up visit on which they were reported and the involved organ system has been summarized in Table 2.

In the causality assessment done using WHO-UMC system for a total of 31 reported ADRs, 6 (19.3%) were categorized as Certain, 6 (19.3%) as Probable/Likely and 19 (61.3%) as Possible. Distribution of ADRs in the WHO-UMC causality categories according to the follow-up visit on which they were reported and the involved organ system has been summarized in Table 3.

Table 1 – The demographic and clinical details of the study participants at the time of enrolment.

Characteristics	Value
Median age in years (IQR)	13.0 (10.0, 16.0)
Female gender (%)	65 (65.7%)
Nutritional status	
• Within normal limits (%)	57 (57.6)
• Moderately underweight (%)	16 (16.2)
• Severely underweight (%)	8 (8.1)
• Moderately stunted (%)	20 (20.2)
• Severely stunted (%)	1 (1.0)
• Moderately wasted (%)	17 (17.2)
• Severely wasted (%)	7 (7.1)
• Overweight (%)	2 (2.0)
Presence of fever (%)	88 (88.9)
Median duration of fever in days (IQR)	21 (14, 28)
Type of Tuberculosis diagnosed	
• Pulmonary (%)	49 (49.5)
• Extrapulmonary (%)	44 (44.4)
• Disseminated (%)	6 (6.1)

Table 2 – Distribution of the Adverse Drug Reactions (ADRs) in the Division of AIDS (DAIDS) severity grading according to the follow-up visit on which they were reported and the involved organ system.

Follow up visit & organ system involved	DAIDS Severity Grading		
	Grade 1	Grade 2	Grade 3
1st follow up visit (2 weeks)	10	5	2
Organ System Involved			
• Hepatobiliary	1	2	1
• Gastrointestinal	7	0	0
• Allergic reactions	2	1	1
• Musculoskeletal	0	2	0
2nd follow up visit (8 weeks)	1	1	4
Organ System Involved			
• Hepatobiliary	0	1	4
• Musculoskeletal	1	0	0
3rd follow up visit (6 months)	0	3	5
Organ System Involved			
• Hepatobiliary	0	0	2[a]
• Gastrointestinal	0	1	0
• Ophthalmological	0	1	3[a]
• Neurological	0	1	0

[a] Two patients had both hepatobiliary and ophthalmological ADRs.

Participants with ADRs had statistically significant presence of malnutrition, lower BMI Z scores and higher doses of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol as compared to those with no ADRs (Table 4). A sub-group analysis according to the different weight bands for the FDC revealed no significant difference in the mean doses for all the 4 drugs in participants with and without ADRs. There was no statistically significant difference with respect to age, age

Table 3 – Distribution of the ADRs in the WHO-UMC causality categories according to the follow-up visit on which they were reported and the involved organ system.

Follow up visit & organ system involved	WHO-UMC Causality Category		
	Certain	Probable/ Likely	Possible
1st follow up visit (2 weeks)	3	5	9
Organ System Involved			
• Hepatobiliary	3	1	0
• Gastrointestinal	0	3	4
• Allergic reactions	0	1	3
• Musculoskeletal	0	0	2
2nd follow up visit (8 weeks)	0	1	5
Organ System Involved			
• Hepatobiliary	0	1	4
• Musculoskeletal	0	0	1
3rd follow up visit (6 months)	3	0	5
Organ System Involved			
• Hepatobiliary	1[a]	0	1[a]
• Gastrointestinal	0		1
• Ophthalmological	1[a]	0	3[a]
• Neurological	1	0	0

[a] Two patients had both hepatobiliary and ophthalmological ADRs.

Table 4 – Comparison of clinical and demographic characteristics of participants with and without ADRs.

Characteristics	Participants with ADR (n = 29)	Participants with no ADR (n = 70)	P value
Median age in years (IQR)	13 (8, 16)	13 (11, 16)	0.817
Age group (%)			0.383
• < 5 years	3 (10.3)	4 (5.7)	
• 6–12 years	8 (27.6)	28 (40)	
• 13–18 years	18 (62.1)	38 (54.3)	
Female gender (%)	17 (58.6)	48 (68.6)	0.343
Mean weight in kg (SD)	28.2 (9.9)	32.2 (10.4)	0.081
Median BMI Z score (IQR)	−1.74 (−2.55, −0.95)	−0.65 (−1.79, −0.15)	**0.002**
Malnutrition (%)	17 (58.6)	25 (35.7)	**0.036**
Socioeconomic status			0.366
• Upper Middle (%)	1 (3.4)	3 (4.3)	
• Lower Middle (%)	3 (10.3)	18 (25.7)	
• Upper Lower (%)	20 (69.0)	40 (57.1)	
• Lower (%)	5 (17.2)	9 (12.9)	
Type of tuberculosis diagnosed (%)			0.591
• Pulmonary	12 (41.4)	37 (52.9)	
• Extrapulmonary	15 (51.7)	29 (41.4)	
• Disseminated	2 (6.9)	4 (5.7)	
Mean drug doses in mg/kg (SD)			
• Isoniazid	8.33 (2.04)	7.46 (1.88)	**0.044**
• Rifampicin	13.84 (2.30)	12.62 (2.01)	**0.010**
• Pyrazinamide	31.27 (3.68)	29.08 (3.12)	**0.003**
• Ethambutol	21.18 (2.49)	19.70 (2.11)	**0.003**

P value< 0.05 mentioned in bold.

group, gender, weight, socio-economic status and type of TB diagnosed in participants with and without ADRs.

Hepatobiliary ADRs were significantly higher in malnourished participants as compared to those with no malnutrition [21.4% vs 3.5% (p = 0.008)]. Malnourished participants also had a higher tendency for more severe ADRs as compared to participants with normal nutritional status [Grade 3 ADRs out of all ADRs: 64.7% vs 0% (p < 0.001)]. Median weight for age z-score of participants with Grade 3 ADRs was significantly lower as compared to those with Grade 2 or Grade 1 ADRs [−2.26 vs −1.60 vs −1.44 (p = 0.027)].

4. Discussion

This longitudinal observational study was conducted in a tertiary care teaching hospital in India with the objective to evaluate the ADRs occurring due to the newly introduced daily regimen and increased dosages of FDC regimen of ATT in children and adolescents. We observed that nearly 30% of participants experienced ADRs. Most common ADRs were hepatobiliary followed by gastrointestinal.

We observed that 29.3% of the participants experienced a total of 31 ADRs. Most of the Indian studies conducted on samples containing predominantly adult participants have reported ADRs ranging from 8 to 19% with only 1 study reporting ADRs in 29% participants.[15–20] All these studies had adopted intermittent regimen of ATT administration according to RNTCP guideline under DOTS. In a study, with pediatric participants on intermittent regimen of ATT, conducted in Pakistan,13.5% ADRs were observed.[21] It appears that there is a significant increase in the percentage of participants experiencing ADRs on daily FDC regimen as compared to the intermittent regimen. This difference can be attributed to the shift in alternate day of ATT intake to daily intake of drugs and with increased doses of the drugs. Similar findings were obtained in a study comparing daily versus intermittent regimen of ATT.[22] The study included 32.6% participants who were less than 20 years old. They had found higher ADRs in patients on daily FDC as compared to those on intermittent ATT (35% vs 25.58%).

In our study 11.1% participants developed hepatobiliary ADRs with more than 80% occurring in the intensive phase of the treatment. More than 80% of those with hepatobiliary ADRs had malnutrition. Previous studies have reported hepatobiliary ADRs in only 1–3% of the participants.[15–19] Amongst the drugs used as ATT, isoniazid, pyrazinamide and rifampicin can potentially cause hepatic injury. The significant increase in the incidence of hepatobiliary ADRs in our study can be attributed to the increased dose of the antitubercular drugs (isoniazid and rifampicin) adopted in the daily FDC regimen. Malnutrition was a significant risk factor for the occurrence of hepatobiliary ADRs. Malnutrition as a risk factor for ADRs due to ATT has been reported in other studies also.[23,24]

In the present study, 4 (4%) patients reported blurring of vision and painless loss of vision. This was attributed to be due to ethambutol-induced optic neuropathy (EON). In studies done on patients on intermittent ATT regimen, EON has been reported in nearly 1–3% participants.[18,20,21] The incidence of EON has been reported to be higher with higher doses of ethambutol.[25] A slightly higher incidence of EON observed in our study can be because of increased duration (intensive and continuation phase) and dose of the ethambutol adopted in Daily FDC regimen.

In our study 35.5% of the ADRs reported were Grade 3 or Severe according to the DAIDS grading of severity. In other studies, on participants taking intermittent ATT regimen, severity was assessed using Hartwig severity scale or modified

Hartwig and Siegel scale, and severe ADRs were seen in 0–8% of the participants.[15,18,20,26] Higher incidence of severe ADRs in our study may be attributed to higher absolute and cumulative dose in daily FDC regimen.

In our study 19.6% of the ADRs reported were classified as certain according to the WHO-UMC causality categories. In another study, only 5.7% ADRs were classified as certain according to the WHO-UMC system.[15] This may reflect the robust system followed in our study for establishment of causality which included regular follow up, detailed history and examination on each visit, maintenance of symptom diary by patients, speciality referrals and laboratory investigations as per requirement.

We found that participants with ADRs had significantly higher doses of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol as compared to those with no ADRs. This suggests that children placed lower in the weight bands used to determine the FDC, according to the NTEP guidelines, may be more prone to develop ADRs. Considering this, a sub group analysis was performed to compare mean drug doses between participants with and without ADRs in each weight band. This however, showed no statistically significant difference in any group. This may be due to the lower number of participants falling in each of the 7 weight bands. Studies with larger sample size and stratified sampling for each weight band may help in better understanding of this association.

In our study more than 60% of ADRs in malnourished participants were severe in grading. Also, participants with Grade 3 ADRs were significantly more underweight than participants with milder ADRs. Malnutrition is an established risk factor for ADRs in patients on ATT.[23,24,27] However, our study also suggests that severity of the ADRs is directly proportional to degree of malnutrition.

To the best of our knowledge, this is the first study in India which monitored the incidence of ADRs in children and adolescents after the introduction of daily FDC of ATT. Strengths of our study were the facts that all participants were followed up prospectively for a period of 6mo after initiation of ATT at regular intervals, which resulted in better reporting of ADRs and participants with known comorbidities were excluded from the study to reduce confounders.

Limitations of our study were smaller sample size and study duration of one year with follow-up of 6mo only. A possibility of recall bias may exist as participants may not have reported mild adverse reactions like nausea and vomiting. We however, tried to diminish this bias by ensuring maintenance of symptom diary and regular checking of the diary on follow up visits. Laboratory investigation to estimate the plasma/tissue drug concentration during ADRs were not done. Also, hematological investigations and liver function tests of only symptomatic patients were done. Larger, multisite and ongoing continuous active monitoring is needed to better understand the ADRs.

To conclude the incidence of ADRs has increased after the introduction of daily FDC of ATT consequent to the increased dose and duration of drugs as compared to alternate day regimen. ATT induced hepatotoxicity was the most common ADR observed in pediatric age group. There was increased incidence of drug induced hepatitis and optic neuropathy as compared to previous studies with participants on alternate day regimen. The severity of the ADRs as estimated by DAIDS was also found to be increased as compared to alternated day regimen. Malnutrition and less weight for age were risk factors for occurrence and severity of ADRs.

Consent to participate

Informed consent and/or assent from all parents and children, as applicable were obtained for inclusion in the study.

Contribution of authors

This study was conceptualized and designed by AA, SHB, BSK and KR. Data analysis and acquisition was done by SHB, SBM, RA and AK. All authors were involved in data interpretation. The manuscript was drafted by SHB and SBM. All authors were involved in critical revision and final approval of the manuscript. All authors are accountable for all aspects of the work.

Conflicts of interest

All authors have none to declare.

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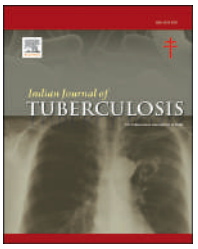

Original article

Perceptions and challenges among health care providers about HIV-TB co-infected children- A qualitative study

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ABSTRACT

Background: Human immunodeficiency virus – Tuberculosis (HIV-TB) co-infected patients have a greater risk of mortality, treatment failure, and recurrence. The significant morbidity and mortality rates associated with tuberculosis and human immunodeficiency virus infection in children cause concern. India aims to enhance the detection and treatment of HIV-TB cases in children and coordinate TB & HIV care.

Objective: To explore the perceptions and challenges of health care providers regarding the diagnosis and treatment of Tuberculosis in HIV-TB co-infected children.

Materials and method: In-depth interviews among 14 health care providers were conducted in ART centers of 5 talukas of Belagavi district to identify health care providers' perceptions and challenges regarding pediatric HIV-TB diagnosis and treatment. Interviews were conducted after receiving informed consent.

Results: Challenges during HIV-TB diagnosis and treatment in children: difficult to get sputum sample for CBNAAT and child was unable to complain about symptoms, caregivers were not able to mention the signs/symptoms correctly, unavailability of a pediatrician in few Taluka ART centers, delay in receiving TB lab report, challenging to feed drugs to an infant, higher loss to follow-up, financial problem, distance from centers, low community awareness, poverty and illiteracy, stigma and death due to TB treatment default.

Conclusion: Efforts such as expanded health care providers, community education, and a constant supply of HIV rapid test kits are required to ensure successful diagnosis and treatment of HIV-TB co-infected children.

Abbreviations: HIV-TB, Human immunodeficiency virus – Tuberculosis; CBNAAT, Cartridge-Based Nucleic Acid Amplification Test; MRI, Magnetic resonance imaging; DOTS, Directly observed treatment short-course; MDR TB, Multidrug-resistant TB; ASHA, Accredited Social Health Activist; PUC, Pre-University Course.

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1. Introduction

According to the world health organization (WHO), human immunodeficiency virus (HIV) prevalence among Tuberculosis (TB) children ranges from 10% to 60% in areas with moderate to high incidence. Among high-prevalence nations, the incidence of Tuberculosis in HIV-positive children is substantially higher. The incidence of tuberculosis among HIV-positive children undergoing HIV treatment in South Africa was 23 per 100 years.[1] Although the prevalence of TB has decreased as antiretroviral therapy (ART) coverage has grown, it remains considerably higher among HIV-positive children than in the overall pediatric population.[1]

The prevalence of Tuberculosis in HIV-positive children is uncertain. The prevalence of HIV-positive children depends on whether the location is TB endemic or not, as well as the availability of antiretroviral medication in that area. The rates of pediatric HIV-TB co-infection fluctuate due to difficulties in obtaining a definite diagnosis of TB in HIV-positive children and under-ascertainment.[1]

India has the world's most significant tuberculosis burden, high HIV prevalence, and high rates of HIV-associated tuberculosis. While tuberculosis is widespread, the HIV epidemic is localized in a few states.[2] In India, HIV co-infection with TB accounted for around 50,000 cases out of 2,155,894 cases, resulting in a TB-HIV co-infection rate of 3.4 percent.[3]

HIV-positive people have a more severe clinical manifestation of Tuberculosis than HIV-negative people.[1] Many pediatric studies have revealed that children with HIV-TB co-infection have a worse illness and are more likely to die than HIV-negative children, showing that two diseases have a combined impact.[4]

Various issues have contributed to the lack of research on HIV-TB co-infection in children. This issue includes the difficulty of diagnosing tuberculosis in small children who are unable to produce sufficient sputum samples for testing, the lack of alternative sensitive diagnostic tests for tuberculosis, the frequent atypical presentation of TB in children, and the lack of a standardized case definition for the diagnosis of tuberculosis in children.[5]

Diagnosing and treating HIV-positive patients who acquire tuberculosis is complex, and there are still gaps in our understanding of the processes of HIV-TB interaction. Adverse medication reactions and drug toxicity that overlaps with ART add to the treatment's complexity.[6]

Different behavioral patterns frequently emerge from health care providers directly impact patients' health-seeking behavior.[7] Nonetheless, the difficulties front-line healthcare workers (HCWs) have in identifying pediatric TB cases, HIV testing, and linking to ART are not widely documented.[8]

Studying the opinions and perceptions of health care providers regarding HIV-TB co-infection may help better diagnose and treat HIV-TB co-infected children. It may also aid in developing new and innovative chemoprophylactic strategies for TB in HIV-affected children. The challenges reflected in this study may be addressed in various programs and policies designed by the Government of India for HIV and TB.

As far as we know, we didn't come across any study conducted on perceptions and challenges among Health Care Providers about HIV-TB co-infected children in India. So, this study aimed to explore the perceptions and challenges of health care providers regarding HIV-TB co-infected children based on diagnosis and treatment of HIV-TB as per the Government of India guidelines.

2. Materials and methods

Qualitative research was conducted for one year (May 2021 to April 2022). An in-depth interview was performed using a semi-structured questionnaire among 14 health care providers (doctors and multi-purpose health care workers) taking care of registered HIV-TB children living at ART centers of 5 talukas of Belagavi district (Belagavi, Gokak, Chikkodi, Saundatti, and Raibag). In-depth interviews were conducted individually till data saturation. Data from health care providers were recorded verbatim using semi-structured questionnaires. Each interview was transcribed and translated verbatim, and data was scrutinized immediately to identify gaps, missing links, or new inquiry requirements. Accordingly, the schedules were modified. Ethical approval was obtained from the Institutional Ethical Committee (IEC) of Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research, Belagavi (**Reference No. MDC/DOME/255 dated 5**th **October 2021**) and from the IEC of Belagavi Institute of Medical Sciences, Belagavi **(Reference No. BIMS-IEC/169/2021–2022 dated 29**th **November 2021)**. Also, the permission was taken from the District Tuberculosis Centre (DTC) **(Reference No. ಜಿಕ್ಷಯನಿಕೇ/ಬಿ/73/2021–22, dated 28**th **December 2021)** and from the administrator of Clinical Services- Academics for Medical Director and Chief Executive, KLES Hospital **(Reference No: KLES/Dr. PK-HOSP/ADM-CS/GEN/21–22/12188 dated 12 January 2022)**. Written informed consent was obtained before interviewing the participants. Voluntariness and confidentiality were assured to the respondents during the consenting process. Audio recordings were done with the respondents' permission, as every participant consented to audio recordings. Data was entered after the data saturation had occurred, and thematic analysis was done. Registered Doctors and Multi-purpose health care workers (Male and Female) taking care of registered children living with HIV-TB (<18 years of age) were included in this study.

3. Results

Overall, 14 participants were interviewed, of whom 7 (50%) of the participants were medical officers, 5 (35.7%) were staff nurses, and 2 (14.3%) were counselors.

The findings from the thematic analysis of the 14 participants using in-depth interviews are reported here. These findings are grouped under four sections known as “themes” and would again contain “sub-themes” based on a semi-structured questionnaire and thorough literature review as listed in Table 1.

Table 1 – Themes and sub-themes of the challenges & perceptions faced by Health Care providers regarding diagnosis and treatment of HIV-TB co-infected children.

SN.	Theme	Sub-theme
1.	Challenges during diagnosis of HIV-TB co-infected children	1.1 Testing HIV-TB children
2.	Challenges during treatment	2.1 Complications faced if any dose of DOTS/ART is missed
		2.2 Loss to follow-up
		2.3 Scarcity of resources
		2.4 Challenges during treatment course and Intervention
		2.5 Challenges due to Caregivers
		2.6 Precautions followed from the patient's side
		2.7 Different patient's behaviors
3.	Stigma towards HIV	
4.	Perception	4.1 Allowing the infected children in regular school and the precautions
		4.1 Testing of family members of HIV-TB patient
		4.2 Approaches

3.1. *Challenges during diagnosis of HIV-TB co-infected children*

3.1.1. *Testing HIV-TB children*

Most participants expressed the difficulties of getting a sputum sample for CBNAAT among small children. The child might not be able to give a proper history of symptoms unless the child were a higher school student. A patient having extrapulmonary TB was diagnosed based on symptoms.

> "*If children are quite young, they don't know how to give sputum samples. So, this is one of the challenges in testing HIV-TB children. So, we have to do gastric lavage. The other challenges include, the child might not be able to tell you that they have a loss of appetite, night sweat, weight loss, and proper history unless the child is a higher school student.*"- *Medical Officer*

> "*For extrapulmonary TB, we do suspect patient based on symptoms.*"- *Medical Officer*

Few participants mentioned other challenges like caregivers were unaware of symptoms; Handling and transporting sputum samples; financial problem; no pediatrician in few Taluka ART centers; difficulty in conducting X-rays in children; and delay in obtaining TB reports which might endanger personnel and other patients, as well as complicate the management of TB-HIV co-infection.

> "*Even the caregiver or the parents might not be able to tell the symptoms and history of the child. There is a tuberculosis unit where the sputum sample is tested. There are challenges to handle sputum sample because the sputum sample need to be handled properly to prevent infection to the lab technicians and need to use proper technique and proper staining to get an accurate result.*"- *Counselor*

> "Financial problem for MRI." - Medical Officer

> "*There is no pediatrician in this ART center. TB report is supposed to give within 24–44 hours, but it extends up to 5–6 days.*"- *Medical Officer*

> "*Child refuses to do chest X-ray because of fear.*" – Nurse

3.2. *Challenges during treatment*

3.2.1. *Complications faced if any dose of DOTS/ART is missed*

The majority of participants stated that if the patients missed DOTS/ART doses for an extended time, the HIV patient's immune system would be impaired, CD4 count would drop, and their viral load would rise. They would develop opportunistic infections, and death might occur. TB patients might have a higher risk of relapse and developing MDR TB and death.

> "*If the patient missed a dose of DOTS/ART for a long period, drug resistance occurs. In HIV, CD4 count decreases, viral load increases, and patients suffer from opportunistic infections. In TB patient, more chance of relapse.*"- *Counselor*

> "*I have a bad experience where few patients died due to TB treatment default.*"-*Medical Officer*

> "*If DOTS doses are missed for 2–3 months, the patient may develop MDR TB and suffer. If ART doses are missed, the patient becomes immunocompromised and will get opportunistic TB, fungal infection, and diarrhea. Finally, death may occur.*" - *Medical Officer*

3.3. *Loss to follow-up*

Few participants responded that distance from the center and financial problems among low-wage caregivers were the barriers resulting in loss of follow-up.

> "*Asking people to come every month to collect the drug is quite challenging because they are poor and depend upon daily wages. If they need to come to collect drugs, then they will lose their daily wages.*"- *Medical Officer*

> "*More loss to follow-up cases in HIV-TB co-infected children. The caregiver cannot afford transportation charge.*" *Medical Officer*

Some participants stated that there might be fewer chances of loss to follow-up in TB treatment. Whereas, in HIV, the healthcare providers could not track the loss to follow-up cases due to stigma, privacy, and confidentiality. But if the patient missed the follow-up for more than three months,

health care providers would file a report to the government, and they dispatched an ASHA worker to track them down and ask them to continue for follow-up.

"*In TB treatment, the patient can't escape follow-up. They constantly monitor and also trace them. So, skipping is not possible in TB treatment unless the person runs away. But we can't do the same concerning ART because of stigma. The HIV patient is socially boycotted. That is why we don't trace or supervise HIV patients, whether they are taking ART. But if the patient misses follow-up for more than three months, we will intervene. We will report it to the government. We will send ASHA workers and try to trace them and ask them to continue for follow-up.*" *Medical Officer*

3.4. *Scarcity of resources*

Few participants stated they had a scarcity of HIV testing kits.

"*We had a scarcity of HIV kits for testing due to COVID-19 lockdown.*" *Medical Officer*

3.5. *Challenges during treatment course and intervention*

Few medical officers stated that feeding medicine to HIV-TB co-infected infants was difficult.

"*For HIV-TB co-infected infants, it is difficult to feed the medicine. Children can easily get infected. They might have multiple co-morbidities and get worse quickly. So, it is one of the biggest challenges.*" *Medical Officer*

Most participants stated that children had complications while on ART and ATT. Treating children according to complications was difficult, and convincing patients to continue ATT was challenging.

"*If medicine has an adverse effect on child-like acidity or multiple things, that would be difficult.*" *Nurse*

"*Depending upon the complications, we need to treat the patient. For example, patients who are taking Zidovudine may suffer from anemia. For severe anemia, we do a blood transfusion. For moderate anemia, we give Haematinic. We change the drugs if the patient has liver or kidney failure.*" *Medical Officer*

"*If patients have mild side effects like gastritis, acidity, nausea, and vomiting, we give them drugs to reduce it. We give alternate medicine if patients have severe side effects like skin rashes. In case of Nephro toxicity and high-level serum creatinine, we referred them to a district hospital.*" *Medical Officer*

"*When HIV-TB cases come to us, it is difficult when we try to modify ART according to case requirement and tell them to stick to Anti Tuberculosis Therapy (ATT).*" *Medical Officer*

3.6. *Challenges due to caregivers*

Few participants responded that poverty and illiteracy among caregivers were challenges to making them understand good adherence to drugs and take proper care of children.

"Poverty and illiteracy are commonly associated with HIV-TB co-infected children." Medical Officer

"*If parents are problematic, then it is a tough task to make parents understand good adherence to drugs and taking care of the child.*" *Counselor*

3.7. *Precautions followed from the patient's side*

Most participants stated that it would be challenging to convince caregivers and patients to take precautions independently because many patients were illiterate.

"*From the patient side, they won't take any precautions because even when we ask them to wear a mask, they won't wear it. An uneducated person doesn't know about taking precautions. So, it is our responsibility to teach them, and whenever we teach and provide proper counseling to the patient about adherence, proper diet, etc., they will follow it. So, there is always a counselor posted in the ART center.*" *Medical Officer*

3.8. *Different patient's behaviors*

Most respondents stated that caregivers' and patients' negative behavior was challenging for a health care provider to provide treatment.

"*Some patients are not co-operative and not regular in their treatment.*" Nurse

"*Some patients are aggressive and argue with us.*" Nurse

"*Some patients are rash, rough, arrogant, unwilling to come to the hospital, and wanted the medicine delivered at their home.*" *Medical Officer*

"*Some patients have fear due to stigma. Some are careless and less motivated due to HIV infection.*" Counselor

3.9. *Stigma towards HIV*

Few respondents stated that the stigma attached to HIV had a tremendous negative impact on HIV patients' everyday lives.

"*I have seen a child who was supposed to be in PUC 11th standard but skipped schooling from 8th standard because his HIV status was revealed to school authorities. So, the school refused to take him back, and even he felt ashamed to attend school with his friends.*" *Medical Officer*

3.10. *Perceptions*

3.10.1. *Allowing the infected children in regular school and the precautions*

The majority of the participants said that infected children should be allowed to attend school generally with precautions.

> *"Yes, but the child needs to take precautions like a mask and social distancing."* Nurse

A few participants stated that TB-infected children must be isolated until the therapy was completed and the child was cured.

> *"HIV patient should be allowed in regular school, but TB patient needs to be isolated till the treatment is completed because TB get transmitted." - Counselor*

> *"Yes. But if a child gets injured, there is a risk of spreading HIV to others through blood. So, the child should be prevented from getting injured. Children in the advanced TB stage need to be isolated at home. After completion of ATT for 2–3 months, the child can attend regular school but need to take precautions." Medical Officer*

3.11. *Testing of family members of HIV-TB patient*

Most participants said it was essential to test family members of HIV-TB patients.

> *"It is compulsory to test the family members living with HIV-TB patients under the same roof."* Nurse

> *"Yes, they must be treated. If an adult sexually harasses a child, the child can get HIV infection, which is less likely. The child does not get infection themselves but through the mother or any adult or drug addict using the same syringes, which is quite less. So it is always good to test parents or people living under the same roof. For TB, it is essential to get tested by each family member." Medical Officer*

3.12. *Approaches*

Most participants responded that the **target-free approach had advantages,** such as high-quality services and screening.

> *"The target-free approach has the quality of screening. We screen only those who have symptoms." Medical Officer*

> *"We can work freely without any compulsion." Nurse*

Most participants said some drawbacks to target-free approaches, such as limited coverage and health care workers being too lazy to locate new cases.

> *"Less coverage because we focus on limited people." Counselor*

> *"Health care workers become lazy and do not put effort to find new cases." Medical Officer*

The majority of the participants said the target approach had some advantages as it made health staff work enthusiastically and honestly.

> *"In target approach, the health care workers work actively with high concentration to find more new cases and are responsible for their job. More coverage within limited time." Medical Officer*

Most participants mentioned some **drawbacks of the target approach, such as inability to reach the target, need to depend on outreach workers, no quality of services, some might conduct CBNAAT among asymptomatic people,** and health staff might get disappointed and discouraged if the target was not reached.

> *"We may not reach the target. We should depend on outreach workers." Medical Officer*

> *"There won't be quality." Medical Officer*

> *"We need to screen the person who does not have symptoms to reach the target." Medical Officer*

> *"Health care workers get disappointed and discouraged if they do not reach the target."* Nurse

4. Discussion

This study reported the challenges during HIV-TB diagnosis and treatment in children, which include difficulty in getting sputum sample for CBNAAT and child was unable to complain about symptoms; caregivers were not able to mention the signs correctly, unavailability of the pediatrician in few Taluka ART center, delay in receiving TB lab report, challenging to feed drugs to an infant, higher loss to follow-up, financial problem, distance from the centers, low community awareness, poverty and illiteracy, stigma and death due to TB treatment default.

Regarding the challenges during diagnosis of TB in children, the study reported difficulties in getting sputum samples for CBNAAT because small children, like infants and under-five children, could not give enough sputum samples; for extrapulmonary TB, they need additional pulmonary TB to diagnose a patient based on symptoms. Similarly, the study conducted in Tanzania also reported that the children could not produce sputum, and the presence of paucibacillary disease was challenged in diagnosing TB.[8]

This research reported that delay in receiving TB lab reports was another challenge due to the lack of resources in laboratories to test sputum samples. The quantity of the sputum samples is more in government laboratories than in private ones. In contrast, the study conducted in northern KwaZulu-Natal, South Africa, also reported delays in receiving lab test results.[9]

This study revealed a scarcity of HIV testing kits due to the COVID-19 lockdown. Similarly, a study conducted in Tanzania also reported a similar finding.[8]

This study revealed poverty, illiteracy, loss to follow-up, a long distance from the center, financial problems due to which caregivers could not afford transportation charges, difficulty in feeding drugs to HIV-TB co-infected infants, side effects of medicine, and missing DOTS doses were the challenges that hinder sustaining treatment adherence which leads to complications and death of a patient. However, the study in Tanzania reported the challenges of taking medications consistently, financial difficulties that hindered their ability to buy nutritious foods, and the lack of pediatric drug formulations for Tuberculosis (TB).[10] Whereas the research in Ethiopia reported that treatment drugs' side effects, drug burden, financial problems, lack of food, stigma, lack of disclosure, and lack of proper communication with health professionals were barriers to treatment adherence.[11] Another study in Swaziland revealed that the barriers to ART initiation were denial, guilt, HIV-related stigma, lack of money, knowledge of Tuberculosis (TB)/HIV co-infection, and distance to clinics.[6]

The present study reported some side effects of ART and ATT, which included: gastritis, nausea, vomiting, skin rashes, anemia, nephrotoxicity, liver failure, and renal failure, whereas a study conducted in the USA reported: fever, nausea, jaundice, loss of appetite, and abdominal pain were the treatment drugs side effects.[12]

This study revealed that the stigma attached to HIV hinders the patient from living everyday life. Due to stigma, the child felt discriminated against and ashamed to go to school after knowing their HIV status, and the school authority also refused to take the HIV-infected child. At the same time, research conducted in Tanzania reported that the stigma caused the care provider to detain bringing children to the health care center.[8] Similarly, another study said that stigma, fear, and discrimination were social challenges to controlling HIV and TB infection and affected health-seeking behavior.[13] Another research conducted in South Africa also reported that stigma among health care providers regarding HIV infection was a barrier to TB and HIV care and treatment.[14]

This study reported that the caregivers' lack of awareness and education hindered the proper treatment of HIV-TB co-infected children because most caregivers who came to these ART centers were illiterate. Similarly, research conducted in Tanzania also revealed that the lack of community awareness obstructs prompt HIV and TB treatment. (8) Another qualitative study in China also reported that low awareness and indifference toward tuberculosis were barriers to proper treatment.[15]

This present study reported no pediatric doctors in a few ART centers as most of these ART centers were located in rural areas, and most of the doctors wanted to work in urban areas. Similarly, the study in South Africa also reported a staff shortage in health facilities.[14]

This study revealed another challenge was negative patient behaviors towards healthcare providers. They would get emotional disturbances due to society's negative attitude towards HIV-TB co-infected patients. They think they might die because of these diseases and get tired of taking lifelong ART. Similar results were found in the study conducted in South Africa.[7]

The current study reported that the health care providers felt the importance of testing family members of HIV-TB patients. Another study by Venturini, E., Turkova, A., Chiappini, E., et al reported similar findings.[1] Health care provider was interviewed about their perspectives, but their work was not observed directly.

5. Conclusion

Challenges during HIV-TB diagnosis and treatment in children: difficult to get sputum sample for CBNAAT and child was unable to complain about symptoms, caregivers were not able to mention the signs correctly, delay in receiving TB lab report, challenging to feed drugs to an infant, unavailability of a pediatrician in few Taluka ART center, distance from centers, low community awareness, higher loss to follow-up, poverty and illiteracy, financial problem, stigma and death due to TB treatment default.

Recommendations

It can be recommended that an awareness campaign should be conducted regarding HIV and TB, the government should supply enough HIV testing kits and resources to perform CBNAAT for TB diagnosis, TB lab reports should be provided in time, and the government should make pediatric doctors available in each ART center.

Ethical approval

Ethical approval was obtained from the Institutional Ethical Committee (IEC) of Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research, Belagavi (**Reference No. MDC/DOME/255 dated 5**th **October 2021**) and from the IEC of Belagavi Institute of Medical Sciences, Belagavi **(Reference No. BIMS-IEC/169/2021–2022 dated 29**th **November 2021)**. Also, the permission was taken from the District Tuberculosis Centre (DTC) **(Reference No. ಜಿಕ್ಷಣಕೇ/ಬಿ/ 73/2021–22, dated 28**th **December 2021)** and from the administrator of Clinical Services- Academics for Medical Director and Chief Executive, KLES Hospital **(Reference No: KLES/Dr. PK-HOSP/ADM-CS/GEN/21–22/12188 dated 12 January 2022)**.

Authorship

Mubashir Angolkar and Ashwini Narasannavar: Conceptualization, Methodology **Snehi Shrestha, Nuha Al-aghbar & Akshata K Ritti**: Data curation, Investigation, Funding

acquisition, Writing- Original draft preparation. **Ramesh Bhandari**: Visualization. **Mubashir Angolkar**: Supervision, Validation **Ramesh Bhandari**: Writing- Reviewing and Editing.

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Conflicts of interest

The authors have none to declare.

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Original article

Epidemiological survival pattern, risk factors, and estimated time to develop tuberculosis after test and treat strategies declared for children living with human immune deficiency virus

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ABSTRACT

Background: Due to their age category and the immune-suppressing effects of HIV, children were more vulnerable to experience endogenous reactivation of latent bacilli in the lung and increased risk of active tuberculosis incidence. The aim of this study is to assess the survival pattern, risk factors, and estimated time to develop TB after children started ART at selected health facilities of North Wollo, Ethiopia, from November 1, to September 30, 2021.

Methods: Facility-based retrospective cohort study was employed from November 1 to September 30, 2021. Cox proportional hazard regression model was used to assess factors associated with incidence of tuberculosis. AHR with 95% CI was used to declare statistical significance for tuberculosis incidence.

Results: During follow-up, 54 (10.9%) new cases of tuberculosis was reported. At the end of follow-up period, overall cumulative survival probability was determined as 43.8% (95%CI: 28.2–54.3). WHO clinical stage III&IV (AHR: 2.4 (95% CI: 1.4, 4.7), Hgb≤10 gm/dl (AHR = 2.2: (95%CI: 1.12–5.8), missed isoniazid preventive therapy (AHR = 2.5 (95%CI: 1.56–10.3) and Viral Load (≥400 cell/ml) (AHR = 2.02 (95%CI: 2.03–6.8) were significant risk factors for tuberculosis incidence.

Conclusion: Nearly ten % of HIV-positive children experienced new cases of tuberculosis with median time of 25(IQR = ±12) months. It would be better to give special attention to children who missed isoniazid preventive therapy with WHO stages III&IV Viral load (≥400 cells/ml), and Hgb≤10 gm/dl to prevent tuberculosis incidence and prolonged quality of life.

Abbreviations: AHR, adjusted hazard ratio; CHR, crude hazard ratio; CI, confidence interval; FMOH, Ethiopian Federal Ministry of Health; MUAC, mid-upper arm circumference; IQR, interquartile range; NGT, nasogastric intubation for feeding; WFH, weight for height; SD, standard deviation.

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1. Introduction

Tuberculosis (TB) and human immunodeficiency virus (HIV) are the leading causes of infectious-disease-related deaths in this globe.[1] The synergy between TB and HIV infection is strong, while TB is the leading cause of death for people living with HIV and HIV is a risk factor for the incidence of active TB through target reduction of immune function.[2,3] The waning of the immune system increased mycobacterium tuberculosis endogenous reactivation of dormant bacilli in the lung[4] and in some Sub-Saharan African populations where 10%–15% suffered from twine epidemic.[2,5]

According to the World Health Organization (WHO) report, in 2019, there were an estimated 10.1 million new cases of TB and 1.7 million new deaths, making TB the leading cause of death from a single infectious agent (ranking above HIV/AIDS).[6] The main reason for the resurgence of TB in Africa is not the deterioration of control programs; it is rather the link between TB and HIV/AIDS coupled with a lack of human resources.[7] Africa is the second TB burden region (25%) next to Southeast Asia (44%)[6,8] and in which responsible for one–third of TB/HIV-associated deaths for children living with HIV.[9,10] Likewise, in 2018, there were about 251,000 deaths from TB among people living with HIV (PLWHIV), which accounts for 33% of the total deaths associated with HIV, which is much higher than the case fatality rate expected ≤5% by WHO.[11,12]

The WHO recent estimation indicates that the risk of developing active TB disease is 21 times higher in people living with HIV (PLHIV) with a 5–10% annual risk and 51% lifetime risk of developing active TB than those without HIV.[1] Ethiopia is one of the 30 high TB- and HIV-burden countries in the world with an incidence of 0.17 cases per 1000 population for HIV and 1.64 per 1000 for TB. Studies conducted in Ethiopia have shown that TB/HIV co-infected individuals have a greater risk of psychosocial problems, low quality of life, and poorer physical health than HIV-infected individuals without active TB.[13] The impact of TB on children is devastating and they are more vulnerable to complications.[2,3] Those living with HIV are particularly at risk, as they are those suffering from malnutrition and common childhood infections, and that is why it demanded global attention.[14,15]

In Ethiopia, estimates and projections for the year 2019 show that from about 669,230 People living with HIV with an overall prevalence of 1.06% and ranging from 0.17% in the Somali region to 4.37% in the Gambela region, 44,229 of people are living with HIV.[14,16] In Ethiopia, in 2016, of an estimated 710,000 people living with HIV among the general population, 62,000 of them were children.[3,14] At least one in four deaths was attributed to TB and many of these deaths were in resource-limited settings.[17,18]

Previous study findings suggested that treatment failure, advanced clinical stage, medical comorbidity conditions, levels of hemoglobin, CD4 count, and orphanage were the most prominent factors associated with episodes of advanced opportunistic infection [16,19–30]including TB incidence.[24] Epidemiologically; extra pulmonary tuberculosis (EPTB) is the most frequently diagnosed and treated TB type among children living with HIV[26,30]; smear-positive and negative cases of PTB type have an overlapping clinical presentation with other upper respiratory infections leading to missed or late diagnosis of the cases.[24,29,31] In general, concomitant administration of isoniazid preventive therapy (IPT) with ART can denote nearly ninety-six percent of new TB incidences[17,25]; however, a completion rate of the six-month IPT is another hot issue in TB prevention and control program of Ethiopia.[25,28]

Despite several small-scale studies have been reported on the incidence of TB among HIV-positive children in many parts of Ethiopia,[1,32] to our knowledge, there are no yet reports on including follow-up characteristics such as current viral load to calculate the incidence rate of TB in our study site. Therefore, this study aimed to assess the survival pattern, risk factors, and estimated time to develop TB after children started ART at selected health facilities of North Wollo, Ethiopia, from November 1, to September 30, 2021.

2. Methods

2.1. Study setting and period

This study was conducted in the public institutions of Woldia hospital and Woldia health center, which are found in the North Wollo Zone, Amhara Region, Ethiopia from July 21–28, 2022. Woldia city is located 521 km away from Addis Ababa, the capital city of Ethiopia, in North East direction and 364 km away from Bahir Dare, the capital city of the Amhara region [33,34]. The main reason for including the two-health facilities is the earlier endorsement and initiation of ART treatment for the catchment population, including children and pregnant women [34,35].

2.2. Study design

A facility-based retrospective cohort study was employed.

2.3. Patient and Public Involvement

No patient involved.

2.4. Source population

All HIV-positive children (under the age of 15) who had been started antiretroviral medication (ART) in Woldia hospital & Woldia health center, from November 1, 2015, to September 30, 2021, North East Ethiopia were source population.

2.5. Inclusion criteria children

All HIV positive children (≤15 years of age) who were enrolled for HIV/AIDS chronic care without SAM at baseline from November 1, 2015, to September 30, 2021, among selected health facilities were included.

2.6. Exclusion criteria

The study excluded HIV-positive children whose ART start dates were unclear and who were hospitalized with

insufficient baseline data (CD4 count, hemoglobin level, clinical stage, weight, and height).

2.7. Sample size determination

The sample size was calculated based on the double population proportion formula using EPI-Info softer using the following significant variables; poor adherence to ART, duration on ART, severe immunodeficiency, advanced clinical stage, and chronic diarrhea.[34] The following assumptions were considered to calculate the sample size. We used P1 (52.1%); which is the percent of exposed with outcome Z $\alpha/2$: 1.96 taking CI 95% and 80% power, P2 (37.7%): is the percent of non-exposed with the outcome, r: is the ratio of non-exposed to exposed which is 1:1. Therefor the final sample size found using advanced clinical stage was 402 and after adding 5% incomplete medical records, the final sample size was found to be 421. However, from November 1, 2015, to September 30, 2021, there were only 498 HIV-positive children enrolled in ART care at both Woldia comprehensive hospital and Woldia health center. Therefore, we included all study subjects without employing a sampling procedure since the files are manageable.

2.8. Dependent variables

Incidence of tuberculosis (Yes/No).

2.9. Independent variable

Socio-demographic characteristics of the children (age, sex, residence, family size, caregiver, parental status), baseline clinical and laboratory factors (clinical stage, level of immune suppression, initial ART regimen, hemoglobin level, functional status, developmental status, opportunistic infections, level of ART adherence, CPT, IPT, viral load, and TB contact history.

2.10. Operational words

Event: Newly diagnosed TB infection after children started ART during their follow-up[23]

Survival time: time in a month from the beginning of treatment (ART) to the diagnosis of TB.[20]

Censored: HIV-positive children on ART, who do not develop an event or TB-HIV death until the last date of the study period, were lost to follow-up and transferred out.[20]

Viral load: persistent viral load ≥400 copies/ml is unsuppressed and viral load <400 copies/ml on ART for at least 24 weeks is suppressed.[31]

Adherence to ART: was classified based on the percentage of drug dosage calculated from the total monthly doses of ART drugs. (Good >95%, fair 85–94%, and poor <85%).[34]

Anemia: was defined as having a hemoglobin level ≤10 mg/dl.[18,24]

CD4 count: CD4 levels below the threshold level were classified based on the child's age (i.e., infant CD4 1500/mm3, 12–35 months, 750/mm3, 36–59 months 350/mm3, and 5 years 200/mm3).[36]

2.11. Data collection instruments and quality control

A standard and pretested data extraction checklist was used to extract the required from individual ART register intake forms; follow-up forms and medical history sheets. The quality of data was ensured at the point of data collection and data entry. Emphasis was given to designing checklists based on the relevance and availability of variables on national pediatrics ART formats.[11,36,37] A pretest was undertaken at Woldia specialized hospital with the prepared checklists before the actual data collection started and the amendment was made to the format. Two data collectors and one supervisor had trained for two days on the objective of the study and the contents of the format. ART provider two BSC nurses did data collection and the principal investigator supervised them. The investigators examined the completeness and consistency of the completed data if incorrect and their amendment was made by going back to patient cards with patients' unique ART numbers.

2.12. Data processing and analysis

Before data analysis, the WHO Anthro-Plus-Version 1.04 and ENA for nutrition smart software were used to generate the Z score (WAZ, HAZ, and WHZ/BAZ) to define the nutritional status of HIV-positive children. The incidence rate of TB is calculated using the total number of people per year (PPY) individual contribution to follow-up as a denominator. Descriptive nonparametric statistical tests such as the Kaplan-Meier plot were used to estimate the median TB free survival time.where SKM = $\pi t(i) \leq t$ pi[38]

$$SKM(t) = 1 \text{ if } t < t(1)$$

The estimate of the failure function, $f_{(t)}$, is computed as $1 - \check{S}_{(t)}$. The standard error reported is given by Greenwood's formula. The Survival function S(t) changes only at failure times, thus for censored times the conditional probability of survival is always one and can estimate when will be half-life or median TB-free survival time both in graphical and statically in association with the log-rank test, which is expressed as follows, the free survival status of children was calculated from the life table estimator of the SF at the jth interval $[t_{j-1}, t_j)$ is given by[38]:

$$S_{LT}(t_j) = \pi_{i=1} \frac{ni - di - (c½)}{ni - (c½)}$$

where:

n_i = # at risk at the beginning of the ith interval $[t_{-1}, t_i)$.

d_i = # events/TB/incidence during the interval $[t_{-1}, t_i)$.

C_i = # censored in the interval $[t_{-1}, t_i)$. Furthermore, we have also assessed whether a real statistically significant survival difference between the two groups for the incidence of tuberculosis and which is estimated by using the log-rank test. The final semi-parametric regression was checked using Cox proportional hazard assumption (**Cox, 1972**), which is checked using a graphical diagnostic based on the scaled Schoenfeld residuals (log-log survival plot) and (observed versus expected graph). The second way is statistical tests (global test estimations which are finally found at end = 0.63).

However, the regression estimation is written as follows for the multivariable Cox proportional hazard regression building of the model for consecutive following of HIV-infected children under clinical observation experiencing events of TB at a point in time. The equation of the Cox model has expressed as follows[38]

$$\text{I. } H(t) = h_0(t) \text{ x } \exp(b_1X_1 + b_2 X_2 + \ldots b_pX_p)$$

where the hazard function h(t) depends on several p covariates (x1, X2, ….XP),

Whose impact is measured by the size of the respective coefficients (b1, b2…, bp).

The term h0 is called the baseline hazard (constant HR) and is the value of the hazard if all xi is equal to zero (the number exp (0) equals. The covariates of the multivariate analysis were selected using the enter method. Variables with P-value <0.25 in the bivariable Cox regression analysis were included in the multivariable Cox regression model to determine the factors associated with TB incidence. The model with the smallest value of the information criterion will be selected as the final model of the analysis and checked using Nelson-Alan and Cox Snell residual test.

3. Result

From November 1, 2015, to September 30, 2021, four hundred ninety-eight (498) children were enrolled for HIV/AIDS care in the two institutions. Of the total children who started ART, three individual files were not available and a card was excluded due to incompleteness.

3.1. *Socio-demographic characteristics of HIV-infected children*

Of the total included participants, two-thirds (69.64%) of them were from urban and more than half (51.01%) were female in gender. The overall mean (±SD) age of the participants was estimated at 9.14 (±3.7) years. Furthermore, more than half (54.45%) of the children have been living with their biological parents, nevertheless, 15 (15.99%) of the participant cases were double orphans (Table 1).

3.2. *Baseline clinical and comorbidity characteristics*

Three hundred twenty-five (65.1%) of the participant children at baseline were WHO clinical stage I and II, one forth (21.26%) of them experienced advanced opportunistic infections (OIs), more than three forth (77.94%) of the participant children had a CD4 count above the threshold, and (37.2%) have anemia. Regarding nutritional status, majority (70.2%) of caregivers in their dyads had nutritional counseling during enrolment for ART. Of the participants, (9.5%), (14.2%), and (16.5%) of the participants experienced severe wasting, stunting, and underweight, respectively (Table 1).

3.3. *Follow up status of HIV-positive children*

During follow-up, four hundred nine (82.7%) and three hundred eighty-one 381 (77.7%) cases received cotrimoxazole preventive therapy (CPT) and isoniazid preventive therapy (IPT) after active TB screening, respectively. Regarding drug adherence, nearly three-fourth (72.8%) of children had good ART adherence (Table 2).

3.4. *Epidemiologic survival patterns of children*

The seven years retrospective cohort study of 494 participant children yielded 1254 persons per year of risk observation (PPY). During the follow-up period, (81.78%) participant children were on follow-up, although, (7.49%) died, (8.91%) experienced loss from the cohort, and nine (1.82%) cases were medically transferred to other institutions. Regarding epidemiology of TB cases, more than half (57.6%) (N = 31) cases were diagnosed and treated as extra-pulmonary TB (EPTB) with respective incidence (IDR) 3.1 (95%CI; 2.2–4.8) Per 100 Person Years (PPY). While the remaining cases were treated as smear-negative pulmonary TB (SNPTB) and Smear positive Pulmonary TB (SPPTB), there were 13 (24.4%) and 10 (18.5%), respectively. The median follow-up time without TB diagnosis was found to be 25(IQR = 12) months with a minimum and maximum of 3 and 79 months, respectively (Table 3).

3.5. *Estimation of time to develop TB*

At the end of follow-up, 54 (10.9%) new cases of TB were reported with an overall incidence density rate (IDR) estimated to be 4.3 (95%CI: 3.2–5.7) Per 100 Person Years (PPY) with a crude proportion of 10.9%. The Majority (68.5%) (N = 37/54) of TB cases occurred within three years of follow-up after ART was initiated. However, a small number of TB cases 2/54 (3.7%) were reported after six years (72 months) of follow-up. Regarding the cumulative survival probability of children without TB and estimated from life at the end of 12, 24, 36, 48, 60, and 72 months after ART was initiated were, 96.6%, 90.6%, 88.4%,81.5%, 76.,2%, and 65.7%, respectively. The overall survival rate at the end of the study period was found to be 43.8% (95%CI 28.1–54.2) (Table 4).

3.6. *Kaplan Meier survival curve for children*

From the Kaplan Meier survival curve, there was a significant survival difference in the time of developing TB. Among the categorical variables; age and sex of the children, residence, family size, level of hemoglobin, CPT, IPT, viral load suppression, and parental status, there were statically significant survival difference in the two groups. There was a significant survival difference in the incidence of TB between children who had hemoglobin ≤10 gm/dl as compared with those who had hemoglobin levels> 10gm/dl and evidence from the log-rank test (Chi2 (1) = 71.6, P = 0.001). Similarly, there was also a significant survival difference for children who had baseline stage I&II compared to those who were in WHO stage III&IV (Chi2 (1) = 95.0, P = 0.001). Likewise, there was a significant survival difference for HIV-positive children who missed isoniazid preventive therapy compared to anyone who ever took it during successive follow-ups (Chi2 (1) = 143.9, P = 0.001). Moreover, there was a significant survival difference among HIV-positive children who had viral load sup-

Table 1 – Baseline socio-demographic characteristics of HIV-positive children in selected health facility at northeast Ethiopia from November 1, 2015, to September 30, 2021.

Variables	Categories	Frequency	Percent
Age	≤5 year	298	40.08
	<5years	296	59.92
Resident	Urban	327	66.19
	Rural	167	33.81
	Both parent alive	268	54.25
Orphan status	At least one orphan	145	29.35
	Double orphan	81	16.4
Family size	<=5	307	62.15
	>5	187	37.8
Dietary counseling	Yes	347	70.2
	No	147	29.7
Treatment failure	Yes	32	6.48
	No	462	93.5
Functional status (age≤5 years)	Appropriate	165	33.40
	Ambulatory	46	9.31
	Bedridden	40	8.10
Developmental History	Working	154	31.17
	Ambulatory	52	10.53
	Bedridden	37	7.49
WHO clinical stage	WHO Clinical stage 1 & 2	325	65.79
	WHO Clinical Stage 3 & 4	169	34.21
Anemia	≤10 gm/dl	311	62.9
	>10 gm/dl	183	37.2
Baseline infection	Yes	105	21.2
	No	389	78.7
Underweight (WFA)	Norma	315	63.7
	WAZ < - 2 Z score	98	19.8
	WAZ < –3 Z score	81	16.5
Stunting (HFA)	Normal	269	54.4
	HAZ < –2 Z score	56	11.5
	HAZ < - 3 Z score	72	14.1
WFH (Wasting)	Normal	392	79.35
	WAZ < –2 Z score	55	11.13
	WAZ < - 3 Z score	47	9.5

pressed as compared with those not suppressed within the past six months (Chi2 (1) = 77.1, P = 0.001) (Figs. 1–3).

3.7. *Predictors for time to develop TB*

To identify predictors of tuberculosis, bivariable and multivariable Cox regression analyses were conducted. Age, sex, residence, parental status, viral load, hemoglobin level, dietary counseling, CPT, IPT, clinical stage, CD4 count, MUAC levels, stunting, underweight, family size were variables entered into the multivariable analysis. Of them, age, sex, residence, levels of hemoglobin, CPT, IPT, viral load suppression, clinical stage III&IV, and parental status were variables which were fitted with the model. Among these only four of them were significantly associated with TB incidence.

The risk of tuberculosis incidence for children whose WHO clinical stage III&IV during follow-up was 4.8 times than children whose WHO clinical stage I and II [AHR: 4.8 (95% CI: 1.6–10.3, P = 0.001). Likewise, HIV-positive children at baseline with (Hgb)≤10 gm/dl were 3.5 times increased risk to develop TB as compared with children with Hgb>10 gm/dl (AHR = 3.5 (95%CI: 1.5–7.9, P = 0.02). Similarly, HIV-positive children with recent (six-month of data collection) follow-ups having a viral load >400 cells/ml were nearly two times increased risk of developing TB than children having viral load (≤400 cells/ml) during follow-up (AHR = 2.02 (95%CI: 2.03–6.8, P = 0.03). Finally, HIV-positive children missing isoniazid preventive therapy (IPT) were 2.5 times increased risk of developing active TB than the counter group (AHR = 2.5, (95% CI: 1.56–10.3, P = 0.001) as shown in (Table 5).

Table 2 – Follow-up status of HIV-positive children in selected health facilities in north East Ethiopia from November 1, 2015, to September 30, 2021.

Variables	Categories	Frequency	Percent
Adherence	Good	360	72.8
	Faire	51	10.3
	Poor	83	16.8
IP	Yes	381	77.13
	No	113	22.8
CPT	Yes	409	82.79
	No	85	17.21
CD4 count or percent	Below threshold	109	22.2
	Above threshold	385	77.9
Viral load	≤400 cell/ml	406	82.12
	>400 cell/ml	88	17.8

3.8. *Overall adequacy model test*

The overall multivariable Cox regression test of model adequacy for TB incidence in HIV-positive children after ART started indicates that the line is on the straight origin (Fig. 4).

4. Discussion

Tuberculosis remains one of the leading causes of morbidity and mortality and is responsible for one-third of deaths among people living with HIV globally, especially in developing countries including Ethiopia.[23] At the end of this study period, the incidence of TB was estimated to be 4.3 (95%CI: 3.2–5.7) Per 100 Person Years (PPY) with a crude proportion of 10.9%.

This finding is higher than previously reported 2.0 Per 100 Person Years in Debre tabor Hospital,[23] 3.78 Per 100 Person Years in Jimma Hospital,[28] 2.63 Per 100 Person Years in Debre Marko's Hospital,[39] and 2. Per 100 Person Years in Southern Ethiopia.[40] This discrepancy could be explained by changes in the study period and setting. Specifically, in developing countries, the treatment standard is typically substandard and varies widely across different health institutions and settings.

On the other hand, our incidence report is consistent with the previous findings of 4.8 per 100 person years of a systematic review and meta-analysis result in Ethiopia[21] and finding reported 4.9 Per 100 person Years (PPY) in North West Ethiopia.[21] This might be due to the use of almost similar guidelines for case ascertainment of tuberculosis diagnosis

Table 3 – Epidemiologic survival pattern of HIV-positive children after ART initiated in a selected health facility in North Wollo Zone, Amhara region, North East Ethiopia.

Variables	Categories	On cohort	Died	Lost-follow up	Transfer out	TB(Case/Non-case		IDR/Year
Age	≤5 years	158 (31.9)	16 (2.8)	19 (3.8)	5 (1.02)	36 (7.2)	162 (32.7)	0.00536
	>5 years	246 (49.7)	21 (4.2)	25 (5.04)	4 (0.8)	18 (3.6)	278 (56.2)	0.00215
Sex	Male	195 (39.4)	24 (4.8)	20 (4.04)	4 (0.8)	30 (6.1)	213 (43.1)	0.00384
	Female	209 (42.3)	13 (2.6)	24 (5.02)	5 (1.02)	24 (4.8)	227 (45.1)	0.00331
Resident	Rural	111 (22.4)	10 (2.1)	40 (8.04)	6 (1.2)	29 (5.9)	95 (19.2)	0.00769
	Urban	293 (59.3)	27 (5.4)	4 (0.8)	3 (0.6)	25 (5.1)	345 (69.8)	0.0022
IPT	Given	324 (65.5)	18 (3.6)	38 (7.6)	5 (1.02)	6 (1.2)	379 (76.3)	0.00050
	Not given	80 (16.2)	19 (3.8)	6 (1.2)	4 (0.8)	48 (9.5)	61 (12.3)	0.01513
Viral load	≤400 cell/ml	336 (68.1)	23 (4.8)	38 (7.4)	7 (1.4)	17 (3.4)	389 (78.7)	0.00136
	>400 cell/ml	68 (13.7)	14 (2.8)	6 (1.2)	2 (0.4)	37 (7.4)	51 (10.3)	0.01445
TB-Epidemiology	EPTB	38 (7.6)	4 (0.8)	1 (0.2)	2 (0.4)			
	SNPTB	10 (2.1)	4 (0.8)	3 (0.6)	0			
	SPPTB	11 (2.4)	1 (0.2)	0	0			

IPT= Isoniazid Preventive Therapy, TB = Tuberculosis.

Table 4 – Survival probability of HIV-positive children after ART initiated on the life table among selected health facilities in North Wollo Zone, Amhara region.

Months	Children at initial	Risky Children	No. TB Cases	Cumulative TB IDR	Survival Rate	95% CI
1 12	494 (100)	480 (76.9)	14 (2.8)	2.8	0.9667	0.9443 0.9802
13 24	480 (76.9)	462 (73.2)	18 (3.6)	6.5	0.9067	0.8696 0.9336
25 36	462 (73.2)	457 (72.2)	5 (1.03)	7.4	0.8847	0.8428 0.9160
37 48	457 (72.2)	447 (70.24)	10 (2.02)	9.6	0.8154	0.7543 0.8627
49 60	447 (70.24)	444 (69.6)	2 (0.4)	9.9	0.7629	0.6821 0.8258
61 72	444 (69.6)	442 (69.2)	3 (0.6)	10.1	0.6576	0.4617 0.6366
≥73 months	442 (69.2)	340 (68.8)	2 (0.4)	10.9	0.4384	0.2818 0.5425

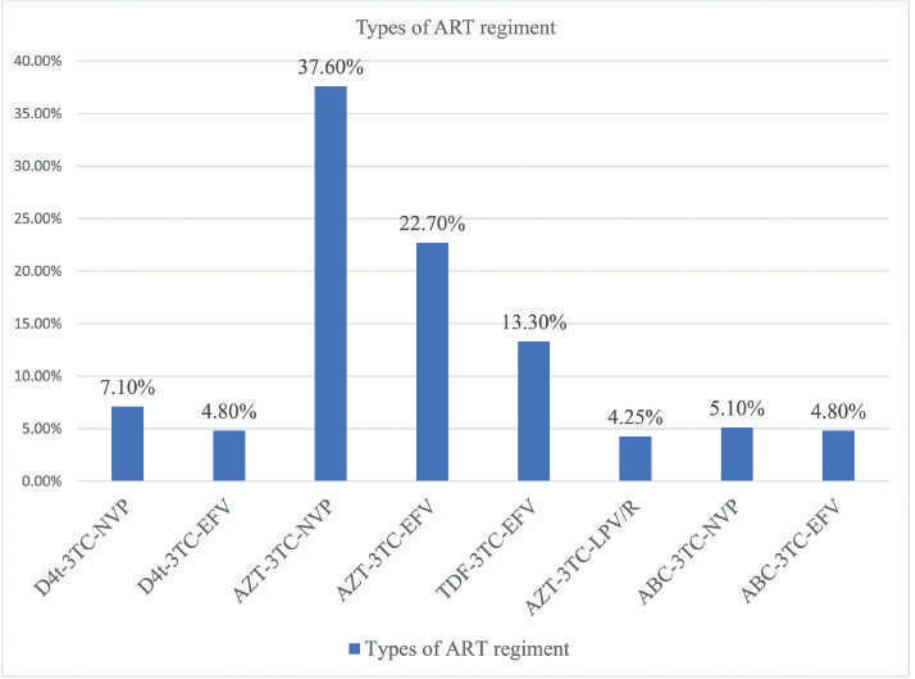

Fig. 1 – Types of ART regiment for HIV-positive children in selected health facility at northeast Ethiopia from November 1, 2015, to September 30, 2021.

and a similar guideline for the provision of HIV/ADIS care for children in addition to socioeconomic and demographic factors and the similarity of the population might contribute.

Conversely, the final report of this study revealed that it is lower than the previously defined results of 7.917 per 100 person years in Southwest Ethiopia,[17] 5.9 Per 100 Person Years

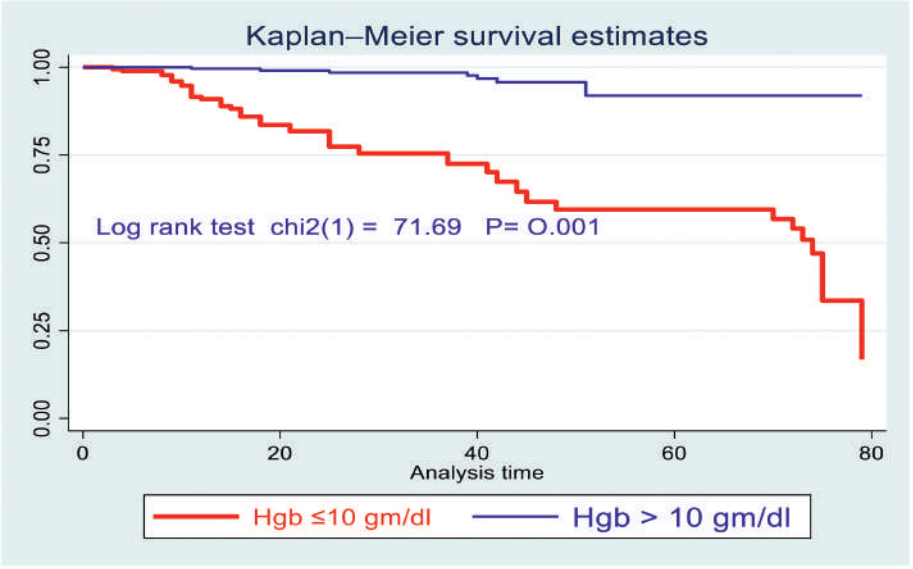

Fig. 2 – Kaplan-Meier survival curve of children living with HIV stratified by levels of hemoglobin after enrolling on ART care in North Wollo zone.

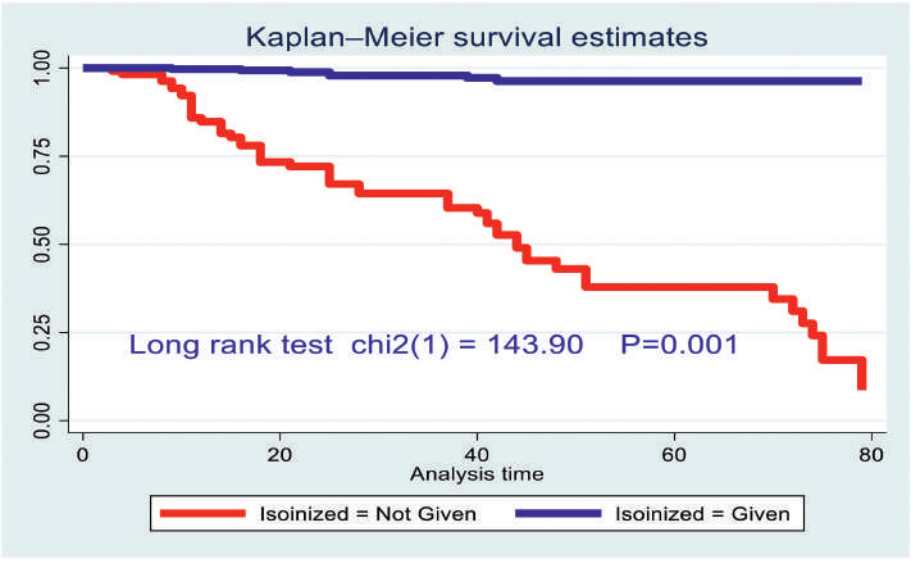

Fig. 3 – Kaplan-Meier survival curve of children living with HV stratified by isoniazid preventive therapy enrolled for ART care in North East Ethiopia.

in Northwest Ethiopia,[26] 6.03 Per 100 Person years in Adam hospital,[16] and 15 Per 100 person years in south Africa.[22] The difference might be due to the quality of care and the ability of healthcare providers to screen and trace for tuberculosis during consecutive follow-ups. What is more, here is the differences in socio-demographic characteristics, quality of treatment, and screening capabilities of the healthcare workers contributed to a spatial variation in each set,[1] and, poverty, overcrowding, family, and living in poor conditions may all contribute to the rocketed incidence rates in Ethiopia.

Regarding predictors for the incidence of TB, the risk of acquiring TB for HIV-positive children who were in clinical stage III & IV at baseline increased 3.4 times as compared with those who had clinical stage I & II (AHR = 3.4 (1.48–7.7, p = 0.004). This is consistent with previously reported finding in Debre Tabor,[36] Debre Markose,[41] Assosa,[42] and Cameron hospitals.[43] This might be because clinical stages III and IV have a major impact on the risk of immunological weakening in HIV/AIDS patients and leads to a worsening prognosis. In addition, immunity loss in advanced clinical stages hastens endogenous reactivation and latent transition of dormant tuberculosis bacilli into active TB infection in the lung.[23]

In this study, low hemoglobin level was found to be a predictor of TB occurrence for children living with HIV. Having low hemoglobin levels (Hgb ≤10mg/dl) at the time of ART initiation was nearly two times increased the risk of TB

Table 5 – Multivariate Cox regression analysis for the incidence of SAM after ART started among HIV-positive children in North Wollo Zone selected health facility.

Variables	Categories	TB incidence		CHR	AHR	P < value
		Yes (%)	No (%)			
Age	≤5 years	36 (7.2)	162 (32.8)	2.8 (1.6–4.7)	0.9 (0.64–2.1)	0.8
	>5 years	18 (3.6)	278 (56.3)	Ref		
Sex	Male	36 (7.2)	162 (32.8)	1.3 (0.82–2.4)	1.27 (0.5–1.7)	0.94
	Female	18 (3.6)	278 (56.3)	Ref	Ref	
Resident	Rural	30 (6.06)	213 (43.1)	5.1 (4.1–12.3)	1.4 (0.89–4.48)	0.15
	Urban	24 (4.8)	227 (45.9)	Ref	Ref	
IPT	Given	6 (1.2)	379 (79.1)	Ref	Ref	
	Not given	48 (9.7)	61 (12.3)	4.8 (2.8–8.1)	4.8(1.6–10.3)	0.001*
CPT	Given	28 (5.6)	381 (77.1)	Ref	Ref	
	Not Given	26 (5.2)	59 (11.9)	3.1 (2.6–8.8)	2.1 (0.9–2.8)	0.17
Levels of hemoglobin	≤10 gm/dl	50 (10.2)	130 (26.3)	7.2 (3.8–13.4)	2.2(1.2–4.6)	0.02*
	>10 gm/dl	4 (0.08)	307 (62.2)	Ref	Ref	
Viral load suppression	≤400 cell/ml	17 (3.4)	389 (78.7)	Ref	Ref	
	>400 cells/ml	37 (7.4)	51 (10.3)	10.3 (5.6–21)	3.4(1.5–7.9)	0.03*
Parental status	Both alive	6 (1.2)	263 (53.2)	Ref		
	At least one orphan	16 (3.1)	167 (33.8)	4.7 (1.8 12.3)	1.2 (0.94–7.1)	0.11
	Double orphan	32 (6.4)	47 (9.7)	2.2 (3.3–7.9)	2.2 (0.97–6.3)	0.13
WHO stage	WHO stage I &II	3 (0.6)	322 (65.1)	Ref		
	WHO stages III &IV	51 (10.3)	118 (23.8)	4.5 (2.6–11.3)	4.8(1.2–18.5)	0.01*
Dietary counseling	Yes	26 (5.2)	321 (64.9)	Ref	Ref	
	No	28 (5.6)	119 (24.2)	2.8 (1.6–4.8)	1.8 (0.8–2.9)	0.08

AHR: Adjusted Hazard Ratio; CHR: Crude Hazard Ratio; *statistically significant at.

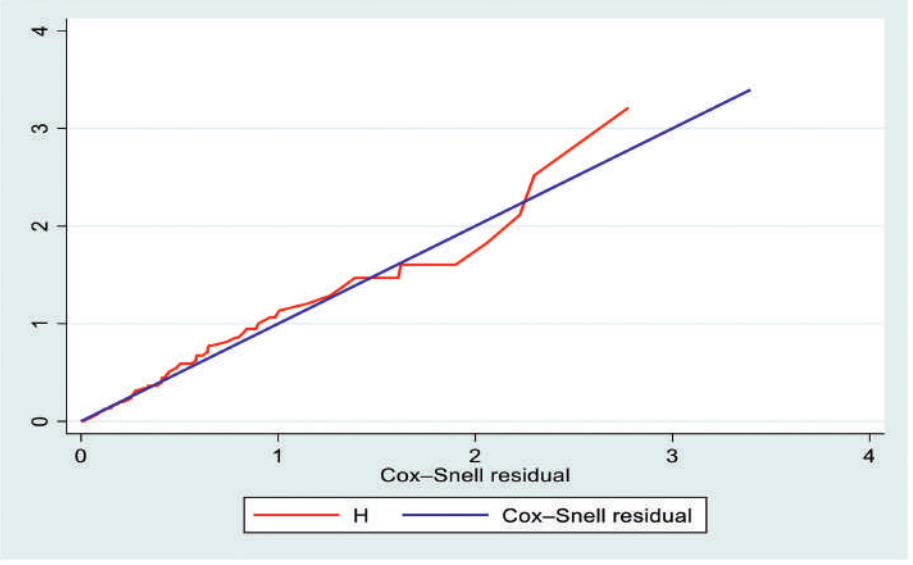

Fig. 4 – Nelson-Alana and Cox-Snell residual test for final model adequacy test.

incidence as compared to children who had Hgb≥10 g/dl (AHR 2.2 (95%CI:1.2–4.6)), P = 0.002). This is similar to previous findings in, Deber Tabor,[36] Adama,[44] Gonder,[45] Northern Ethiopia,[20] Dar es Salaam, Tanzania,[46] and England hospitals.[47] This is due to hemoglobin levels having a high predictive value for the incidence of active TB and poor clinical outcome.[48] Consistent with previous research finding at Adama hospital,[44] Pawe and Assosa Hospitals,[42] Zaire and Cameron hospitals,[4,49,50] HIV-positive children who missed IPT after starting HIV/AIDS care increased nearly three-time risk of developing active TB as compared with who had IPT. This might be in some instant, however, ART alone is not adequate for reducing the load of TB infection and the implementation of other TB-specific interventions for risk alleviation is highly needed. Based on previous research findings in[25,28] concomitant administrations of IPT with ART have the power to denote ninety-six percent of active TB among children living with HIV. Children presenting with viral load not suppressed (viral load ≥400 copies/ml) were significantly associated with TB incidence as compared to their peers did have (<400 copies/ml). Previous findings reported by the Harvard school of public health,[29] advanced opportunistic

infection incidence increased significantly when the viral load increased and was inversely related to increased CD4 count. Viral suppression is a marker of HIV treatment success, thus reducing onward transmission of HIV.[31] However, several factors are associated with a lack of achieving HIV viral suppression, and individuals with unsuppressed HIV viral load (viral load ≥400 copies/mL), continue to sustain the epidemic incidence of advanced OIs including incidence.[31]

4.1. *Limitations of the study*

This study had inherent limitations resulting from its retrospective study nature and was exposed highly for missing significant variables like caregivers' household economic assets and information lacking caregivers' educational status on the ART follow-up form; all this might bias the final interpretation of the result.

5. Conclusion

Nearly one in every ten (10.9%) of HIV-positive children experienced new cases of TB within a median time of 25 (IQR = ±12) months. It would be better to give special attention to children who missed IPT, clinical stages III&IV, viral load ≥400 copies/ml, and anemic cases to prevent early tuberculosis incidence and to ensure prolonged survival outcomes.

Ethical consideration

The ethical review board of Woldia university college of health Science Research, And Community Service, Technology-Transformation, And University-Industry Linkage office ethically cleared this research to conduct with refill number (RCS, TT & UIL; 0015/2015) on 09/12/2015 E.C. In addition, IRB board of Woldia University waived Patient written informed consent since the principal investigators had no physical contact with patients during data collection, only on medical charts of recorded files retrospectively.

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Conflicts of interest

The authors have none to declare

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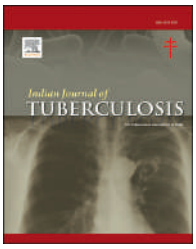

Original article

Role of line probe assay in detection of *Mycobacterium tuberculosis* in children with pulmonary tuberculosis

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ABSTRACT

Background: India is the highest TB burden country in the world with almost 27 lakh cases reported in 2019. Pediatric tuberculosis in India accounts for almost 31% of global TB burden. Despite such high mortality and morbidity in children, diagnosis of pulmonary TB in children still remains very challenging.

Material and methods: This cross-sectional study was conducted in a tertiary care hospital in Delhi, India. Children between 1 and 12 months with clinical suspicion of pulmonary tuberculosis who had not previously taken ATT were included.

Early morning gastric aspirate samples were collected after overnight fasting, on two days. Both days sputum sample were subjected to sputum smear microscopy and one of the two samples was subjected to line probe assay (LPA), cartridge based nucleic acid amplification (CBNAAT) and mycobacterium growth in tube (MGIT-960).

Results: 84 children with pulmonary tuberculosis were enrolled. The most common presenting complaint was fever seen in 83 patients (98.8%). Only 17 (20.24%) were sputum smear positive by Ziehl- Neelsen (ZN) staining. LPA was positive in 47 (55.95%) samples and among these positive samples both INH and RIF resistance was detected in 2 (4.26%) samples. CBNAAT was positive in 53 patients (63%). Growth in liquid culture media (MGIT 960) was observed in 44 (52.38%) samples. Among 17 smear positive samples, LPA was detected in 14 (82.35%) samples and among 67 smear negative sample LPA was detected in 33 (49.25%) samples. LPA had 63.46% sensitivity, 100% specificity in detecting *mycobacterium tuberculosis*.

Discussion: WHO's recommendation for using LPAs has been limited to culture isolates or smear-positive sputum specimens. New data has since been generated on the use of LPAs as newer versions of LPA have been developed over past few years.

Previous studies conducted using LPA version 1.0 reported much lower detection rate of mycobacterium tuberculosis in smear negative specimens.

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With the availability of newer versions such as Hain GenoTypeMTBDR*plus*version 2 and Nipro NTM + MDRTB detection kit 2, the diagnostic utility of LPA may be enhanced.

1. Introduction

India is the highest tuberculosis burden country in the world with almost 27 lakh cases reported in 2019.[1] Pediatric TB in India accounts for almost 31% of global TB burden.[1] India has set the ambitious target to end TB by 2025, 5 years ahead of sustainable developmental goal (SDG) goal for 2030. This can be achieved only by accurate and early diagnosis followed by prompt treatment. Diagnosing pediatric pulmonary tuberculosis has always been challenging. This is primarily because children are unable to expectorate and give a good quality sputum sample. Further, the traditional method of diagnosing TB (Ziehl – Neelsen staining) which already has low sensitivity, diagnoses even few pediatric patients, as pediatric disease is paucibacillary.[2] With the emergence of universal drug sensitivity testing (U-DST) of every patient with tuberculosis upfront, an attempt is now being made to give a tailor made regimen for the patient depending upon the drug sensitivity testing.[3] Hence the National tuberculosis elimination programme (NTEP) now recommends a nucleic acid amplification test (NAAT) for every patient with clinical suspicion of tuberculosis.[4]

2. Methods

This cross sectional study was conducted in a tertiary care pediatric hospital in Delhi, India catering to children <14 years of age. The study was approved by Institutional ethics committee. 84 consecutive children (1month–12 years) with clinical suspicion of pulmonary tuberculosis were included in the study.[4] Children who were taking anti-tubercular drugs and those who were deemed treated and later relapsed were excluded. Detailed history regarding symptom onset, contact history and clinical examination was done for each patient and details were recorded in a predesigned proforma. Tuberculin skin test and chest X ray was done for all patients. After taking informed consent from parents/guardians and assent from children >7 years, early morning gastric aspirate was collected after overnight fasting, on two days. Both days' sample was subjected to sputum smear microscopy by ZN staining and one of the two samples was subjected to Line Probe Assay (LPA; Genotype MTBDR plus version 2, Haines's Life sciences), Cartridge based nucleic acid amplification test (CBNAAT, Gene Xpert/RIF assay) and liquid culture (MGIT-960). Other investigations were planned depending upon the clinical condition of the child.

The collected sample from all the candidates was divided in two parts, one for Gene Xpert/RIF assay and the second part was processed by the conventional method of N-acetyl-L-cysteine NaOH (1% final NaOH concentration). After decontamination, the concentrated sediment was resuspended in 2 ml phosphate buffer solution (pH 6.8) and the smears were prepared and stained by Ziehl- Neelsen staining. 0.5 ml of decontaminated specimen was inoculated on MGIT 7H9 broth medium for MGIT- 960 liquid culture. These vials were then inoculated at 37 °C in MGIT -960 system. Also DNA extraction for performing LPA was carried out by using Genolysis kit (Haines Life Science, Nehren, Germany) according to manufacturer instruction.[5]

The data entry was done in the Microsoft excel spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software version 21.0. For statistical significance, p value of less than 0.05 was considered as significant.

3. Results

Results were compiled for 84 diagnosed pulmonary tuberculosis patients. The baseline demographic and clinical characteristics are summarized in Table 1. The most common presenting complaint was fever seen in 83 (98.8%) patients. According to Kuppuswamy scale, 69 (82.14%) patients belonged to lower socioeconomic class. Only 38 (45.24%) patients were fully immunized as per age. BCG scar was present

Table 1 – Demographic and clinical characteristics of the enrolled patients.

Characteristic	N (%)
Age in years (mean age ± SD)	7.35 ± 3.3 years
Male: Female	34: 50
Weight (kg) (mean ± SD)	19.25 ± 7.97
Height (cms)	116.52 ± 24.32
Distribution of type of household	
Overcrowding	57 (67.86)
Adult contact	26 (30.95)
Immunization status	
Fully immunized	38 (45.2)
Partially immunized	44 (52.3)
Not immunized	2 (2.3%)
Presenting complaints	
Fever	83 (98.8%)
Cough	78 (92.86)
Weight loss	75 (89.29)
Chest X ray findings	
Hilar lymphadenopathy	22 (26.1)
Miliary shadows	19 (22.6)
Pleural effusion	16 (19.0)
Tuberculin skin test (mean ± SD)	13.3 ± 5.8

Table 2 – Comparative results of LPA with MGIT in positive ZN staining.

LPA	Growth (n = 15)	No growth (n = 2)	Total	P value
Detected	13 (86.67%)	1 (50%)	14 (82.35%)	0.331
Not detected	2 (13.33%)	1 (50%)	3 (17.65%)	
Total	15 (100%)	2 (100%)	17 (100%)	

Table 3 – Comparative results of LPA with MGIT in sputum smear negative patients.

LPA	Growth (n = 29)	No growth (n = 38)	Total	P value
Detected	24 (82.76%)	9 (23.68%)	33 (49.25%)	<.0001
Not detected	5 (17.24%)	29 (76.32%)	34 (50.75%)	
Total	29 (100%)	38 (100%)	67 (100%)	

in 67 (79.7%) patients. Tuberculin skin test was positive (more than 10 mm) in 64 (76.19%) patients. The mean age of study participants was 7.3 years and 17 (20.2%) patients were sputum smear positive by ZN staining, LPA detected *mycobacterium tuberculosis* in 47 (55.9%) patients, while CBNAAT detected *mycobacterium tuberculosis* in 53 (63.1%) patients. Growth on liquid culture (MGIT) was seen in 44 (52.3%). Comparative results of LPA with sputum smear microscopy and MGIT are summarized in Tables 2 and 3. Specificity, sensitivity, positive predictive value and negative predictive value of LPA are summarized in Table 4.

4. Discussion

Our study demonstrates that when compared to MGIT, LPA (MTBDR plus VER 2.0) has a sensitivity and specificity of 63% and 100% respectively in sputum smear negative pulmonary tuberculosis patients.

The road to eliminating tuberculosis requires universal drug testing[4] and reducing time to diagnosis. Molecular line probe assays were approved by WHO for detecting *mycobacterium tuberculosis complex* (MTBC) and rifampicin resistance in sputum smear-positive specimens (direct testing) and in cultured isolates of MTBC (indirect testing) in 2008 (6). In 2011, WHO approved CBNAAT to diagnose and detect rifampicin resistance in patients suspected of pulmonary tuberculosis.[7] Line probe assays were approved by the WHO in 2008 with the available literature on Haines Life sciences (MTBDRplus Ver 1).[6] Evidence for generation of these guidelines was primarily generated from the INNO-LiPA Rif. TB assay (Innogenetics, Ghent, Belgium) and the GenoType MTBDRplus (VER 1) However, with improving technology, improved versions of LPAs have been released and hence it is only prudent to reevaluate their use.

One of the major factors which differentiate Genotype MTBDR plus VER 2 from VER1 is the presence of a genolyse kit which aids in DNA isolation which was done mechanically in VER 1 hence reducing the risks of cross-contamination.[8]

A number of studies have evaluated the role of LPA (MTBDRplusVER2). Most of these studies have been in adults and the results have been contrasting.

Ninan et al.[9] evaluated the role of LPA in ninety-one suspected cases of MDR – TB (>18 years of age) and demonstrated a high sensitivity and specificity of 81.5% (95%CI 67.4–91.1%) and 87.5% (95%CI 71–96.5%) respectively for the detection of tuberculosis. Similar results have been shared by Barnard et al.[10] and Meaza et al.[11]

Bernard et al.[10] subjected 282 consecutive sputum samples irrespective of AFB status to both Xpert/RIF assay and GenoType MTBDRplus (version 2) and found that both molecular assays have similar diagnostic performance. Both tests were able to detect *Mycobacterium tuberculosis* in 56–58% of smear negative but culture positive samples. They concluded that both assays had similar sensitivities (73.1% and 71.2% respectively), while both had specificity reaching 100%.

A study conducted by Singh et al.[8] subjected 572 sputum smear negative samples to LPA (MTBDRplusVER2) and found a sensitivity and specificity of the assay were 68.4% and 89.3% respectively. In their study, the sensitivity and specificity increased further on comparing their results to a composite reference score (a new concept to evaluate a diagnostic test, in this case taking into account clinical symptoms, and other tests and response to treatment to label a patient as tuberculosis). Further, in this study the authors had used larger volumes of decontaminated sputum samples as compared to that recommended by the manufacturer.

The WHO commissioned a systematic review[12] to guide a policy update on molecular tests for the diagnosis and drug sensitivity testing for tuberculosis. To detect mycobacterium tuberculosis, the review found marked heterogeneity in pooled sensitivity estimates [44.4% (95% CI 20.2–71.7%)]while specificity estimates were homogenous. There was scarcity of information with regard to condition of sample, sputum smear status and whether the patients who were being tested were previously on antitubercular drugs or not.

However, irrespective of varying results, performance of newer versions line probe assays is markedly improved and

Table 4 – Sensitivity, specificity, positive predictive value and negative predictive value of LPA in sputum smear negative and positive patients.

LPA	Tuberculosis in negative ZN staining	Tuberculosis in positive ZN staining
Sensitivity(95% CI)	63.46%(48.96% to 76.38%)	82.35%(56.57% to 96.20%)
Specificity(95% CI)	100%(78.20% to 100.00%)	-
Positive Predictive Value(95% CI)	100%(89.42% to 100.00%)	100%(76.84% to 100.00%)
Negative Predictive Value(95% CI)	44.12%(27.19% to 62.11%)	0%(0.00% to 70.76%)

the limited data available with newer versions appears encouraging.

The advantage of using GenoTypeMTBDRplus (v2.0) is additional information it provides with regard to Isoniazid resistance. It can also help in validating a Rifampicin resistance detected on Xpert/RIF assay.

However, we need to generate more robust data before making any conclusions with regard to use of this molecular assay for the diagnosis and universal drug testing in patients who have not taken ATT previously.

Our study has been able to generate data with regard to use of line probe assay in children who are largely sputum smear negative. However, the limitation of our study was a small sample size.

5. Conclusion

Line probe assay may play a role in diagnosis of paucibacillary tuberculosis.

Conflict of interest

The authors have none to declare.

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Original article

Outcome of tuberculous meningitis in children aged 9 months to 12 years at the end of intensive phase of treatment

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ABSTRACT

Background: Tuberculous meningitis (TBM) is associated with high morbidity and mortality. Most of the literature focuses on outcomes at the end of therapy when it may be too late for intervention to improve the outcomes. So, the present study addresses outcomes by the end of intensive course of therapy.

Methods: It was a prospective cohort observational study that enrolled 80 patients with TBM between 9 months and 12 years of age. Participants were classified into Definite, Probable and Possible TBM using Marais criteria. Survival/Mortality was evaluated at the end of hospital stay. Demographic, clinical, cerebrospinal fluid and radiological parameters were evaluated for predictors of morbidity and mortality. Standardized tools were used to assess possible impairments in different domains at the end of intensive phase of treatment, namely Gross Motor Functional Classification System for motor functional ability, Pediatric-Mini Mental score examination (MMSE), Blantyre Coma Scale (BCS) score and Vineland Social Maturity Scale (VSMS) for cognitive outcome, Auditory Brainstem Evoked Responses for hearing outcome and using Teller's/Snellen's visual acuity charts to assess visual impairment.

Results: A high Mortality rate of 42.5% was seen in the enrolled patients. Out of the total 80 patients, 20% recovered completely while 36.25% survived with disability (morbidity). Motor, Hearing, Cognitive and Vision impairment was present in 33.3%, 4%, 33.3% and 48.9% of the survivors respectively. On multivariate regression, raised intracranial tension and stage III disease were significantly associated with mortality. Morbidity was significantly associated with Stage III disease on multivariate analysis.

Conclusions: Despite advances in treatment, Tuberculous meningitis is associated with high burden of deaths and devastating neurological sequelae. Timely diagnosis and intervention of neurological impairments is needed to improve the outcome of TBM in survivors.

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1. Introduction

According to WHO, India has one of the highest loads of tuberculosis (TB), accounting for around 20% of all new TB cases annually.[1] Approximately, 25% of childhood TB cases are extra-pulmonary, with Tuberculous Meningitis (TBM) being the most severe form, responsible for majority of the deaths due to TB worldwide.[1]

Timely treatment may dramatically improve the outcome in children with TBM. WHO rapid advice for TB treatment in children recommends a 12 month regimen of anti-tubercular therapy (ATT) along with systemic corticosteroids as an adjunctive treatment for TBM.[2]

The availability of effective central nervous system (CNS) penetrating antitubercular drugs and neuroimaging have contributed to better management and early diagnosis. This has been however paralleled by a steep rise in number of survivors with varying degree of neurological sequelae such as hemiplegia, quadriplegia, cognitive impairment, seizures, cranial nerve palsy, hearing loss, vision impairment and behavioural problems.

So far, the major outcome studies have focussed on long term outcome at 6 months or 1 year of diagnosis. However, there is a paucity of studies that have focussed on outcome at the completion of intensive phase of treatment. The present study was planned to find the outcome of children between 9 months and 12 years of age with TBM at discharge and at completion of intensive phase of treatment. Such a study can provide information for prognosticating patients regarding expected trajectory of recovery and also improve compliance to treatment. The present study would also enable to identify impairments sooner and initiate timely rehabilitation.

The primary objective of this study was to determine the proportion of deaths in children with TBM aged 9 months to 12 years before the completion of intensive phase of treatment. The secondary objectives of this study were to determine the predictors of mortality and morbidity in children with TBM aged 9 months to 12 years before the completion of intensive phase of treatment and to determine the motor outcome, cognitive outcome, vision impairment and hearing impairment of children with TBM aged 9 months to 12 years at completion of intensive phase of treatment.

2. Methods

Venue of study: Department of Paediatrics, Safdarjung Hospital, New Delhi.

Type of study: Prospective cohort observational study.

Duration: September 2020 to March 2022.

Patients: Consecutive patients admitted in the Paediatric wards of Safdarjung hospital during the course of the study with ages between 9 months and 12 years with a diagnosis of TBM were included while patients with co-existence of major cardiac anomaly/chronic liver disease/chronic renal failure/malignancy were excluded.

A set of clinical findings typical for meningitis were used as the entry criteria. Diagnosis of TBM was made in accordance with the standardized clinical case definitions mentioned by **Marais et al**[3] as depicted below.

2.1. *Diagnostic Classification of CNS TB patients: MARAIS et al*[4] *CRITERIA*

- CSF AFB or CSF CBNAAT Positive: **Definite TBM**
- Diagnostic ≥12 score (when imaging available) or Diagnostic score ≥10 (when imaging is not available): **Probable TBM**
- Diagnostic score is 6–11 (when imaging available) or Diagnostic score is 6–9 (when imaging is not available): **Possible TBM**
- Alternative diagnosis established, without a definitive diagnosis of tuberculous meningitis or other convincing signs of dual disease: **Not TBM**

2.1.1. *Diagnostic score*

(I) Clinical criteria (Maximum Category score = 6)
- Symptom duration of more than 5 days (score = 4)
- Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children), night sweats, or persistent cough for more than 2 weeks (score = 2)
- History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age) (score = 2)
- Focal neurological deficit (excluding cranial nerve palsies) (score = 1)
- Cranial nerve palsy (score = 1)
- Altered consciousness (score = 2)

(II) **Cerebral imaging criteria (Maximum category score = 6)**
- Hydrocephalus (CT and/or MRI) (score = 1)
- Basal meningeal enhancement (CT and/or MRI) (score = 2)
- Tuberculoma (CT and/or MRI) (score = 2)
- Infarct (CT and/or MRI) (score = 1)
- Precontrast basal hyperdensity (CT) (score = 2)

(III) CSF criteria (Maximum category score = 4)
- Clear appearance (score = 1)
- Cells: 10–500 per μl (score = 1)
- Lymphocytic predominance (>50%) (score = 1)
- Protein concentration greater than 1 g/L (score = 1)
- CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2·2mmol/L (40 mg/dL) (score = 1)

(IV) Evidence of tuberculosis elsewhere (Maximum category score = 6)
- Chest radiograph suggestive of active tuberculosis: signs of tuberculosis = 2; miliary tuberculosis = 4 (score = 2/4)
- CT/MRI/ultrasound evidence for tuberculosis outside the CNS (score = 2)
- AFB identified or *Mycobacterium tuberculosis* cultured from another source—i.e., sputum, lymph node, gastric washing, urine, blood culture (score = 4)

- Positive commercial M tuberculosis NAAT from extra-neural specimen (score = 4)

2.1.2. Exclusion of alternative diagnoses

- An alternative diagnosis must be confirmed microbiologically (by stain or culture when appropriate), serologically (eg, syphilis), or histopathologically (e.g., lymphoma).
- The list of alternative diagnoses that should be considered, dependent upon age, immune status, and geographical region, include: pyogenic bacterial meningitis, cryptococcal meningitis, syphilitic meningitis, viral meningoencephalitis, cerebral malaria, parasitic or eosinophilic meningitis (Angiostrongyluscantonesis, Gnathostomaspinigerum, toxocariasis, cysticercosis), cerebral toxoplasmosis and bacterial brain abscess (space-occupying lesion on cerebral imaging)and malignancy (eg, lymphoma)
- TST = tuberculin skin test. IGRA = interferon-gamma release assay. NAAT = nucleic acid amplification test. AFB = acid-fast bacilli.

The demographic data of these patients was recorded along with other clinical, epidemiological, CSF and radiological parameters. Staging of the disease was done at presentation using the refined MRC scale. Assessment of neurological status was also done at admission using the Glasgow coma scale (GCS) and the Blantyre coma scale (BCS). BCS was used in children below three years of age while GCS was used in older children (aged 3 years or above).

All children received standard anti-tubercular therapy (ATT) as recommended by.

DOTS: Isoniazid (10 mg/kg/day), Rifampicin (10 mg/kg/day), Pyrazinamide (25 mg/kg/day), and Ethambutol (20 mg/kg/day) for 2 months of intensive phase followed by 10 months of continuation phase with Isoniazid (10 mg/kg/day), Rifampicin (10 mg/kg/day) and Ethambutol (20 mg/kg/day) along with steroids for 6–8 weeks.

Other supportive measures (anticonvulsants, mannitol, acetazolamide or VP shunt) were given as per clinical requirement. The patients were followed till discharge/death.

2.1.3. Details of participant evaluation procedures

1) **Paediatric MMSE (Jain et al):** It is a screening test used in children more than 3 years of age to assess cognitive dysfunction. It may be used to pick up early encephalopathy and to check the progression of neurological diseases. It also gives a standardized score, which can be used to compare two groups when a particular therapy is being tried.[5]

A good correlation was found between the paediatric MMSE and Glasgow coma scale using Pearson's correlation coefficient. In paediatric MMSE cut off scores 2 SD below mean for different ages can be used as an indicator of early cognitive dysfunction. The average time to administer the test is nearly 6 minutes which is comparable with 5–10 minutes required for the adult MMSE.[5] In our study, we used paediatric MMSE to assess cognitive dysfunction in children more than 3 years of age with GCS 15/15.

2) **Blantyre Coma Scale (BCS):** The **Blantyre coma scale** is a modification of the Paediatric Glasgow Coma Scale and is a measure of the state of consciousness in children. The score assigned by the Blantyre coma scale is from 0 to 5. The minimum score is 0 which indicates poor results while the maximum score is 5 which is suggestive of good results. Scores are determined by adding the results from three groups: Motor response, verbal response, and eye movement. Best responses in each of the three categories are noted and the scores are added together as mentioned below.

VERBAL	MOTOR	EYE
0: no cry	0: non specific or no response	0: not directed
1: inappropriate cry or moan	1: withdrawal from pain	1: directed eye movements
2: appropriate cry	2: localises pain	

3) **The Vineland Social Maturity Scale (VSMS) (Malin AJ et al):** This scale is a measure social competencies of an individual. VSMS can be used for the age group of 0–15 years. It measures Social Age (SA) and Social Quotient (SQ). It was found that social quotient shows high correlation (0.80) with intelligence.[6]

The scale consists of 89 test items grouped into year levels. The examiner gains information on VSMS test items regarding child's abilities through direct observation and may supplement it by questioning the mother/guardian.

The child's responses are noted on a record sheet. If the child is able to perform an item correctly, it is marked pass and fail if otherwise. Half credits are given if it can be presumed that the child could have passed the item if the opportunity was present. These half credits receive full credit if they lie between two passed items. Passed scores (full and half) are added up.

Find out the Social Age (SA) from VSMS manual based on the total score as shown below. Social Quotient (SQ) is then calculated by dividing Social Age by Chronological Age and multiplying by 100.

VSMS TEST ITEMS ACCORDING TO AGE

S.No.	Test Items	S.No.	Test Items
0-1 Year		30.	Discriminates edible substances from non-edibles
1.	"Crows", Laugh	31.	Uses names of familiar objects
2.	Balance head	32.	Walks upstairs unassisted
3.	Grasps objects within reach	33.	Unwraps sweets, chocolates
4.	Reaches for familiar persons	34.	Talks in short sentences
5.	Rolls over, (unassisted)	**2-3 Years**	
6.	Reaches for nearby objects	35.	Signals to go to toilet
7.	Occupies self-upright	36.	Initiates own play activities
8.	Sits unsupported	37.	Removes shirt or frock if unbuttoned
9.	Pulls self-upright	38.	Eats with spoon/hands (food)
10.	"Talks", imitates sounds	39.	Gets drink (water) unassisted
11.	Drinks from cup or glass assisted	40.	Dries own hands
12.	Moves about on floor (creeping, crawling)	41.	Avoids simple hazards
13.	Grasps with thumb and finger	42.	Puts on short or frock unassisted (need not button)
14.	Demands personal attention	43.	Can do paper folding
15.	Stands alone	44.	Relates experience
16.	Does not drool	**3-4 Years**	
17.	Follows simple instructions	45.	Walks downstairs, one step at a time
1-2 Years		46.	Plays cooperatively at kindergarten level
18.	Walks about room unattended	47.	Buttons shirt or frock
19.	Marks with pencil or crayon or chalk	48.	Helps at little household tasks
20.	Masticates (chews) solid or semi-solid food	49.	"Performs" for others
21.	Pulls off clothes	50.	Washes hands unaided
22.	Transfers objects	**4-5 Years**	
23.	Overcomes simple obstacles	51.	Cares for self at toilet
24.	Fetches or carries familiar objects	52.	Washes face unassisted
25.	Drinks from cup or glass	53.	Goes about neighborhood unattended
26.	Walks without support	54.	Dresses self except for trying
27.	Plays with other children	55.	Uses pencil or crayon or chalk for drawing
28.	Eats with own hands (biscuits, bread, etc.)	56.	Plays competitive exercise games
29.	Goes about hours or yard		

2.2. Outcome measures

Primary outcome under evaluation was mortality. Demographic, clinical, CSF, radiological and all other parameters were evaluated to look for predictors of morbidity and mortality.

Disability was defined as impairment in any of the following domains: motor, cognitive, hearing and vision domain. Vision, hearing, motor and cognitive function assessment was done under secondary outcome evaluation at the end of intensive phase of treatment using standardised tools. **Statistical analysis** was done using the latest version of Statistical Package for Social Sciences, Windows, licensed version 21.0 SPSS.

Details of Secondary outcome measures:

a) The **extent of cognitive dysfunction** was gauged using the **Paediatric Mini Mental State Examination**[5] in children more than 3 years of age with GCS 15/15, where score <10 will be taken as poor outcome and **Blantyre coma scale** wherever GCS was less than 15 or age less than 3 years, where score <2 was considered as a poor outcome and **Vineland Social Maturity Scale (VSMS)**[6] where cognitive impairment was defined as Social Quotient <70 as per the Indian version of VSMS. Other scales (Paediatric MMSE and BCS) were measured and their numerical value was used for quantification.

b) The **level of motor impairment** was judged using the **Gross Motor Function Classification System (GMFCS)**[4]. The GMFCS is an objective tool to classify child's current gross motor function. On this scale, function is divided into five levels: Level I to level V. Children in Level I have the most independent motor function and children in Level V have the least. In this study, GMFCS level III to V was considered as a measure of poor outcome.

c) **Vision impairment** was defined as **Teller's visual** acuity <2 lines below expected for age in children between 9 months and 3 years of age and **Snellen's visual** acuity <2 lines below expected for age in children above 3 years of age. The Teller's acuity card procedure is a quantitative measure for assessing visual acuity used in infants, toddlers and nonverbal children.[7]

d) **Hearing impairment** was defined as failed diagnostic auditory brainstem responses (ABR).

VSMS TEST ITEMS ACCORDING TO AGE

S.No.	Test Items	S.No.	Test Items
5-6 Year		**9-10 Years**	
57.	Uses hoops. flies kites. or uses knife	75.	Cares for self at meals
58.	Prints (writes) simple words	76.	Makes minor purchase
59.	Plays simple games which	77.	Goes about home town freely
60.	require talking turns Is trusted with money	**10-11 Years**	
61.	Goes to school unattended	78.	Distinguishes between friends any playmates
6-7 Year		79.	Makes independent choice of shops
62.	Mixed rice "properly unassisted	80.	Does small remunerative work. makes articles
63.	Uses pencil or chalk for waiting	81.	Follows local current events
64.	Bathes self assisted	**11-12 Years**	
65.	Goes to bed unassisted	82	Does simple creative work
7-8 Years		83.	Is left to care for self or others
66.	Can differentiate between AM & PM	84.	Enjoys reading books, newspapers and magazines
67.	Helps himself during meals	**12-15 Years**	
68.	Understands and keeps family secrets	85.	Plays difficult games
69.	Participants in pre-adole- scent	86.	Exercises complete care of dress
70.	Combs or brushes hair	87.	Buys own clothing accessories
8-9 Years		88.	Engages of adolescent group activities
71.	Uses tools or utensils	89.	Performs responsible routine chores
72.	Does routine household tasks		
73.	Reads on own initiative		
74.	Bathes self unaided		

The flowchart of the study is described below in Fig. 1.

3. Results

Eighty patients with TBM were enrolled in this study. Diagnosis of the participants was made using the Marais criteria. 20.0% of the participants were diagnosed as Definite TBM. Majority (52.5%) of the participants were diagnosed as Probable TBM and the remaining 27.5% as Possible TBM as shown in Fig. 2.

Participants were staged using the refined MRC scale. 62.5% of the participants were identified as Stage III TBM. 16.2% of the participants had stage IIA and 12.5% of the participants had Stage IIB, while the remaining 8.8% of the participants were identified as Stage I as shown in Fig. 3.

The mean age of presentation was 5.91 years. Males were commonly affected than females. The demographic profile of the patients have been highlighted in Table 1.

On clinical history, majority of the participants had an acute mode of onset. Nearly 75% received treatment within a month of onset of symptoms but 26.2% of the participants had a delay of more than 1 month. The other prominent findings in history have been recorded in Table 2.

The patients in the present study had variable clinical presentations, the most common being fever, which was present in 69/80 (86.2%) of the participants. 66/80 of the participants had signs of raised ICT.12/80 patients also showed features of hypothalamic involvement, such as anorexia, persistent fever or SIADH. The other significant presentations have been shown in Table 3.

Out of the 80 patients who were evaluated in our study, 25/80 (31.25%) of the participants had associated TB. The most commonly associated TB was found to be Miliary TB, found in 14 (17.5%) patients. Other types of associated TB were pulmonary TB, abdominal TB and lymph node TB, respectively in decreasing order of frequency.

26/80 (32.5%) of the participants had presence of Focal Deficits. Type of focal deficit with their occurrence has been highlighted in Table 4. More than one focal deficit was also found in some patients.

In this study, on clinical examination, there was an almost equal distribution of patients with low BCS (<2) and those with good BCS (>2) at admission, in the ratio of 11:12. BCS was primarily used for children <3 years of age. The mean BCS ±SD at admission was 2.96 ± 1.30 as depicted in Table 5.

Whereas, GCS was primarily used for children >3 years of age, the mean GCS ±SD at Admission was 11.31 ± 2.90. 12.1% of the participants had poor GCS at admission (GCS <8), whereas majority had GCS more than equal to 8, as depicted in Table 5.

In the present study, abnormal CSF findings were reported in all the patients. Majority of the patients had a cellular CSF. These results demonstrated lymphocytic predominance in CSF (>50% lymphocytes) in 73.8% of the participants along with raised CSF protein (>100 mg/dL) present in 75.0% and low CSF glucose (<40mg/dL) in 58.8% of the total participants. 16/80 (20.0%) of the participants had positive result for CSF CBNAAT. The findings have been summarised in Table 6.

CONVERSION OF VSMS TOTAL SCORES INTO SOCIAL AGE

SCORE	SA	SCORE	SA	SCORE	SA	SCORE	SA
1	0.06	**21**	1.24	**44**	3	**67**	7.4
1.5	0.09	**22**	1.30	**45**	3.2	**68**	7.6
2	0.12	**23**	1.35	**46**	3.3	**69**	7.8
2.5	0.15	**24**	1.41	**47**	3.5	**70**	8.0
3	0.18	**25**	1.47	**48**	3.7	**71**	8.3
3.5	0.21	**26**	1.53	**49**	3.7	**72**	8.5
4	0.24	**27**	1.59	**50**	4.0	**73**	8.8
5	0.30	**28**	1.65	**51**	4.2	**74**	9.0
6	0.35	**29**	1.71	**52**	4.3	**75**	9.3
7	0.41	**30**	1.77	**53**	4.5	**76**	9.7
8	0.47	**31**	1.83	**54**	4.7	**77**	10.0
9	0.53	**32**	1.89	**55**	4.8	**78**	10.3
10	0.59	**33**	1.94	**56**	5.0	**79**	10.5
11	0.65	**34**	2.00	**57**	5.2	**80**	10.8
12	0.71	**35**	2.1	**58**	5.4	**81**	11.0
13	0.77	**36**	2.2	**59**	5.6	**82**	11.3
14	0.83	**37**	2.3	**60**	5.8	**83**	11.7
15	0.89	**38**	2.4	**61**	6.0	**84**	12.0
16	0.94	**39**	2.5	**62**	6.3	**85**	12.6
17	1.00	**40**	2.6	**63**	6.3	**86**	13.2
18	1.06	**41**	2.7	**64**	6.8	**87**	13.8
19	1.12	**42**	2.8	**65**	7.0	**88**	14.4
20	1.18	**43**	2.9	**66**	7.2	**89**	15.0

Mantoux was found positive in 66.2% of the participants. 57/80 of the participants had an elevated WBC count (TLC >11,000). An elevated ESR count was found in 93.8% of the participants. 82.5% of the participants had a normal Chest X-ray. Abnormalities were detected in only 14/80 17.5% patients with 4 of those participants showing opacity/consolidation and the rest 10 showing the presence of miliary pattern. 8.8% of the participants also had positive results for gastric aspirate AFB/CBNAAT.

Neuroimaging was done in 75 out of 80 participants. An abnormal neuroimaging was reported in all the scanned patients.

The most common finding was hydrocephalus, established in 59/75 of the participants. The other commonly encountered findings have been demonstrated in Fig. 4 as shown below. Most of the patients had multiple findings.

Primary outcome evaluation was done at the end of hospital stay. A mortality rate of 42.5% and a survival rate of 57.5% was reported as shown in Fig. 5. On univariate analysis, the following variables were significantly associated ($p < 0.05$) with the mortality as shown in Table 7.

On multivariate logistics regression the presence of raised ICT ($p = 0.040$) and stage III disease ($p = 0.005$) had a significant association with mortality as shown in Table 8.

Evaluation for secondary outcomes was done at the completion of intensive phase of treatment. Complete recovery was reported in 20% of the participants and survival with disability was reported in 36.25% of the total participants. Survival with disability was considered as a measure of morbidity. On univariate analysis, the following variables were significantly associated ($p < 0.05$) with morbidity as shown in Table 9 below.

Detailed assessment for disability was done using standardised scales as mentioned. A poor motor outcome was reported in 33.3% of the survivors. On univariate analysis, the following variables were significantly associated ($p < 0.05$)

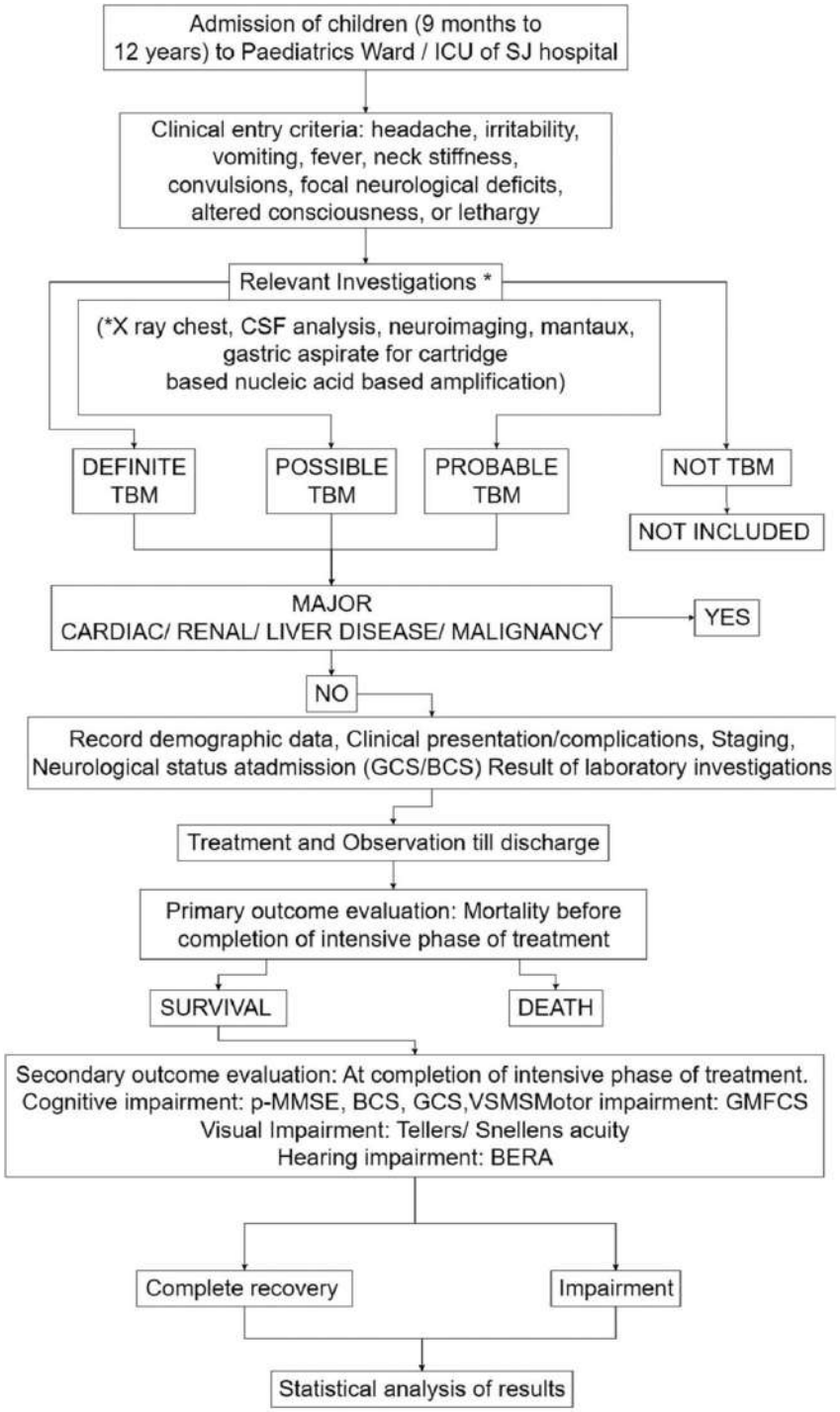

Fig. 1 – Flowchart of study.

with motor impairment: Presence of convulsions, acute mode of onset, CSF pleocytosis, CSF lymphocytic predominance, CSF CBNAAT positive status, presence of infarct on neuroimaging, focal deficit and low GCS score ($p < 0.05$).

A poor cognitive outcome in 33.3% of the survivors. On univariate analysis, the following variables were significantly associated ($p < 0.05$) with cognitive impairment: Presence of convulsions, presence of altered sensorium, CSF CBNAAT positivity, low GCS at admission, presence of infarct, absence of leptomeningeal enhancement on neuroimaging and Stage III disease.

A poor hearing outcome was reported in 4% of the survivors. On univariate analysis, a significant association was found between hearing impairment and the presence of cranial nerve palsies.

A poor vision outcome was reported in 48.9% of the survivors. On univariate analysis, the following variables were significantly associated ($p < 0.05$) with vision

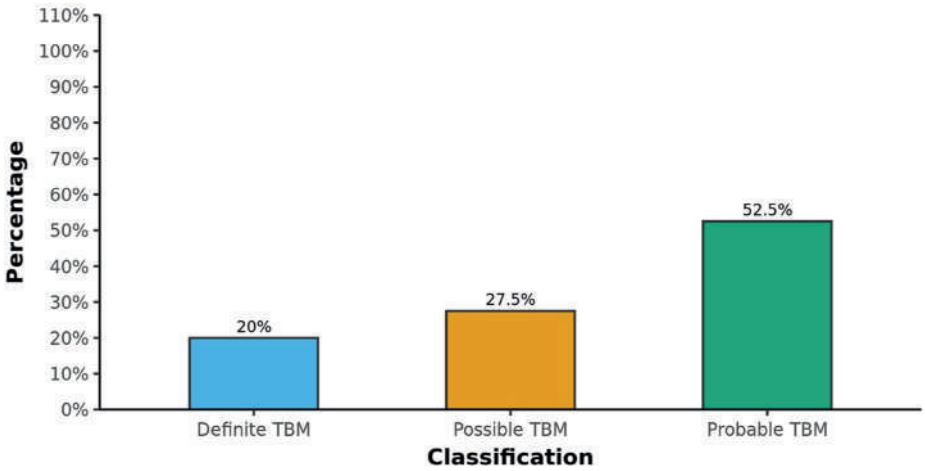

Fig. 2 – Diagnostic classification of study cohort (n = 80).

impairment: meningeal irritation (p = 0.022) and Low GCS (p = 0.046).

4. Discussion

This prospective cohort observational study examines the predictors of morbidity and mortality in children with tuberculous meningitis (TBM).

One of the most significant predictors of morbidity and mortality was Stage 3 disease on univariate and multivariate analysis. These findings were consistent with other studies which demonstrated a significant association between TBM Stage 3 and mortality.[8–10]

On univariate and multivariate logistics regression analysis, raised ICT was also found to have a significant association with mortality. Similar observation was made by another prospective study.[8]

Other predictors were also identified. Altered consciousness, was found to be significantly associated with mortality (p = 0.032) and morbidity (p = 0.010) on univariate analysis. These findings were consistent with other studies that reported a significant association between altered consciousness and mortality.[9–11]

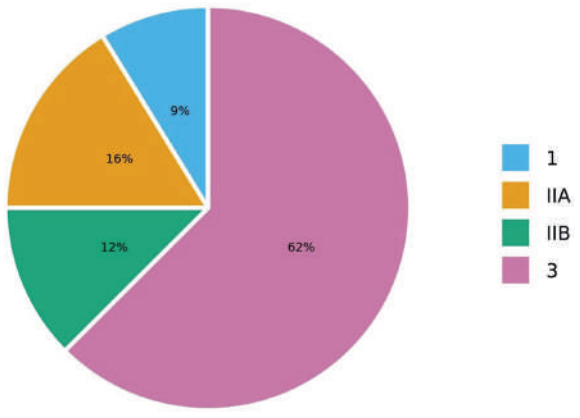

Fig. 3 – Distribution of stage in study cohort (n = 80).

Low BCS (≤2) and low GCS (<8) were also found to be statistically associated with morbidity and mortality on univariate analysis. Various studies have reported similar findings. Bang et al did a prospective descriptive study, where low BCS (i.e. BCS score 1) was demonstrated to be significantly associated with death and disability.[10] Israni et al also found a significant association between low GCS (score <7) and mortality.[8]

Low GCS has also been found to be a significant predictor of poor outcome (death/disability) in multiple other studies done.[8,11–14]

In the present study, the results showed an abnormal CSF finding in all the patients. On univariate analysis, a significant association was found between CSF pleocytosis and lymphocytic predominance with mortality. CSF pleocytosis has also been previously associated with mortality in a study done by Israni et al.[8] Some studies have also reported a significant association between raised CSF protein and morbidity.[9,10,15]

Hydrocephalus was another predictor of mortality in the present study. This result was comparable to other studies

Table 1 – Basic Demographic Profile of study participants (N = 80).

Basic Details	Frequency (%) (n = 80)
Age	
Mean ± SD (years)	5.91 ± 3.62
Range (years)	0.75–12.00
Gender	
Male	42 (52.5%)
Female	38 (47.5%)
Socioeconomic Status	
Lower	49 (61.3%)
Middle Lower	22 (27.5%)
Upper Lower	9 (11.2%)
Weight	
Mean ± SD (kg)	17.12 ± 8.09
Range (kg)	3.50–38.00
Head Circumference	
Mean ± SD (cm)	47.79 ± 3.82
Range (cm)	37.00–56.00

Table 2 – Clinical History of study participants (N = 80).

Clinical history	Frequency (%) (n = 80)
BCG Vaccination given	60 (75.0%)
TB Contact present	32 (40.0%)
Mode of Onset	
Insidious	34 (42.5%)
Acute	46 (57.5%)
Complain Duration	
<7 Days	24 (30.0%)
7–30 Days	48 (60.0%)
>30 Days	8 (10.0%)
Time Lag for Treatment	
<15 Days	28 (35.0%)
15–30 Days	31 (38.8%)
>30 Days	21 (26.2%)

which reported significant association between hydrocephalus and disability.[11,15]

However, in the present study, no significant association could be established between TBM Diagnostic Classification with morbidity or mortality which is in contrast to few of the other previously reported studies. A study done by Jin Et Al found that patients with Definite TBM had a poor outcome and their mortality was significantly higher than in Possible TBM patients (42.4% vs. 17.8%, $p < 0.05$).[11]

The rate of mortality in our study was significantly higher as compared to the other prospective Indian studies. A study done by Israni et al showed a mortality rate of 24.3%,[8] another study done by Karande Et al showed a mortality rate of 23%.[15] Various other studies have shown mortality rates ranging from 8% to 38%.[10,16] The high rate of mortality in the present study can be explained due to referral bias, this study was done in a tertiary level referral hospital where extremely sick and non-salvageable patients were referred from all over the country. Majority of our patients had stage 3 disease, thus increasing the chances of poor outcome.

In the present study, 45/46 survivors were followed up and were assessed for the extent of motor dysfunction, cognitive dysfunction, vision and hearing impairment.

In the present study, 16/80 (20%) of the participants had complete recovery, while 29/80 (36.25%) of the total survived With Disability. A prospective Indian study which evaluated the outcome to TBM patients showed that 20% of the children recovered completely while 23% survived with disability.[15]

Table 3 – Clinical Presentation of study participants (n = 80).

Presentation	Frequency (%) N = 80
Fever	69 (86.2%)
Headache	47 (58.8%)
Vomiting	45 (56.2%)
Convulsions	55 (68.8%)
Altered Consciousness	43 (53.8%)
Personality Changes	4 (5.0%)
Other Symptoms	25 (31.2%)
Co-Morbidity/Co-Infection	9 (11.2%)
Meningeal Irritation	63 (78.8%)
Associated TB	25 (31.2%)
Focal deficit	26 (32.5%)

Table 4 – Type of Focal Deficit in study participants (n = 80).

Type Of Focal Deficit	Frequency (%) n = 80
No Deficit	54 (67.5%)
Hemiparesis	5 (6.2%)
Quadriparesis	4 (5.0%)
Hemiplegia	3 (3.8%)
Cranial Nerve palsy	17 (21.2%)
Aphasia	1 (1.2%)

In the present study, the findings showed a good motor outcome in 66.7% of the survivors. A retrospective Indonesian cohort study done enrolled 139 TBM patients, out of which only 29 could be followed up and motor functions were assessed in these children using the GMFSCS. The results of their study demonstrated a good motor outcome in 65.5% of the patients. This data was consistent with the findings of our study.[4]

Cognitive dysfunction was assessed using the pediatric MMSE scale, BCS score and VSMS scale.

According to the study done by Jain et al, a score less than 10 on pediatric MMSE scale in children with encephalopathy predicted poor cognitive outcome with a sensitivity of 35% and specificity of 100%.[5] However, an abnormal cutoff score <2 SD below mean for age was also established for children in different age groups which suggested a cognition lower than expected. On using the given cut off scores by Jain et al to the present cohort, 26.5% of the participants had an abnormal score. We realized that this value could have been a better predictor for assessing cognitive outcome.

Very limited studies have been done previously for establishing the effect of TBM on cognitive function. One such retrospective study was done by Schoeman et al where they reported a median cognitive development of 69.9% (95% CI = 59.1%–73.2%). They also found a significant association between cognitive impairment and stage of TBM with a median difference of 28.7% between stage 2 and stage 3 TBM ($p = 0.02$).[17]

Another retrospective study done by Nataprawira et al evaluated mental development outcome on 29 children with TBM using Wechsler intelligence scale for children (WISCIII) more than 8 years of age and Griffiths general DQ for children less than 8 years of age. Their study revealed mental impairment in around 78% of the patients.[18]

Table 5 – Neurological status of study participants at admission (n = 80).

Findings	Mean ± SD	Median (IQR)	Min-Max
		Frequency (%)	
BCS at Admission	2.96 ± 1.30	3.00 (2.00–4.00)	1.00–5.00
BCS Category at Admission			
<2		11 (47.8%)	
>2		12 (52.2%)	
GCS at Admission	11.31 ± 2.90	12.00 (9.00–13.75)	5.00–15.00
GCS Category at Admission			
<8		7 (12.1%)	
8 to 12		30 (51.7%)	
>12		21 (36.2%)	

Table 6 – CSF Studies in study participants (n = 80).

CSF Cytology	Mean ± SD || Median (IQR) || Min-Max || Frequency (%) (n = 80)
Number of Cells	80.84 ± 75.64 || 67.50 (11.50–130.00) || 0.00–320.00
Cells	
<10	18 (22.5%)
10-100	38 (47.5%)
>100	24 (30.0%)
% Lymphocytes	61.10 ± 36.92 || 71.00 (43.75–90.50) || 0.00–100.00
Lymphocytes	
<50%	21 (26.2%)
>50%	59 (73.8%)
CSF Biochemistry	**Mean ± SD || Median (IQR) || Min-Max || Frequency (%)**
Protein (mg/dL)	169.98 ± 150.36 || 142.00 (99.50–180.75) || 5.00 –1179.00
Protein	
<100 mg/dL	20 (25.0%)
>100 mg/dL	60 (75.0%)
Glucose (mg/dL)	44.35 ± 22.66 || 41.50 (24.00–60.00) || 10.00–109.00
Glucose	
<40 mg/dL	33 (41.2%)
>40 mg/dL	47 (58.8%)
	Frequency (%)
CSF: Culture	
Sterile	77 (96.2%)
Contaminants	3 (3.8%)
CSF AFB (Positive)	3 (3.8%)
CSF CBNAAT (Positive)	16 (20.0%)

In the present study, Social Quotient was studied as another indicator for cognitive impairment and was assessed using the Vineland Social Maturity Scale (VSMS).

Another prospective study was done by Wait Et al compared behaviour profiles of Stage II and Stage III patients.

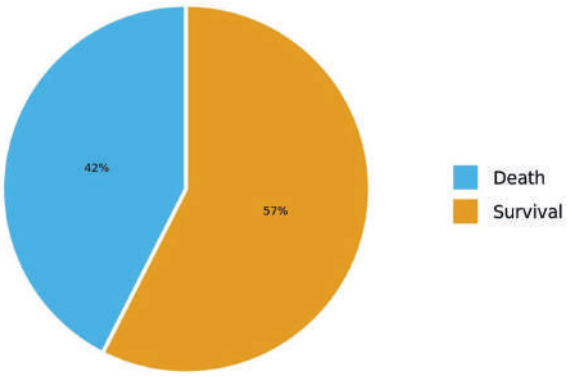

Fig. 5 – Distribution of primary outcome of study cohort (n = 80).

The results showed social problems score in clinical range in 20.6% of TBM patients and a significant difference was found with regard to social problems between the patients of stage II and stage III disease ($p < 0.01$).[19]

In the present study, 43/45 (95.6%) of the participants had passed the BERA test, while 2 (4.4%) of the participants failed the test, suggestive of hearing impairment. These results were in contrast to the previous retrospective studies where hearing loss was reported in 39% of the participants.[18]

In the present study, a significant association was found between hearing impairment and the presence of cranial nerve palsies.

TBM has been discussed as an important etiological cause of SNHL in a few case reports.[20,21] The theoretical association of cranial nerve palsies was statistically proven in our study.

In the present study, Vision assessment was done using Tellers and Snellens visual acuity cards.[23] The results of our study showed that 48.9% participants had Vision impairment.

Another prospective study done by Sinha et al in Lucknow, India assessed vision status of TBM survivors after 6 months

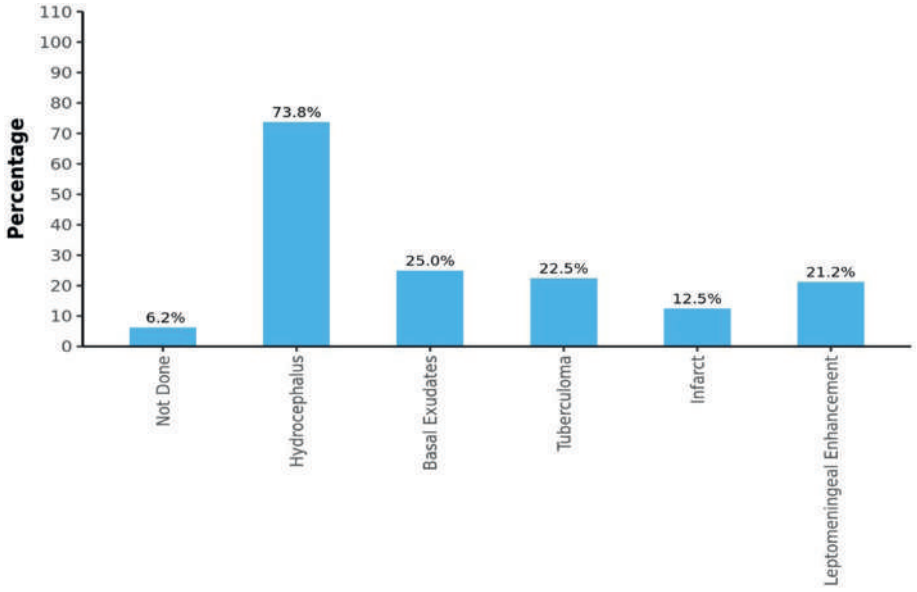

Fig. 4 – Neuroimaging findings in the study cohort (n = 80).

Table 7 – Association between Primary outcome (Death/Survival) and various parameters: Predictors of Mortality.

Parameters	Outcome		p value
	Death (n = 34)	Survival (n = 46)	
Age (Years)[a]	4.65 ± 3.28	6.85 ± 3.62	0.008[1]
Altered Consciousness (present)[a]	23 (67.6%)	20 (43.5%)	0.0322
CSF Cytology: Number of Cells[a]	95.71 ± 64.46	69.85 ± 81.90	0.0281
CSF Cytology: Lymphocytes[a]			0.0442
<50%	5 (14.7%)	16 (34.8%)	
>50%	29 (85.3%)	30 (65.2%)	
Neuroimaging Findings: Hydrocephalus (Present)[a]	29 (85.3%)	30 (65.2%)	0.0442
BCS at Admission[a]	2.25 ± 1.06	3.73 ± 1.10	0.0061
BCS Category at Admission[a]			0.0062
<2	9 (75.0%)	2 (18.2%)	
>2	3 (25.0%)	9 (81.8%)	
GCS at Admission[a]	8.74 ± 2.14	13.00 ± 1.94	<0.0011
GCS Category at Admission[a]			<0.0014
<8	7 (30.4%)	0 (0.0%)	
8 to 12	16 (69.6%)	14 (40.0%)	
>12	0 (0.0%)	21 (60.0%)	
Raised ICT (present)[a]	32 (94.1%)	34 (73.9%)	0.0192
Stage[a]			<0.001[5]
1	0 (0.0%)	7 (15.2%)	
IIA	0 (0.0%)	13 (28.3%)	
IIB	5 (14.7%)	5 (10.9%)	
3	29 (85.3%)	21 (45.7%)	
Onset of Oral Feeds (Days)[a]	0.15 ± 0.61	12.04 ± 14.14	<0.0011

[a] Significant at p < 0.05, 1: Wilcoxon-Mann-Whitney U Test, 2: Chi-Squared Test, 3: t-test, 4: Fisher's Exact Test.

Table 8 – Multivariate logistics Regression for outcome: MORTALITY

Dependent: Outcome		Survival	Death	OR (univariable) (95% CI, p value)	OR (multivariable) (95% CI, p value)
Raised ICT	Present	34 (51.5)	32 (48.5)	5.65 (1.17–27.22, p = 0.031)	6.00 (1.09–33.14, p = 0.040)
Stage	I/IIA/IIB	25 (83.3)	5 (16.7)	–	–
	III	21 (42.0)	29 (58.0)	6.90 (2.27–21.00, p = 0.001)	5.37 (1.65–17.49, p = 0.005)

Multivariate analysis by bidirectional stepwise selection was done for variables found to be significantly associated with outcome on univariate analysis.
MODEL FIT: $\chi^2(4) = 23.26$, p = <0.001 Pseudo-R^2 = 0.21, Number in dataframe = 80, Number in model = 80, Missing = 0, AIC = 95.8, C-statistic = 0.79, H&L = Chi-sq(8) 2.60 (p = 0.957).

Table 9 – Association between Type of Recovery and Parameters: Predictors of morbidity (n = 45).

Parameters	Recovery (n = 45)		p value
	With Disability (n = 29)	Complete (n = 16)	
Altered Consciousness (Present)[a]	17 (58.6%)	3 (18.8%)	0.010[2]
Co-Morbidity/Co-Infection (present)[a]	1 (3.4%)	4 (25.0%)	0.047[3]
GCS at Admission[a]	12.29 ± 2.03	14.00 ± 1.22	0.0104
GCS Category at Admission[a]			0.0162
<8	0 (0.0%)	0 (0.0%)	
8 to 12	12 (57.1%)	2 (15.4%)	
>12	9 (42.9%)	11 (84.6%)	
Type Of Focal Deficit: Cranial Nerve palsy (present)[a]	10 (34.5%)	0 (0.0%)	0.0083
Stage[a]			0.0023
1	2 (6.9%)	5 (31.2%)	
IIA	6 (20.7%)	7 (43.8%)	
IIB	2 (6.9%)	2 (12.5%)	
3	19 (65.5%)	2 (12.5%)	
Onset of NG Feeds (Days)[a]	9.00 ± 9.87	2.88 ± 3.63	0.0024
Onset of Oral Feeds (Days)[a]	15.86 ± 15.85	5.75 ± 7.33	0.0184
Duration of Hospital Stay (Days)	27.93 ± 23.50	15.94 ± 7.08	0.0524

[a] Significant at p < 0.05, 1: t-test, 2: Chi-Squared Test, 3: Fisher's Exact Test, 4: Wilcoxon-Mann-Whitney U Test.

follow up visit. They reported papilledema, cranial nerve palsy and raised CSF protein to be significant predictors of low vision and blindness. TBM stage III was found to be an additional predictor of blindness in this study.[22]

In conclusion, TBM continues to be associated with high rates of mortality and morbidity, especially in developing nations. The present study highlighted the important predictors associated with morbidity and mortality.

5. Conclusion

TBM continues to be associated with high rates of mortality and morbidity, especially in developing nations. In the present study, a mortality rate of 42.5% and a survival rate of 57.5% was reported. Complete recovery was reported in 20% of the participants and survival with disability was reported in 36.25% of the total participants. The present study also assessed vision, hearing, motor and cognitive function in TBM survivors and evaluated the proportion of dysfunction in the above mentioned domains. Motor impairment was reported in 33.3% of the survivors, cognitive impairment in 33.3% of the survivors, hearing impairment was reported in 4% of the survivors and vision impairment in 48.9% of the survivors. Even with the advancement in scientific knowledge, diagnostic technologies and advancement in treatment facilities, a large proportion of survivors of this disease suffer devastating sequelae. Our study highlighted the important predictors associated with morbidity and mortality.

Conflicts of interest

The authors have none to declare.

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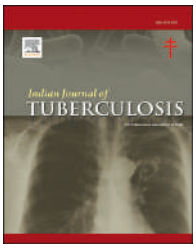

Letter to the Editor

Irrational prescription of ATT for children with abdominal symptoms: Annoying revelation from a survey

Dear Editor,

Tuberculosis (TB) is still a major public health problem in India. According to the India TB Report 2020, there were an estimated 2.69 million cases of TB in India, accounting for a quarter of all global TB cases.[1,2] The diagnosis of tuberculosis in children is extremely challenging, especially abdominal tuberculosis. The gold standard tool for the diagnosis is the demonstration of Acid-Fast-Bacilli (AFB), which is difficult to isolate from the specimen. Abdominal complaints in children, such as poor appetite, failure to gain weight and height, pain and constipation, are very common, primarily non-specific or functional. Similarly, the early stage of abdominal TB may have a similar presentation which can lead to overdiagnosis of abdominal TB. Children with such non-specific symptoms are often started on anti-tubercular therapy (ATT) by quacks or treating pediatricians/physicians without thorough investigations. Appropriate guidelines are available for treating tuberculosis, but irrational practices of ATT are still widespread in India. Many patients are erroneously diagnosed and treated for tuberculosis without confirmation because of a lack of awareness, knowledge, and the availability of ATT "over the counter" facilitates their indiscriminate use. Inappropriate use of ATT can result in life-threatening side effects, drug resistance, and increase the disease burden as well as the cost of healthcare.

We report our observation on 28 children presenting to our Pediatric Gastroenterology OPD from April 2020 to April 2022 at a tertiary referral center with abdominal symptoms and either on ATT or received ATT in the last six months. All children who had been prescribed ATT by their referring physicians were identified, and the basis of starting the treatment was assessed with their previous records and by detailed history, examination, lab parameters with or without imaging, and tissue biopsy, wherever available, were done for each case. Inappropriate therapy was defined when (1) an alternative clinical diagnosis can be attributed to the symptoms, (2) no clinical response to ATT after at least two months could be documented.

During a 2-year study period, 28 children (range, 3–17 y; 18 boys) had been prescribed ATT prior to coming to us. These children were divided into two groups.

Group 1 (n = 21): These consisted of apparently healthy children with normal anthropometry who received ATT, just based on minor abdominal signs and symptoms without detailed evaluation. Among them, subcentric (6 ± 3 mm) mesenteric lymphadenopathy on sonography (n = 15, 71%) was the most common reason, followed by functional abdominal pain (n = 4, 19%) and 1 (4.7%), each with chronic non-specific diarrhea and bleeding per rectum due to rectal polyp.

Group 2 (n = 7): This group consisted of patients with confirmed abdominal tuberculosis [lymphocytic ascites with AFB stain positive in the omental biopsy, n = 3; conglomerate mesenteric lymphadenopathy with central necrosis, n = 2; Ileocecal ulcers with biopsy suggested granulomas and Gene-X-pert positive for mycobacterium, n = 2].

Our approach to the case of abdominal tuberculosis is individualized, based on clinical evaluation and investigations, including microbiological, imaging, endoscopy, and histopathological tests. We start ATT only in those cases who have micro or pathological confirmation or where the index of suspicion is high; those with abdominal lymphadenopathy with central necrosis, conglomerated; clumped bowel loops, bowel wall thickening/stricture/ulcer, and lymphocytic or high adenosine deaminase (ADA) ascites not explained by alternate diagnosis.

Our observations indicate that the irrational use of ATT is highly prevalent in our community (3/4th). Unwarranted use of these drugs is not uncommon in India and has been prevalent for many decades, but the scenario has not changed.[3] Recurrent abdominal pain and constipation, without any red flags, are widespread problems in childhood. These symptoms are usually clubbed under functional gastrointestinal disorders, encompassing chronic conditions without serious gastrointestinal or intra-abdominal pathology. Also, vague symptoms like poor appetite, poor weight

gain (constitutional), and over-reporting of mesenteric lymphadenopathy (insignificant) are not uncommon in developing countries like India, where worm infestations/infections are a considerable concern. Starting ATT in these children just based on subtle symptoms is not justified. We have to be more vigilant and cautious while prescribing ATT. The empirical use of ATT should not be equated to its inappropriate use. However, unwanted ATT use is not safe because of its propensity to cause hepatotoxicity and even death by causing acute liver failure.[4,5]

To conclude, irrational prescription of ATT is highly prevalent. All efforts should be made to confirm the diagnosis before starting ATT. We as Pediatrician/Physician have this responsibility to sharpen our knowledge and be more rational and scientific with the use of antitubercular drugs.

Author's contribution

AB - collection of data, revision and provided intellectual inputs; DP - collected the data and drafted the manuscript; AV - provided intellectual inputs.

Conflicts of interest

None.

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Case report

Indolent ulcer and discharging sinus on the eyelid of healthy Indian children and an adult: Orbital tuberculosis, a spot-diagnosis

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ABSTRACT

Five cases of tuberculous osteomyelitis of the fronto-zygomatic (F-Z) region presented with a non-healing ulcer or discharging sinus in the eyelid skin in healthy children and an adult. Lack of awareness about peri-ocular manifestations of extra-pulmonary tuberculosis and delayed referral to specialists, along with poor compliance to long-term ATT, could be the reason for its underreporting in India.

1. Introduction

India has a *high burden* of tuberculosis.[1] As per the WHO's 2022 report, undiagnosed and untreated cases have been expected to be higher in India.[1] This implies more community transmission, and indolent tubercular diseases have emerged in immunocompetent or vaccinated healthy young persons. Five instances of chronic non-healing ulcers and discharge from the sinus of the eyelid, both of which were diagnosed clinically as tuberculosis, have been described by the author in this article.

2. Case description

The five out-patients described here (Table 1) share many common features, viz., young age of presentation, prior BCG vaccination, long-standing non-healing lid ulcer or sinus, previously undiagnosed F-Z sclerosis, on-and-off sticky-watery foul-smelling discharge, absence of any other ocular symptom, non-yielding microbiology of the discharge, and Mantoux reading of 15 mm or more. All were using non-specific antibiotic creams prescribed by themselves or their primary care physicians. Cervical lymph nodes were enlarged and matted in two patients (Fig. 1f–j), of whom one developed skin ulceration over the submandibular node a few days after its FNAC (Fig. 1i–j) and later confirmed acid-fast bacilli in the node biopsy. Apart from a history of recurrent fever in patients with cervical lymphadenopathy and Pott's spine, no other systemic complaint was mentioned by the patients (Fig. 1g, i, and 2). Two patients (cases 2 and 5) adhered to the ATT until complete healing. Rest three patients either deferred treatment or were lost to follow.

3. Discussion

It's not uncommon to encounter periocular tuberculosis in India, either in an outpatient department or in the literature.[3,4] However, the author believes it has been 'under-reported,' possibly due to late diagnoses or misdiagnoses.[5]

F-Z periostitis, bone thickening, sclerosis, and hyperostosis are most common manifestation of long-standing orbital tuberculosis, unlike a typical 'sequestrum-involucrum' or 'bone erosion' in 'cold abscess' or 'tuberculosis of long bones'.[6] Overlying long-standing sinus or ulcer with sloping edges sometimes heaped up, on-and-off foul-smelling non-purulent discharge, and skin scarring represent ongoing bone necrosis with simultaneous incomplete healing. All skin lesions developed just posterior to the bony orbital margin or over the posterior edge of the bony orbital margin. This could be due to the relatively thin roof (frontal plate), thin skin, scarcity of subcutaneous fat, and less bulky muscle layer of the eyelids compared to the thicker soft-tissue layer anterior to the orbital margin. This could lead to a shorter path to the nearest skin for spontaneous, naturally draining sinuses (which might appear as a skin ulcer) containing the necrosed products of underlying osteomyelitis. The dual vascular supply and watershed areas of this region could be more attractive for the aerobic bacteria.

Cicatricial lid retraction and a single lesion were seen in all cases except the first, in which mechanical ptosis and proptosis could be due

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Table 1
Case description.

Patient (figure)	Age/ Sex	BCVA	Affected eye	Discharge[a]	Duration (months)	Lid lesion (s)[b]	Adjacent skin excoriation or scarring	Lid retraction (R)/Ptosis (P)/ Proptosis (Pr)	Orbital NCCT; CXR (±)[c]	Past/treatment history	Systemic findings, BCG[d]	Microbiology[h], Mantoux (mm)	Follow up till[i]	Surgery performed after ATT
1 (1a-1c)	23/ M	6/6, 6/6	Left, UL + LC	+	36	Multiple pinhead size black holes	+	P+, Pr+	F-Z sclerosis, bone thickening; CXR-	Antibiotic ointments	Nil, V	-, 20	2 mo	-
2 (1d-1e)	12/ M	6/6, 6/6	Right, UL	+	12	Ulcer with sloping edges	+	R+	F-Z sclerosis, CXR-	Antibiotic ointments	Nil, V	-, 15	6 mo	-
3 (1f-1g)	13/F	6/6, 6/6	Left, LL + LC	+	24	Punched-out ulcer	+	R+	F-Z-M bone and soft-tissue thickening; CXR-	Antibiotic ointments	Fever[e], enlarge node[f], V	Deferred	0 mo	-
4 (1h-1j)	8/M	6/6, 6/6	Left, UL	+	18	Deep sinus	+	R+	F-Z periostitis, sclerosis; CXR-	Antibiotic ointments	Fever[e], enlarged (later ulcerated) node[g], V	-, 30 Node[g] FNAC- Node[g] Biopsy+	4 mo	-
5 (2a-2e)	4/F	6/6, 6/6	Right, UL	+	12	Slit on lid crease	+	R+	F-Z periostitis, sclerosis; CXR-	Antibiotic ointments, systemic antibiotics, Spine surgery	Fever[e], Pott's spine, V	-, 20 Spine Biopsy+	12 mo	Full thickness skin graft elsewhere

UL, Upper Lid; LC, Lateral Canthus; NCCT, non-contrast computerized tomogram.

[a] On-and-off, foul smelling, non-purulent, watery, and sticky discharge from the lesion(s).

[b] A hole, ulcer, sinus, or slit, is based solely on appearance. Pathologically, all are draining sinuses from underlined necrosed bone.

[c] CXR, chest x-rays (plain, PA view), presence (+) or absence (−) of cavitation, prominent septal markings (Kerley B lines), hilar lymphadenopathy, consolidation, fibrosis, pleural effusion, etc.

[d] BCG vaccination at birth as informed by parents or scar of the vaccination[2]; V, Vaccinated.

[e] Recurrent, low-grade fever.

[f] Left pre-auricular node.

[g] Left submandibular lymph node.

[h] Smear (Gram stain, KOH stain, Z-N stain), culture (Blood agar, Chocolate agar, L-J media), and PCR of the discharge samples to detect *M. tuberculosis*; FNAC and biopsy results (±) are based on detection of AFB, *acid-fast bacilli*.

[i] Since months (mo) after starting ATT; Case 2, parents were very reluctant for ATT, they stopped ATT in the second month, then restarted after counseling and continued for a total of six months course; Case 3, parents deferred treatment in the first visit without citing any reason; Cases 1 and 4 could not be tracked after a few months of ATT; Case 5 improved and later got operated on lid retraction at their native place.

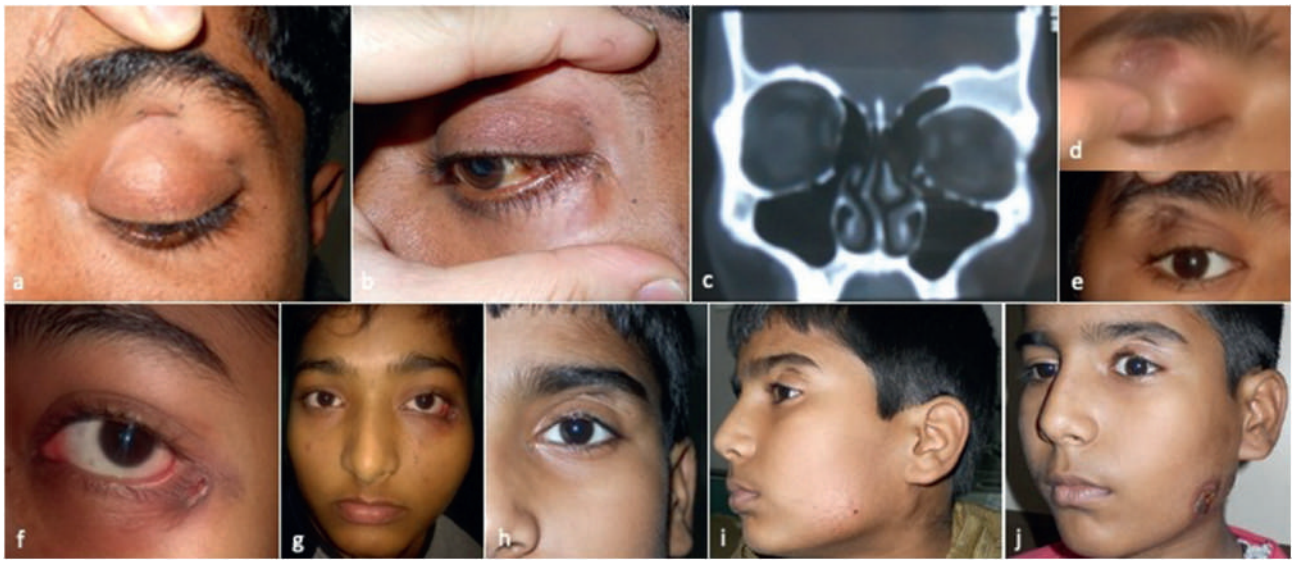

Fig. 1. (a–j): a, fullness in the superior sulcus (temporal) with adjacent multiple black pinhead size holes in different stages of healing or activity; b, skin excoriation and scarring; c, coronal NCCT, bone window, showing F-Z region bony thickening or sclerosis; d, sloping edges of ulcer with wet glistening surface; e, dry, epithelialized ulcer with pigmented scarring, after two months of ATT; f, punched-out ulcer with wet base; g, lower lid retraction with fullness over the temple; h, sinus deep to superior orbital rim, after a month of ATT; i, cervical node enlarged, blood spot indicates FNAC site; j, necrosed submandibular node.

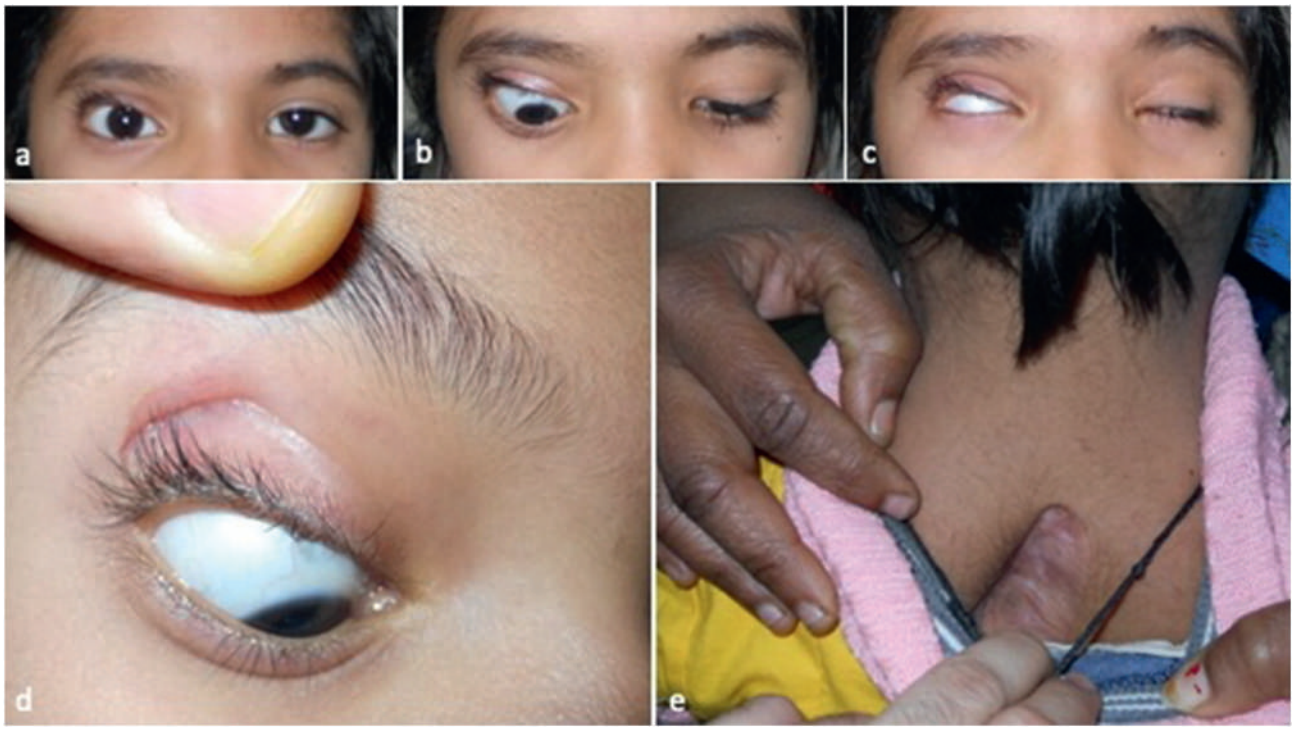

Fig. 2. (a–e): a, lid retraction; b, lid lag; c, lagophthalmos; d, deeply fixed, temporal lid crease with wet, excoriated slitted skin; e, interscapular scar of Pott's spine.

to the direct effect of bone thickening and expansion-related orbital volume decrease, respectively (Fig. 1c). In the same patient (Case 1), the black color of the lesions could be due to pigments produced by *M. tuberculosis*.[7]

M. tuberculosis is a very fastidious organism to grow on culture media, especially when inoculum volume is scanty, as in paucibacillary disease. Apart from PCR, CBNAAT (GeneXpert) or IGRA (interferon-gamma release assays, e.g., QuantiFERON-Gold) could be used if available.[2,8] Due to inconsistent results, the author does not favour diagnostic curettage or biopsy, as it might lead to aggravation of inflammation (Fig. 1f–j), poor wound healing, and more scarring; in addition, it might need to be performed under general anaesthesia. Apart from poor yield, these microbiological investigations, or biopsy procedures, might increase the burden on patients for costs and number of visits in a country where follow-up is a major issue.[1] It might be justified in cases of cold abscess, 'atypical' clinical presentation, complicated cases such as intracranial involvement with increased morbidity, or poor response to ATT. Despite being the gold standard to confirm a diagnosis, a negative microbiology and pathology do not rule out active tuberculosis in the presence of a high index of clinical

suspicion.[5] In the majority of cases, a clinico-radiological diagnosis supported by a Mantoux reading of 15 mm or more, irrespective of prior BCG, is sufficient for starting ATT with dosages as per WHO guidelines.[2,5] BCG vaccinations protect from severe disease but are not effective in preventing tuberculosis.[1] Clinical improvement is usually noticed within one to two months of starting ATT. Usually, ATT alone is curative, and skin grafting may be required to correct residual cicatricial retraction after completion of ATT.[3]

'Lost-to-follow' is the most unfavorable outcome.[1] The author has encountered various reasons off-record, such as unawareness to the ophthalmic manifestations of extra-pulmonary TB, unacceptance of the diagnosis in the absence of positive microbiology and respiratory symptoms, the relative asymptomatic and slow-progressive nature of the disease, unwillingness for invasive procedures (e.g., FNAC or biopsy), or unwillingness to take long-term medication in the presence of slow healing and social stigma.[9]

4. Conclusion

Tuberculous osteomyelitis is a commonly overlooked and potentially treatable pathology in Indian teenagers and young adults presenting with a long-standing skin ulcer or sinus of the eyelid near the orbital margin close to the F-Z region. In this case series, 'loss-to-follow' was the main reason for incomplete treatment. As a result, the author believes that all cases with strong clinic-radiological suspicion should begin ATT as soon as possible, without waiting for microbiology reports or scheduling a biopsy.

Contributions to authors

Preparation and editing of manuscript (GL); Literature search (GL); Review or approval of the manuscript (GL).

Statement about research and ethics

The research complies with the Declaration of Helsinki's ethics criteria.

Statement about conformity with author information

An informed consent was taken from the patients for publishing data including clinical photographs.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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