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REVEIW ARTICLE

Spotlights on Rapid Noninvasive Diagnostic Approaches for Pediatric Tuberculosis

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ABSTRACT

Tuberculosis (TB) is one of upmost infectious agents with a great impact on the global health services worldwide. Annually, millions of children; referred as under fifteen years of age; had TB. Pediatric TB represents about twelve percent of TB global burden and seventeen percent of all deaths by tubercle bacilli infection. Furthermore, in 2024, WHO reported that at least 30% of children with new and previously treated TB had rifampicin (RIF) resistant; that accounted the double incidence reported in 2016. Infected children act as reservoirs of M. tuberculosis *from whom, it can spread. Therefore, without successful detection and effective treatment, elimination strategies will be difficult to be achieved. It is important to highlight pediatric TB to know how far we are. In Egypt, researchers predict a static or increasing level especially with increased population growth and obvious risk factors e.g overcrowding and immunodeficiency. In 2023, WHO TB country profile estimated that the total TB incidence in Egypt was about ten per one hundred thousand population with six percent were children. Therefore, the unique vulnerabilities and different clinical presentations make the early and highly sensitive diagnostic procedures in this age, one of the most important challenges.*

INTRODUCTION

Pediatric tuberculosis (TB) is considered a great staggering problem. Co-infection of *M. tuberculosis* with other bacterial pathogens, such as *Klebsiella peunomaiae* and *Pseudomonas aeroginosa*, can worsen the severity of pediatric TB and masking proper diagnosis. In fact, childhood TB is a hidden epidemic; a great scale of tuberculous children remained undetected or not reported; and what we know is only the top of the iceberg. Risk factors for pediatricTB include the environmental conditions, living conditions, exposure to tobacco and other pollutants, neglected BCG vaccination, malnutrition and immunodeficiency.[1]

M. tuberculosis is the main causative agent in pediatric TB through airborne route. However *M. bovis* infection may occurs primarily after unpasteurized milk or dairy products ingestion; representing about one to two percent of TB childhood cases. COVID-19 pandemic has great squeal on pediatric TB by affecting the immune status and decreasing health care services available to this vulnerablegroups to target this infection. It was reported an elevated TB death rate during three years of COVID-19 pandemic.[2]

Resistant TB strains represent an obstacle for achieving control of TB disease worldwide. These strains defined as having at least rifampicin (RIF) and isoniazid (INH) resistance. While extensively TB drug resistant is resistance to INH, RIF plus any resistance to fluoroquinolones and injectable antituberculous drugs e.g kanamycin, amikacin, capreomycin. Although, Mycobacteria can be prevented and cured, pediatric TB is often neglected and unexpected by healthcare providers. Children are more susceptible to life-threatening TB disease *e.g.* TB meningitis hampering their life quality compared to any other age[3].

Rapid progression of pediatric TB from infection to disseminated disease gets a limited chance for intervention to prevent disease complications particularly in low and middle income countries. Infected TB children often have variable nonspecific clinical presentations that look likes common childhood illnesses so that they haven't advised to perform the needed diagnostic tests and radiography services. Additionally, children are more prone to have TB extrapulmonary, which in turn aggravate the variability in their clinical symptoms and sings with subsequent transmission through community. Non invasive samples like urine, stool and nasopharyngeal aspirates (NPA) are preferable in children; however these specimens have fewer mycobacteria with lower sensitivities. Clinicians should always expected childhood TB to estimate a proper clinical decision on TB evaluation[4].

On 24th March each year, World TB Day, is themed to get awareness that Mycobacteria infection is an

epidemic, causing the deaths of nearly one and a half millions people each year, mostly in developing countries. It referred to 1882 when Robert Koch had recognized tubercle bacilli. The theme of World TB Day 2024 called 'Yes! We can end TB!' carrying a hope message of possibility for complete eradication against the TB epidemic[5].

Pathogenesis of Pediatric TB:

Primary TB is often occurred in childhood on the first exposure to tubercle bacilli. Moreover, any age is susceptible particularly if was unexposed. Tubercle bacilli are settled down in alveoli often at lower part of the upper lobe or upper part of the lower lobe, where they are phagocytosed by alveolar macrophages. Macrophages are first immune cells responsible for an effective destruction of this intracellular pathogen. Balance action between bactericidal ability of the alveolar macrophage and bacterial virulence, it is the key factor. Destruction by macrophages release tubercle bacilli products and chemokines stimulating further immune response. Other inflammatory cells are attracted to the area forming what known as the Ghon's focus.[6]

Alveolar lymphangitis and lymphadenitis is a common squeal as a result of drainage of tubercle from the Ghon's focus hilar lymph nodes. Ghon's focus, lymphangitis and lymphadenitis together form the primary tuberculous complex. T-lymphocytes, within the lymph node, develop acquired immune response against tubercle bacilli. Immune response in children may not sufficient to prevent multiplication of bacilli, so bacilli may disseminate by lymphatics then blood and throughout the body causing military disease within a few months. Meninges, bone marrow, skeletal bones, liver and spleen are usually seeded with mycobacteria. [7]

TB meningitis especially common in infants younger than 5 years usually present with non-specific symptoms and if not diagnosed leads to sever sequels affecting central nervous system extensive brain injury and neurodisability. Latent pediatric TB infection isn't clear targeted only to who have a history of contact with a patient suffering from active TB disease. It is an evidence of immunological response to *M. tuberculosis* without evidence of clinical manifestation.[8]

Risk of reactivation of latent tuberculosis infection oscillate between five to fifteen percent across the lifetime of the infected child. Meanwhile, this risk varies depending on the species, environmental factors and host relationship. In fact, it was estimated that with the existence of risk factors; children develop TB disease within one year following mycobacteria infection. Earlier detection and screening with subsequent proper evaluation of latent tuberculosis in children in largely improve the prognosis and reduce the morbidity and mortality substantially reduces the community transmission of the disease[9].

One of the main immunological hallmarks of pediatric tuberculosis infection is a dynamic status of *M. tuberculosis* infected macrophages. Macrophages heterogeneity is referred to existence in two phenotypes. In the early phase of *M. tuberculosis* infection M1phenotype macrophages were with pro-inflammatory attributes is predominate. Whereas the M2 phenotype, later on, encourage tissue remodeling facilitating granuloma formation and *M. tuberculosis* survival (figure 1). [10]

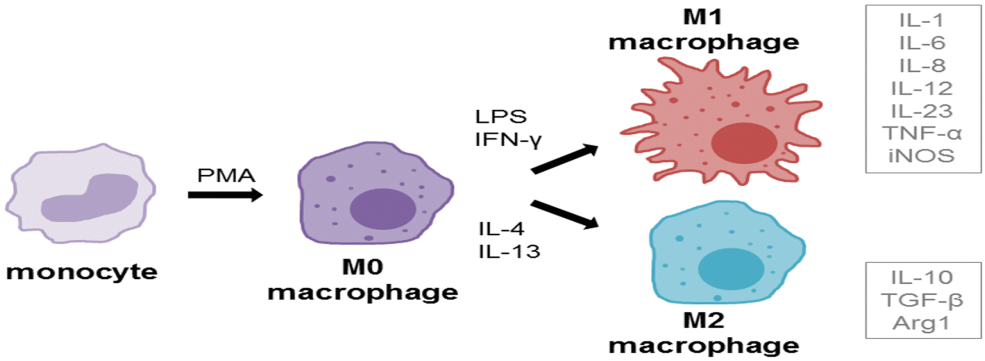

Figure (1): Macrophage phenotypes (M1 and M2)

Gut eubiosis has a tipping point for children immune immune response against pediatric TB. Gut microbiota and metabolites in form of short chain fatty acids and secondary bile acids emphasis a great impact on both child innate and adaptive immunity. It promote Th1/Th2 balance. *Bacteroides* abundance and decreased of *Lachnospiraceae* in gut microbiota result in increases susceptibility to pediatric TB. Furthermore, gut microbiota metabolites produced, transported to the lungs affecting inflammatory lung response and granuloma formation upon mycobacteria infection. Metabolites, such as propionate and butyrate, could decrease lung production of IL-17, suppressing Th1 and consequently influencing the outcome of *M. tuberculosis* infection.[11]

Meanwhile, gut-lung axis could influence the host immune homeostasis against *M. tuberculosis*; lung microbiota also affects gut microbiota through

microorganisms translocation of into blood. Probiotics could modulate gut microbiota through interference with pathogenic bacteria, secreting antibacterial effects, enhancing epithelial cell growth, and improving barrier function. Therefore, they could be applied as strategies in TB mangment.[12]

Protein and zinc deficiency has been identified as an important risk factor enhancing bacterial growth with disseminated fatal tuberculosis. Protein deficiency impairs T-cell functions and decrease production of IL-2 and IFN-γ which in turn affect bacteria elimination. It is important to calculate the child protein requirements during treatment[53]**.** Plasma zinc status is likely a marker for monitoring the severity of disease and the response to tuberculosis therapy. Cellular killing by macrophages was found to be reduced during zinc deficiency and rapidly restored after zinc supplementation. Regular monitoring of zinc micronutrients during treatment of chronic TB infection is essential.[13]

Spotlights on non invasive accurate and rapid diagnostic approaches of pediatric TB:

1- Microscopical examination of smear and culture:

Low incidence of cavitary disease in the pediatric population make the bacterial load within sputum is low. However, if endobronchial caseating lesions are present or caseating lymph nodes have eroded into a bronchus, the bacterial load and likelihood of smear positivity increases. Early morning gastric aspirate washings are preferred in children as they tend to swallow sputum rather than expectorate it. This method requires overnight fasting with collection on three consecutive days and is often best performed during a hospitalization.[14]

If gastric specimens do not yield positive acid-fast bacilli results, bronchoscopy for lavage of airways can often increase the detection of *M. tuberculosis*. Sputum samples can be collected successfully by induction procedures using nebulization of hypertonic saline in children who are old enough to cooperate with huff coughs and expectoration maneuvers. However, this is a procedure that can also generate aerosol of bacterial particles, so it is impractical if tried outside of hospital settings. Moreover, the health care provider performing this procedure requires strict adherence to infection control procedures.[15]

Ziehl-Neelsen staining is a specific stain in microbiology laboratories to identify acid-fast bacteria (AFB). A fluorescent staining e.g auramine- O stain can improve the sensitivity but involves higher costs. Nowadays, these conventional manual screening smear preparation techniques may be aided by using the artificial intelligence-based methods for identifying tubercle bacilli in stained smears. This is an automated microscope and specialized software to demonstrate a detection limit of 102 bacilli/mL of sputum. *Mycobacteria other than tuberculosis* (MOTT) also may produce positive sputum smear results mimicking *M. tuberculosis*, so culture is essential to distinguish between *them*. Disease due to MOTT is usually unresponsive to first-line anti-TB drugs. Automated liquid cultures have improved turnaround times and detection rates.[16]

Culture is the gold standard for diagnosis providing a highly positivity rate in cases of negative sputum smear microscopy. Culture allows a definitive confirmation of *M. tuberculosis* and provide an accessibility to do antibiotic susceptibility testing. Lowenstein-Jansen medium (LJ), solid conventional culture; is a selective medium with results available after an incubation of 4–6 weeks. Middlebrook media, agar-based; is superior over egg based one in transparency, which enables earlier detection of growing colonies. The BACTEC MGIT 960 System technique (Mycobacteria Growth Indicator Tube 960) is an advanced and automated method for the detection and cultivation of the bacterium *M. tuberculosis*. It is a sensitive, safe and automated liquid culture solution. [17]

Sample decontamination is a crucial step in the preparation of biological samples before they are inoculated on culture media. *M. tuberculosis* culture requires significant training, infrastructure, strict infection control measures and on-going quality assurance to ensure hazards of transmission.[18]

2- Molecular Methods:

These tests are essential for the rapid and accurate detection; Nucleic Acid Amplification Tests (NAAT). GeneXpert MTB/RIF is a real PCR to detect tubercle bacilli and its resistance to rifampicin by targeting mutations in the rpoB gene. It can be used directly on sputum sample and results become available within 2 hours. It is recommended to use Xpert MTB/RIF as the initial diagnostic test for Childhood TB in place of conventional approaches such as microscopy and culture.[17]

Multiplex PCR is another molecular method with simultaneous detection of several pathogens or different genotypes. Line Probe Assays (LPA) uses nucleic acid amplification PCR and reverse hybridization to rapidly detect drug resistance mutations. The 1st line FL-LPA detect mutation in Rna polymerase Beta (rpo B) gene of rifambicin and Inah in the promotor region of isoniazid and the 2nd line SL LPA detect mutations in gyr A and B in fluoroquinolones resistance and in injectable drugs. LPA can be used directly on sputum specimen or isolates and the results were obtained within 1-2 days.[15]

Molecular Methods can act on samples of sputum, gastric aspirate, bronchoalveolar lavage and/or pleural fluid, nasopharyngeal swabs and tissue biopsy specimens and are highly sensitive and specific.[14]

3- Gold nanoparticles:

They used for rapid diagnosis of the tuberculosis when applied on a vitro culture. This technique uses the most unique optical and electrical properties of gold

nanoparticles. These particles have synthesized at specific shapes, sizes, and surface chemistries stabilities in the presence of biological fluids becoming one of the most suitable materials for diagnostic purposes. In this method, either bacterial antigens or specific antibodies are used as immunoreagents and are immobilized onto paper chromatography strips. The biological sample potentially containing the target is allowed to interact through a highly specific antigen–antibody reactions at which nanogold represent an optical visible labeling.[18]

4- Circulating Free DNA (cfDNA):

Bacteria derived cell free DNA (cfDNA) in blood sample; namely circulating cfDNA, has been used to detect Mycobacteria in children. *M. tuberculosis* fragmented gene derived from necrotic human cells and tissues are released into the acellular fraction of blood and easily detected. This method depend on the convential PCR. [19]

5- Detection of Breath Metabolites:

Volatile organic molecules (VOC) can be identified as biomarkers in expired breath of children with TB e.g aldehydes, methylated aromatics, alkanes, and alkane derivatives. Most of these molecules are also general products of oxidative stress. This method need highly infection policies to prevent aerosol's transmission hazards.[15]

6- Urinary mycobacterial lipoarabinomannan antigen:

Lipoarabinomannan is a specific component of mycobacterial cell envelope that is excreted in the urine with active both pulmonary and extrapulmonary tuberculosis. It is considered a non-invasive rapid biomarker. Pediatric ipoarabinomannan urinary testing provides earlier diagnosis decreasing mortality.[20]

CONCLUSION

Pediatric TB is different from adult one due to its paucibacillary nature. Therefore, there is a growing priority to establish accurate noninvasive approaches to estimate actual rate of pediatric TB. These approaches will be able to decrease morbidity and mortality in children.

Exquisite blend of different approaches should applied for TB screening at health care settings where children seeking care to prevent disease transmission and complication.

Multidrug drug resistant TB in children is disappeared by early diagnosis with appropriate treatment.

Gut eubiosis influences pediatric TB prevention, pathogenesis, and treatment mainly by affecting efficacy of different immune cells. Therefore, establishment balanced eco between gut and lung is essential in pediatric TB management.

RECOMMENDATION

Current diagnostic abilities for the detection of pediatric tuberculosis are suboptimal. Efforts should be continued to update pediatric TB control programs. TB once diagnosed, treatment outcomes are excellent. Effective and feasible diagnostic and screening approaches is challenging.

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