Original Article

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Serum Levels of Interleukin-12 and Interferon Gamma in Pediatric Tuberculosis: A Clinico-Microbiological Correlation

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Abstract:

Objective: The interleukin-12/interferon-γ (IL-12/IFN-γ) pathway is the most validated cytokine pathway regulating *Mycobacterium tuberculosis* infection. The role of IL-12/IFN-γ axis in protecting against tuberculosis (TB) is exhibited in people having mutations in genes encoding these cytokines. We aimed to study the serum levels of IL-12 and IFN-γ in pediatric tuberculosis and their correlation with clinical and microbiological features.

Material and Methods: A case-control study was conducted on 60 microbiologically confirmed (smear and/or culture and/or cartridge-based nucleic acid amplification test) or clinically diagnosed (based on clinical features and radiography and/or contact history and/or Mantoux test with/without microbiological confirmation) pediatric TB patients ≤12 years. Serum interleukin-12 and interferon-gamma levels were estimated using enzyme-linked immunosorbent assays.Thirty age- and sex-matched controls were also included in the study.

Results: The median IL-12 levels were lower in our pediatric TB patients (488.1 pg/ml) compared to controls (784.8 pg/ml). However, the IFN-γ/IL-12 ratios were significantly higher among the TB patients as compared to the controls. Moreover, the levels of interleukin-12 and interferon gamma were significantly lower in cases with no evidence of TB on chest radiography. IL-12 was significantly lower in patients with hydrocephalus and enlarged ventricles. Higher levels of IL-12 and IFN-γ were associated with positive results by conventional microbiological techniques.

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Conclusion: The serum IFN-γ level and the IFN-γ/IL-12 ratio were significantly higher in children with TB compared to the controls in this study. Higher IL-12 and IFN-γ levels as well as IFN-γ/IL-12 ratios were associated with positive results by conventional microbiological techniques. Further studies on larger sample sizes could help evaluate the usefulness of interleukin-12 and interferon-γ as potential markers of severity and prognosis in pediatric TB.

Keywords: IFN-γ, IL-12, pediatric, tuberculosis

Introduction

Pediatric tuberculosis (TB) accounts for 10-20% of all TB cases in high-burden countries[1]. In 2016, the incidence of TB in India was estimated at 2,790,000 of which pediatric cases were around 227,000[2]. Children usually present with non-specific clinical symptoms for TB leading to a delay in diagnosis. In addition, the positivity of smear microscopy and culture tests remains low in children due to suboptimal samples and paucibacillary disease[3]. The World Health Organization (WHO) has recommended upfront Xpert MTB/RIF for the diagnosis of TB in presumptive TB cases in the pediatric population. Recent studies have found increased sensitivities when applying the Xpert MTB/RIF and Xpert MTB/RIF Ultra tests[4,5]. However, due to limitations related to finances and infrastructure in many settings,clinicians mostly rely on medical history, contact tracing, tuberculin skin tests (TST), chest radiography, and the observed lack of response to antibiotics when diagnosing TB in children[3].

As TB is considered a disease of a weak immune system, biomarkers reflecting various stages may prove to be of utility for the efficient management and control of the disease, especially among children. Interferon-γ (IFN-γ) and interleukin-12 (IL-12) act as central cytokines in the regulatory and effector phases of the immune response to *Mycobacteriumm tuberculosis*[6]. IL-12 is synthesized by macrophages infected with intracellular microorganisms. IL-12, in turn, induces the production of IFN-γ by T cells and natural killer (NK) cells. IFN-γ is mainly involved in stimulating the transcription of major histocompatibility complex class II genes within macrophages, co-stimulatory molecules like CD80, or enzymes like nitric oxide synthase, which lead to the production of microbicidal products and IL-12[7].

The IL-12/IFN-γ pathway has been extensively studied and is the most validated cytokine pathway regulating *M. tuberculosis* infection. The role of IL-12/IFN-γ axis in protecting against TB is exhibited in people having mutations in various genes encoding these cytokines. Such people exhibit Mendelian susceptibility to mycobacterial disease (MSMD)[8,9]. Various studies from different parts of the world have identified the association of defects in the IL-12/IFN-γ pathway with the severe and disseminated forms of TB in children[10,11]. Despite being so important in the pathogenesis of TB, there are not many studies on these cytokines in pediatric TB. Furthermore, to our knowledge, the levels of these cytokines have not been studied in association with various clinical and microbiological features of pediatric TB. We aimed to study the serum levels of interleukin-12 and interferon gamma in pediatric TB and their correlation with clinical and microbiological features.

Material and Methods

A prospective case-control study was conducted at Guru Teg Bahadur Hospital from November 2016 to October 2017 after approval from the Institutional Ethics Committee-Human Research, University College of Medical

Sciences, Delhi (Reference no. IECHR/2016/27/54). Sixty newly diagnosed pediatric TB patients were included in the study. Written informed consent from the legal guardians of the study participants and assent from the patients were obtained wherever applicable.

Inclusion criteria

All clinically suspected pediatric TB cases up to 12 years of age during the study period were recruited. The relevant specimens were subjected to conventional microbiological tests and the cartridge-based nucleic acid amplification test (CBNAAT) as a part of the routine investigations from the hospital's Department of Pediatrics as per the Revised National Tuberculosis Control Program (RNTCP) recommendations[12].

Cases

From all the clinically suspected pediatric TB cases, 60 children, who were microbiologically confirmed (smear and/or culture and/or cartridge-based nucleic acid amplification test) or clinically diagnosed (based on clinical features and radiography and/or contact history and/or the Mantoux test with/without microbiological confirmation) to have active pulmonary or extrapulmonary TB and whom the clinician had decided to treat with a full course of anti-tubercular therapy, were enrolled in the study.

Patients with asymptomatic and latent TB infection were not considered for enrollment in the study.

Definitions

Pulmonary TB: a clinically diagnosed/micro-biologically confirmed case with the involvement of lung parenchyma or the tracheobronchial tree, miliary TB, and cases with both pulmonary and extrapulmonary features[12].

Extrapulmonary TB: an either clinically diagnosed or microbiologically confirmed case with the involvement of organs other than lungs[12].

Clinical presentation of pulmonary TB: fever and cough for ≥2 weeks, unexplained recent weight loss, a history of contact with an infectious case, and/or suggestive features on chest X-ray, and/or a reactive TST[13].

Clinical presentation of extrapulmonary TB: according to the site involved along with non-specific symptoms like fever, weight loss, anorexia etc., with/without suggestive radiography features, and/or a reactive TST, and/or contact history[13].

Exclusion criteria

A previous history of TB or anti-tubercular treatment and compromised immunity disqualified thepatients from enrollment in the study.

As per the past hospital records of around 300 annual samples were received from suspected pediatric TB cases, a microbiological positivity rate of 10% was estimated. Hence, we recruited until we obtained at least 30 microbiologically diagnosed pediatric TB cases and continued the enrollment till the end of the study period.

Relevant samples (pulmonary TB-sputum or gastric lavage aspirate wherever sputum was not available; extrapulmonary TB as per the site involved) for microbiological tests were collected according to the site of involvement and were subjected to Ziehl Neelsen (ZN) staining for acid-fast bacilli (AFB), culture in Lowenstein-Jensen (LJ) media for *M. tuberculosis*, and CBNAAT. All specimens were handled in a biosafety cabinet class II B2[14]. The inoculated LJ media were incubated at 37 °C for up to 8 weeks. Cultures were identified by morphology and growth rate analysis and confirmed via niacin, p-nitrobenzoic, and MPT64 antigen detection tests[15]. CBNAAT was performed as per the standard operating procedure (SOP) developed by RNTCP at the directly observed therapy, short course (DOTS) center[12].

Blood specimens (1-2 ml) were obtained from all cases for biomarker level estimation.

Controls

Thirty age- and sex-matched apparently healthy children up to 12 years of age with no history of any recent or chronic illness were included as controls, and pre-vaccination blood specimens were collected at the time of routine vaccination.

The tests were performed as per the protocol of the commercially available interleukin-12p40 assay (Diaclone Human IL-12p40 ELISA kit, France) and interferon-gamma assay (Diaclone Human IFN-γ ELISA kit, France) based on the principle of the solid-phase sandwich enzyme-linked immunosorbent assay (ELISA). For IL-12p40, the ELISA test was valid if the concentration of the controls was 1287±322 pg/ml and the minimum detectable dose (sensitivity) was 20 pg/ml. For IFN-γ, the ELISA test was valid if the concentration of the controls was 235±59 pg/ml and the minimum detectable dose (sensitivity) was 5 pg/ml. The cytokine level in the sample was determined by extrapolating the optical density (OD) values against the standard cytokine concentrations using a standard curve.

Statistical analysis

The data were analyzed using the SPSS 20 software. The median levels and interquartile ranges (IQR) of IL-12 and IFN-γ were calculated for various groups among the TB patients and controls using the HAVERAGE or weighted average (definition 1) method in the SPSS software. Non-parametric tests were applied to compare cytokine levels between different groups and report their statistical significance. A p-value<0.05 was considered to indicate a statistically significant difference. The Mann-Whitney U test was used for two-group comparisons, whereas the Spearman rank correlation coefficient was used to test the correlation between serum IL-12 and IFN-γ levels. The receiver operating characteristic (ROC) curve analysis was also done for the biomarkers under study.

Results

The age of the children in this study ranged from 2 months to 12 years; themean age was 6.8 years. The male-to-female ratio was 1.2:1. Of the 60 participants, 40 children (66.66%) were found to have a weight-for-age and body mass index <-2 standard deviations and were diagnosed as being malnourished or underweight as per the WHO child growth standards[16].

Thirty-five participants (58.3%) were diagnosed with pulmonary TB, while 25 (41.7%) were classified as having extrapulmonary TB. Twenty-two patients (36.7%) were found to have disseminated or miliary TB involving both pulmonary and extrapulmonary sites. They were categorized as pulmonary TB cases as per the RNTCP guidelines[12]. The mean age of children with extrapulmonary TB (8±3.48 years) was significantly higher than that of children with pulmonary TB (6±4.09 years) (p-value=0.046). Regarding site involvement, the pediatric TB cases in this study comprised pulmonary TB (58%), tubercular meningitis (20%), skeletal TB (8%), pleural TB (5%), tubercular empyema (1%), tubercular liver abscess (2%), tubercular lymphadenopathy (2%), abdominal TB (2%), and tubercular psoas abscess (2%). Tubercular meningitis constituted the majority (12/25) of extrapulmonary cases.

The most common symptoms at presentation were fever, loss of appetite, weight loss, and pallor. All the cases had no apparent co-morbidities or underlying diseases. Forty-seven children (78.3%) had a history of BCG administration. The TST for 30/54 (55.5%) tested cases resulted reactive. A history of close contact with an infective TB case was found in 32 (53.3%) patients. Fifty-one of the participants (85.0%) had ≥1 radiological finding suggestive of TB.

Thirty-three cases (55%) were microbiologically confirmed via microscopy and/or culture and/or CBNAAT, while the rest were clinically diagnosed. AFB were detected by ZN staining in 12/60 (20.0%) cases. *M. tuberculosis* was

isolated in culture among 17 patients (28.3%), and 50% of the study sample was CBNAAT positive.

The median serum levels of IL-12 were higher among controls compared to the confirmed TB patients, but the difference was not significant, while the IFN-γ level was significantly higher in pediatric TB cases (p-value=0.000) compared to their control counterparts (Table 1).

Table 1 shows the ROC curve analysis of circulating markers under study for pediatric TB. The area under the curve (AUC) of IL-12 levels was not able to discriminate between the two study groups, while the AUC for IFN-γ was found to discriminate between pediatric TB cases and healthy controls at a statistically significant level (p-value=0.000).

Serum IL-12 was significantly higher among males. Moreover, serum IL-12 levels were significantly higher among patients with cough (p-value=0.011) and dyspnea (p-value=0.015), but it was lower among those with headache at presentation (p-value=0.000). IFN-γ levels were also found to be higher among cases presenting with cough compared to those who did not have cough (p-value=0.006). Serum IL-12 and IFN-γ concentrations were significantly higher in cases with pulmonary TB (Table 2).

Serum IL-12 levels were significantly lower (p-value= 0.013) in children having a normal chest radiograph as well as in children resulting with hydrocephalus on a brain scan (p-value=0.014) as compared to children who did not have these characteristics. Meanwhile, serum levels of IFN-γ were found to be significantly lower (p-value=0.041) in pediatric TB cases with a normal chest radiograph (Table 3).

Table 4 shows that the median IL-12 levels were significantly higher among culture-positive as compared to culture-negative cases (p-value=0.019). IL-12 levels were also higher in patients resulting positive for TB via microscopy and/or isolation on culture media as compared to those who were found to be negative by both of these tests (p-value=0.015). The median serum levels of IFN-γ were significantly higher in pediatric TB cases that were AFB-positive on microscopy testing as compared to AFB-negative cases (p-value=0.007), culture-positive for *M. tuberculosis* as compared to culture-negative cases (p-value=0.018), and positive by either the direct demonstration of AFB in microscopy and/or culture as compared to those who were negative by both testing methods (p-value=0.007).

Table 1 Median serum levels of biomarkers among pediatric tuberculosis cases and healthy controls with the analysis of receiver-operating-characteristic curve of biomarkers for their diagnostic efficacy in pediatric tuberculosis.

Biomarker under study	Median serum levels (IQR)		p-value	ROC curve analysis			
	Pediatric TB cases (IQR)	Healthy controls (IQR)		AUC (95% CI)	Best cut-off	Sensitivity (%)	Specificity (%)
IL-12 (pg/ml)	488.10 (218.33-893.62)	784.80 (476.05-1027)	0.055	0.624 (0.510-0.739)	NA	NA	NA
IFN-γ (pg/ml)	10.17 (2.30-73.86)	0.00 (0.00-2.34)	0.000	0.832 (0.748-0.916)	4.28	68.3%	86.7%

IQR=interquartile range, pg/ml=picogram/milliliter, ROC=receiver operating characteristic, CI=confidence interval, NA=not applicable, AUC=Area Under the Curve

Table 2 Median serum levels of IL-12 and IFN-γ associated with clinical presentation

Clinical presentation	Median levels of IL-12 in pg/ml (IQR)	p-value	Median levels of IFN-γ in pg/ml (IQR)	p-value
Gender				
Female (n=27)	418.50 (130.70-631.20)	0.018	9.78 (3.25-77.37)	0.911
Male (n=33)	729.70 (237.30-1232.60)		10.57 (1.22-80.15)	
Type of TB				
Extra pulmonary (n=25)	418.50 (162.45-680.45)	0.015	7.46 (0.00-15.87)	0.002
Pulmonary (n=35)	615.50 (323.50-282.40)		26.29 (3.74-162.40)	
Disseminated/miliary TB				
Present (n=22)	438.65 (191.23-815.90)	0.266	8.42 (2.64-70.47)	0.764
Non-disseminated (n=38)	584.10 (231.90-1000.55)		12.42 (1.52-79.48)	
Fever				
Present (n=52)	520.15 (218.33-893.62)	0.965	11.37 (2.30-83.69)	0.452
Absent (n=8)	418.75 (214.58-1101.82)		7.26 (1.22-21.86)	
Evening fever				
Present (n=13)	402.60 (171.05-630.20)	0.175	6.85 (1.92-73.83)	0.529
Absent (n=47)	575.60 (228.50-1023.20)		12.67 (3.25-77.37)	
Chills				
Present (n=8)	495.05 (155.15-1610.77)	1.000	48.25 (0.70-224.4)	0.527
Absent (n=52)	488.10 (218.33-893.62)		9.63 (2.42-61.36)	
Cough				
Present(n=34)	593.85 (385.42-1407.85)	0.011	20.87 (4.39-136.56)	0.006
Absent (n=26)	237.30 (129.90-861.35)		5.09 (0.00-19.84)	
Dyspnea				
Present (n=15)	868.70 (428.20-1925.80)	0.015	22.07 (0.96-161.40)	0.238
Absent (n=45)	419.00 (209.35-828.60)		9.43 (2.47-59.48)	
H/o recurrent pneumonia				
Present (n=4)	503.35 (289.50-1588.25)	0.658	4.50 (0.93-200.32)	0.658
Absent (n=56)	488.10 (213.12-893.62)		11.37 (2.30-73.85)	
Loss of appetite				
Present (n=49)	575.60 (223.45-914.50)	0.335	9.78 (2.47-88.86)	0.559
Absent (n=11)	349.20 (159.80-895.10)		19.08 (0.00-56.27)	
Weight loss				
Present (n=46)	538.55 (225.98-960.65)	0.631	10.97 (2.03-93.19)	0.462
Absent (n=14)	446.60 (195.42-875.30)		10.00 (3.65-28.60)	
Abdominal pain				
Present (n=7)	631.20 (228.50-868.70)	0.702	9.48 (2.14-243.72)	0.928
Absent (n=53)	431.10 (214.85-917.45)		12.17 (2.48-70.34)	
Lymphadenopathy				
Present (n=10)	756.80 (483.53-1333.15)	0.150	15.75 (2.03-211.71)	0.585
Absent (n=50)	427.75 (216.58-875.30)		10.17 (2.81-62.85)	
Vomiting				
Present (n=18)	223.45 (114.55-960.58)	0.087	10.17 (2.10-21.11)	0.293
Absent (n=42)	593.85 (303.20-901.85)		10.82 (2.03-103.60)	
Headache				
Present (n=8)	30.80 (14.85-114.55)	0.000	1.72 (0.00-6.75)	0.006
Absent (n=52)	593.85 (329.92-1002.35)		19.11 (3.37-90.39)	
Seizures				
Present (n=15)	218.40 (127.50-868.70)	0.082	9.78 (3.44-26.29)	0.462
Absent (n=45)	575.60 (280.85-981.50)		10.57 (1.92-94.45)	

IQR=interquartile range, pg/ml=picogram/milliliter, TB=tuberculosis

Table 3 Comparison of median serum levels of IL-12 and IFN-γ with important radiological features in pediatric tuberculosis cases (n=60)

Radiological feature	Median levels of serum IL-12 in pg/ml (IQR)	p-value	Median serum levels of IFN-γ in pg/ml (IQR)	p-value
Lung consolidation				
Absent (n=35)	419.00 (165.10-868.70)	0.101	9.78 (0.00-35.93)	0.164
Present (n=25)	615.00 (360.50-1103.00)		19.38 (2.79-145.84)	
Miliary mottling				
Absent (n=54)	488.10 (218.38-890.68)	0.914	9.45 (2.03-58.69)	0.153
Present (n=6)	472.15 (136.32-2124.15)		77.62 (9.13-193.70)	
Pleural effusion				
Absent (n=51)	474.70 (207.30-895.10)	0.301	10.57 (2.14-91.92)	0.604
Present (n=9)	501.50 (335.25-1392.20)		9.37 (2.35-38.51)	
Normal chest X-ray				
Absent (n=48)	584.10 (262.60-1002.35)	0.013	15.62 (3.51-95.72)	0.041
Present (n=12)	191.75 (88.65-578.15)		6.14 (0.00-19.09)	
Lymphadenopathy on radiology imaging				
Absent (n=48)	452.90 (222.90-928.62)	0.789	11.62 (2.91-73.85)	0.739
Present (n=12)	558.50 (181.48-817.95)		8.41 (1.82-98.08)	
Hydrocephalus and enlarged ventricles				
Absent (n=52)	584.10 (239.22-1002.35)	0.014	10.02 (2.30-90.39)	0.383
Present (n=8)	191.70 (39.37-458.70)		11.22 (0.86-19.30)	

IQR=interquartile range, pg/ml=picogram/milliliter

Table 4 Comparison of levels of IL-12 and IFN-γ with various diagnostic techniques (n=60)

Diagnostic technique	Median serum levels of IL-12 in pg/ml (IQR)	p-value	Median serum levels of IFN-γ in pg/ml (IQR)	p-value
Direct demonstration of AFB				
Positive (n=12)	740.90 (398.78-658.75)	0.096	81.58 (19.66-191.63)	0.007
Negative (n=48)	452.90 (180.45-866.25)		8.41 (1.54-33.52)	
Isolation of *M. tuberculosis*				
Positive (n=17)	644.90 (400.05-533.30)	0.019	77.37 (5.86-182.22)	0.018
Negative (n=43)	418.50 (165.10-798.30)		7.46 (0.96-22.07)	
Direct demonstration and/or isolation				
Positive (n=19)	644.90 (402.60-1784.20)	0.015	77.37 (6.85-163.41)	0.007
Negative (n=41)	427.30 (168.30-833.50)		7.46 (0.71-24.18)	
CBNAAT				
Positive (n=30)	593.85 (234.42-1336.70)	0.206	20.30 (4.32-132.92)	0.071
Negative (n=30)	429.65 (196.75-815.90)		6.36 (0.83-30.62)	

IQR=interquartile range, pg/ml=picogram/milliliter, AFB=acid fast bacilli, CBNAAT=cartridge-based nucleic acid amplification test

There was a significant positive correlation between serum levels of IFN-γ and IL-12 (rho=0.648, p-value=0.000). The IFN-γ/IL-12 levels were significantly higher in pediatric TB cases [median (IQR)=0.03 (0.004-0.107)] compared to healthy children [median (IQR)=0.00 (0.000-0.003)] (p-value=0.000). The AUC for IFN-γ/IL-12 was found to statistically significantly discriminate between pediatric TB cases and healthy controls (p-value=0.000); the best cut-off value was 0.003, with a sensitivity of 76.7% and a specificity of 83.3% [AUC (95% confidence interval (CI))=0.839 (0.755-0.923)].

The IFN-γ/IL-12 ratio/was significantly higher in children with pulmonary TB (p-value=0.005). The median values of IFN-γ/ IL-12 ratio were significantly higher in pediatric TB cases who were smear-positive (p-value=0.037) and TB-positive by at least one of the three microbiological techniques (p-value=0.013).

Discussion

There is a paucity of work done on biomarkers in pediatric TB, especially with reference to IL-12 and IFN-γ levels. Therefore, it was not possible to compare all our findings with those from previous studies.

More than 60% of the children under study were found to be underweight or malnourished. On one hand, TB can lead to significant weight loss through the production of inflammatory cytokines, while on the other hand, significant malnutrition may affect the inflammatory response and aggravate the outcome of TB[17]. Since most of our cases belonged to households with a lower socioeconomic status, who are more prone to malnourishment, and since data about parameters like weight and height before the onset of symptoms in our study setting was largely dependent on the recall of the pediatric patients or their guardians,without much documentation, it was difficult to assess theacute onset of weight loss, and whether the disease had caused it, or it was present before the onset ofthe disease.

The median serum levels of IL-12p40 were not significantly different among our pediatric TB patients and the healthy children enrolled in our study, which was similar to what was reported in another Indian study[18]. Though the IL-12p40 levels in pediatric TB cases were lower than those detected among healthy controls, the IL-12p40 levels in both our cases and controls were much higher than what has been reported among healthy children in a previous study (67.42±20.58 ng/l)[19]. A Tanzanian study on TB lymphadenitis that compared cytokine levels between adult and pediatric patients reported lower levels of IL-12p40 among children as compared to adults with active TB and adult healthy controls; however, the unavailability of sera from age-matched healthy children was a limitation of that study[20].On the other hand, Deveci et al. found that the median IL-12p40 levels were significantly higher in active TB cases compared to the inactive TB patients and healthy controls[6]. Despite our extensive search for relevant data, we could not document any study with Indian participants reporting the mean or median IL-12 levels in TB cases of a pediatric age. However, with the limited data available to us for comparison, we inferred that the higher levels of IL-12 in both confirmed TB cases and healthy controls in our study was probably indicative of a higher baseline level of IL-12 in the Indian population due to an increased exposure to various tropical diseases like protozoan, viral, bacterial and mycobacterial infections[21]. Most of these infections remain at the subclinical stage in endemic areas, which may result in higher levels of some cytokines in apparently healthy controls. Another reason may be genetic polymorphisms. Certain genotypes may have been more prevalent in the population we studied, which would have resulted in higher IL-12 levels among both TB patients and controls in our investigation.

An increase in the levels of IL-12p40 has been reported to be significantly different between different genotypes in Indian active TB patients[22]. In a study

conducted in Delhi, among 5 variants of the IL-12 gene, only 2 genotypes (AA, GG) showed a correlation with the serum IL-12 levels[23]. Another study conducted in North India on the polymorphism of the IL12β promoter region in TB cases and controls found that significantly more individuals were homozygous for the deletion of the allele of the IL12β promoter among the TB group, and that this genotype was associated with the highest levels of IL-12 production by dendritic cells following CD40 ligation. It also confirmed that the genotype for the highest known IL-12 producer is found in ~30% of Indian TB subjects and in only ~9% of controls[24]. Further genotypic studies in this regard are required to elucidate whether these factors are responsible for the variation in IL-12 levels. Males had a significantly higher median level of IL-12p40 than females in our study, while Mustafa et al. reported higher IL-12p40 levels in adult females with active TB compared to their male counterparts[20].

The median levels of IL-12p40 were found to be significantly higher among pulmonary pediatric TB cases in this study than among extra pulmonary TB participants, yet another study from India by Kumar et al. reported no significant differences between pediatric pulmonary and extrapulmonary TB cases[18]. The findings of our study suggest that serum IL-12 can be used to differentiate between pulmonary and extrapulmonary TB.

Disseminated or miliary TB signifies a severe form of the disease. The median serum levels of IL-12 were not found to vary significantly between disseminated and non-disseminated pediatric TB cases. Though we could not find any study comparing cytokine levels in disseminated and non-disseminated TB, an earlier Indian study reported that IL-12 was not significantly associated with the severity of pulmonary TB[25].

The median levels of IL-12p40 were significantly higher in children having cough or dyspnea at presentation as compared to those who did not present with cough. Since cough and dyspnea are predominant symptoms of pulmonary TB, this finding further strengthens the significance of higher IL-12 levels in pulmonary TB cases as compared to patients with extrapulmonary TB.

However, the median levels of IL-12p40 were lower in cases with central nervous system (CNS) symptoms like headache, vomiting, and seizures compared to the children who did not present with symptoms related to CNS; nevertheless, the difference was statistically significant only among cases having headache. This finding is further supported by the fact that the levels of this biomarker were also lower in cases having hydrocephalus and enlarged ventricles according to brain imaging results, pointing towards a compartmentalized immune response in tuberculous meningitis. The median levels of IL-12p40 were significantly higher in culture-positive as compared to culture-negative pediatric TB cases. A similar finding was reported in a previous study among culture-positive adult TB patients. No significant difference, however, was observed among culture-positive andculture-negative pediatric TB cases in that study. Since, being culture-positive means having a higher bacillary load, it is difficult to explain this finding considering the protective immune response of IL-12 to TB[20]. However, higher IL-12 levels in culture-positive cases appearto be due to an early immune response to the high bacillary load, which in turn, induces IFN-γ production that stimulates Th1 responses, which are important in the control of TB disease[26,27].

IFN-γ plays a pivotal role in the cellular immune response against TB and overcomes the inhibition of phagolysosome maturation. IFN-γ levels were found to be low or below the detection limit in healthy children in our study—the same was reported by Sack et al. Thus, IFN-γ appears to be a genuine mediator and a potential marker of immunity and inflammation in children[28].

Similar to our findings, previous studies have also reported higher serum levels in patients with active TB as

compared to healthy controls[6,20,29]. IFN-γ seems to play an important role in the Th1-mediated cellular immunity and the stimulation of macrophages to produce TNF-α and 1,25-dihydroxyvitamin D, both of which help inhibit mycobacteria[6].

Serum IFN-γ was significantly higher among our pulmonary as compared to extrapulmonary TB cases. Yet, a previous study conducted on a pediatric population reported no significant difference in serum IFN-γ levels between pulmonary TB, extrapulmonary TB, and healthy controls[18]. However, the median serum levels of IFN-γ were not found to vary significantly between disseminated and non-disseminated pediatric TB cases. In another Indian study, IFN-γ was found to be associated with the severity of pulmonary TB and have significantly higher levels in bilateral disease[25].

The serum levels of IFN-γ were also found to be significantly higher in cases having cough, which is the predominant symptom in pulmonary TB at presentation; this lends further support to the significance ofthe finding of higher levels of serum IFN-γ in pulmonary TB.

Serum levels of IFN-γ were also significantly higher in cases where the presence of AFB could be demonstrated by ZN staining and/or a positive culture result. A similar finding was reported by Hussain et al., who observed higher levels of IFN-γ among TB patients confirmed by ZN staining and histopathological findings as compared to clinically diagnosed cases[30]. Though there are conflicting reports about the correlation of IFN-γ with bacillary load in TB, various studies have reported a progressive decrease in the levels of IFN-γ with treatment; hence, IFN-γ might prove useful in monitoring the efficacy of antitubercular therapy[31].

The median values of the IFN-γ/IL-12 ratio were found to be significantly higher among pulmonary as compared to extrapulmonary TB cases. Despite our extensive research of literature, we failed to find any study mentioning this ratio with respect to pediatric TB. The IFN-γ/IL-12 ratio might be more reliable as a biomarker as it involves two important cytokines that play an important role in the pathogenesis of TB. The IFN-γ/IL-12 ratio could probably be utilized to study the course of the disease; however, no study could be found to support this deduction. MSMD is a rare disorder associated with impaired immunity against mycobacterial pathogens, for which genetic etiologies, including mutations in the previously mentioned genes, have been described[9]. Such mutations may be predominantly present in the Indian subcontinent making India an endemic country for TB. Further investigations in such cases at the molecular level may help answer some questions regarding the underlying mechanisms at play and identify genetic defects affecting the IL-12/IFN-γ pathway in these pediatric TB cases. There is a continued need to reconsider the genetic theory of infectious diseases like tuberculosis to ensure the timely development of novel preventive and therapeutic treatments.

The levels of IL12p40 and IFN-γ were found to be significantly lower in pediatric TB cases with a normal chest radiograph, hence proving the role of inflammation as a marker in TB patients. It was observed in this study that the median level of IL-12p40 was significantly lower among those with concomitant hydrocephalus and enlarged ventricles. In a previous study, biomarker analysis was done on both the cerebrospinal fluid and serum of patients with tuberculous meningitis. The levels of all biomarkers tested, including IL-12p40 and IFN-γ, were not elevated in serum, suggesting that biomarker levels in serum do not reflect injury tothe CNS, and that the immune response is compartmentalized to the site of disease[32].

Unlike our study, which found no significant difference in the serum levels of biomarkers in patients with and without pleural effusion, another study by Sharma et al. on patients with pleural TB reported that their pleural fluid analysis revealed 25-fold higher IFN-γ levels in serum

as compared to their blood; this finding serves as further support for the compartmentalized nature of the immune response in TB[33].

Conclusion

The serum levels of IFN-γ and the IFN-γ/IL-12 ratio were significantly higher among pediatric TB patients as compared to the healthy controls. IL-12 and IFN-γ levels and the IFN-γ/IL-12 ratio were found to significantly differentiate between the pulmonary and extrapulmonary types of TB. Since higher values for IL-12 and IFN-γ levels and IFN-γ/IL-12 ratio were found to correlate with positive TB results by conventional microbiological techniques, further studies employing larger sample sizes could help evaluate their role as potential markers of disease severity and prognosis. The biomarkers associated with pediatric TB combined with the patient's clinico-radiological and microbiological profile could play a role in the development of new algorithms for the diagnosis of pediatric TB.

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Conflict of interest

No conflict of interest related to this research to declare.

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