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ORIGINAL RESEARCH

Critical involvement of interleukins in pulmonary tuberculosis pathogenesis

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ABSTRACT

Introduction: Pulmonary Tuberculosis (pTB) remains a significant global health concern, with 10 million new cases and 1.6 million deaths reported in 2021. Interleukins (ILs), particularly IL-1β, IL-6, and IL-10, play pivotal roles in the cellular immune response to *Mycobacterium tuberculosis* (MTB). Understanding their complex interactions is crucial for developing targeted therapies. Hence, we aimed to explore the nuanced roles of IL-1β, IL-6, and IL-10 in pTB.**Methodology:** In this study, 300 participants were categorized into 100 individuals as healthy controls and 200 diagnosed with pTB. Blood specimens were gathered, and the concentrations of ILs in the serum were assessed through Enzyme-Linked Immunosorbent Assay (ELISA), and the results were statistically analysed.**Results:** The demographics revealed significant TB-IgG and TB-IgM antibodies in pTB patients. Immunological parameters show marked increases in pTB cases across genders and ages. Serum cytokine correlations unveil a strong correlation between IL-1β, IL-6, and IL-10. Gender-specific analyses reveal positive correlations in males but not females, suggesting variations in immune responses. Age-specific correlations highlight nuanced interactions, with age-related differences observed. These findings underscore the complex cytokine interplay in pTB, influenced by gender and age.**Conclusion:**Our findings conclude that these cytokines can serve as indicators of disease activity and the progression of inflammation in pulmonary tuberculosis.

Keywords: Mycobacterium tuberculosis, Immune response, Inflammation, T lymphocytes, Interleukin-1 beta

INTRODUCTION

Pulmonary Tuberculosis (pTB), a historic respiratory ailment, persists with 10 million new cases and 1.6 million deaths globally in 2021. [1,2] Clinical symptoms include fever, anorexia, and T lymphocyte recruitment to granulomas. *Mycobacterium tuberculosis* (MTB) elicits a cellular immune response involving interleukins (ILs) such as IL-1β, IL-6, and IL-10. These ILs play pivotal roles in shaping the immune landscape during TB infection, highlighting their significance in understanding and managing the disease. [3] IL-1β, a pro-inflammatory cytokine, is produced in response to infection and acts as a crucial mediator of innate immunity. Its role in TB involves initiating and amplifying the early inflammatory response, recruiting immune cells to the site of infection, and influencing the formation of granulomas. [4] While an appropriate IL-1β response is essential for effective anti-mycobacterial defence, an excessive or dysregulated production may contribute to tissue damage and pathology. [5]IL-6, another multifunctional cytokine, participates in the acute-phase response and inflammation. [6] During TB, IL-6 is implicated in promoting the differentiation of T-helper cells and activating macrophages. Elevated levels of IL-6 have been associated with disease severity, suggesting its potential as a biomarker for monitoring TB progression. However, the precise balance of IL-6 expression is crucial, as excessive inflammation can lead to immunopathology. [7,8]In contrast, IL-10, an anti-inflammatory cytokine, plays a complex role in TB. [9] While it is generally considered an immunosuppressive factor, limiting inflammation and preventing tissue damage, its overexpression can impede protective immune responses. Elevated IL-10 levels are associated with increased susceptibility to TB, as they may hinder the ability of immune cells to control bacterial replication. [10]Understanding the intricate interplay between IL-1β, IL-6, and IL-10 in TB is vital for developing targeted therapeutic interventions. Striking a delicate balance in the immune response is crucial to harness the protective aspects of inflammation while mitigating the risk of

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immunopathology. Further research is needed to elucidate the nuanced roles of these interleukins in TB pathogenesis and to explore their potential. Therefore, we aimed to study the role of interleukins in the development of pulmonary tuberculosis.

MATERIAL AND METHODS

This study was conducted for three years, where 300 participants aged 20 to 60 were enrolled and categorized into 100 healthy controls and 200 diagnosed with pTB. Ethical clearance and informed consent were meticulously secured from participants at the Medicine Outpatient Department of Gandhi Medical College in Bhopal, Madhya Pradesh. Thorough documentation of demographic information preceded the aseptic collection of 5 ml blood samples from the anticubital vein of individuals diagnosed with pTB. Post-clotting, the blood samples were carefully transferred to plain vials, and the obtained supernatant serum underwent centrifugation at 3000 rpm for 5 minutes.Immunological assessments were conducted in the Department of Microbiology, focusing on various serum cytokine levels crucial for understanding TB's immunological parameters. The specific targets included Interleukin-1 Beta (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Tuberculosis Immunoglobulin-G and Immunoglobulin-M (TB-IgG, IgM). Each analysis employed ELISA kit-based methods, with distinct kits and catalogue numbers for IL-1β (BD OptEIA, 559603, USA), IL-6 (ACCUCYTE ASSAY ELISA KIT, UK), IL-10 (8D OptEIA, 555252, USA), and TB-IgG, IgM (OMEGA KIT, Scotland, UK).

Statistical Analysis

The data obtained from the study were subjected to statistical analysis using SPSS version 26.0 for further evaluation at the significance level of p-value=0.05. Continuous variables were presented as Mean ± Standard Deviation (SD), and categorical variables were expressed in terms of frequency and percentage. For categorical data, Chi-square statistical analysis was performed, while for continuous data, Student's t-test was employed. Additionally, correlation coefficients (r) were calculated to explore relationships among variables.

RESULTS

The demographics have been depicted in Tables 1 and 2, showing control and pTB cases. We observed a significant presence of detectable TB-IgG and TB-IgM antibodies among individuals with pTB. Notably, the absence of positive results in the control group serves as a baseline, indicating a strong association between the presence of these antibodies and pTB. Both male and female subjects exhibit substantial proportions with detectable levels of TB-IgG and TB-IgM antibodies, with slight variations in percentages but similar overall patterns. (Figure-1 and 2) Immunological parameters showed significant increases ($P<0.001$) in individuals with pTBcompared to controls. This pattern holds across both male and female subjects. (Table-3) Moving to age-specific analyses, all immunological parameters in age groups I and II for both genders showed significant increases compared to controls. (Table-4) Further investigation into serum cytokine correlations unveils strong positive associations between IL-1β IL-6 and IL-10. A moderately strong positive correlation (0.41) is observed between IL-6 and IL-10, suggesting concurrent increases in IL-1β levels correspond to elevated IL-6 and IL-10 levels.Among males, positive correlations exist between IL-1β and IL-6 (0.24*) and IL-1β and IL-10 (0.31**), implying potential immune interactions. Conversely, no significant correlations are found for females between IL-1β, IL-6, and IL-10, suggesting gender-specific variations in immune responses.Examining age-specific correlations for males in age group I, non-significant correlations between IL-1β and IL-6 (0.11NS) and IL-1β and IL-10 (0.04NS) coexist with a strong positive correlation between IL-6 and IL-10 (0.41**), indicating age-related differences. In age group I for females, non-significant IL-1β and IL-6 correlation (0.13NS) contrasts with a significant IL-1β and IL-10 correlation (0.06***), and a significant IL-6 and IL-10 correlation (0.31*), implying age-specific immune responses. There were non-significant correlations between IL-1β and IL-6 (0.12NS) and IL-1β and IL-10 (0.002NS). A significant positive correlation is observed between IL-6 and IL-10 (0.20*) in age group II of males. Further, a significant positive correlation between IL-1β and IL-6 (0.35**) in females of age group II suggests an interconnected response, while no significant correlations between IL-1β and IL-10 or IL-6 and IL-10 indicate nuanced immune interactions. (Table-5) Overall, these findings underscore the intricate interplay of cytokines in pTB, influenced by gender and age.

Table-1: Demographic distribution of subjects.

Study Group	Male	Female	Age group I	Age group II
Normal healthy control subjects (n=100)	50	50	48	52
Pulmonary tuberculosis subjects (n=200)	130	70	72	128

Table-2: Distribution of subjects by gender and study group in different age groups.

	Study Group	Age group I	Age group II
Male	Normal healthy male control subjects (n=50)	22	28
	Pulmonary tuberculosis male subjects (n=130)	40	90

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Female	Normal healthy female control subjects (n=50)	26	24
	Pulmonary tuberculosis female subjects (n=70)	32	38

Table-3: Serum Interleukin levels in control and pulmonary tuberculosis subjects stratified by gender.

Parameters			**Interleukins**		
			IL-1β (pg/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**
Study Groups	**Control subject (n=100)**	**Mean±SD**	1.20±0.19	1.35±0.25	1.29±0.22
	Pulmonary tuberculosis subjects (n=200)		3.45±0.85***	3.35±0.85***	3.30±0.74***
Male	**Control subject (n=100)**		1.25±0.20	1.40±0.26	1.30±0.22
	Pulmonary tuberculosis male subjects (n=130)		5.05±1.25***	3.65±0.92***	3.45±0.98***
Female	**Control subject (n=50)**		1.19±0.16	1.24±0.22	1.22±0.21
	Pulmonary tuberculosis female subjects (n=70)		3.98±0.60**	2.91±0.41*	2.85±0.39*

***P<0.05, **P<0.01, ***P<0.001, NS= non-significant**

Table-4: Serum Interleukinin control and pulmonary tuberculosis subjects stratified by gender and age groups.

Parameters			**Interleukins**		
			IL-1β (pg/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**
Male	**Control subject age group I (n=22)**	**Mean±SD**	1.21±0.19	1.30±0.24	1.26±0.18
	Pulmonary tuberculosis subjects age group I (n=40)		4.21±1.14**	2.99±0.69**	3.01±0.71**
	Control male subject age group II (n=28)		1.36±0.22	1.54±0.20	1.47±0.19
	Pulmonary tuberculosis male subjects age groups II (n=90)		6.14±1.75***	4.19±1.06***	4.07±1.02***
Female	**Control female subject age group I (n=26)**		1.78±0.20	1.20±0.22	1.21±0.24
	Pulmonary tuberculosis female subjects age group I (n=32)		3.14±0.50**	2.75±0.30**	2.80±0.31**
	Control female subject age group II (n=22)		1.30±0.18	1.30±0.21	1.27±0.20
	Pulmonary tuberculosis female subjects age groups II (n=40)		4.50±0.70***	3.54±0.47***	3.15±0.41***

***P<0.05, **P<0.01, ***P<0.001, NS= non-significant**

Table-5: Correlation analysis of serum interleukin levels in pulmonary tuberculosis subjects stratified by gender and age groups.

Study groups	**Variables**	**IL-6**	**IL-10**
Pulmonary Tuberculosis	IL-1β	0.74***	0.65***
	IL-6	-	0.41***
Pulmonary Tuberculosis Male Subjects	IL-1β	0.24*	0.31**
	IL-6	-	0.03^{NS}
Pulmonary Tuberculosis Female Subjects	IL-1β	0.13^{NS}	0.12^{NS}
	IL-6	-	0.14^{NS}
Pulmonary Tuberculosis Male Subjects of Age Group I	IL-1β	0.11^{NS}	0.04^{NS}
	IL-6	-	0.41**
Pulmonary Tuberculosis Female Subjects of Age Group I	IL-1β	0.13^{NS}	0.06***
	IL-6	-	0.31*
Pulmonary Tuberculosis Male Subjects of Age Group II	IL-1β	0.12^{NS}	0.002^{NS}
	IL-6	-	0.20*
Pulmonary Tuberculosis Female Subjects of Age Group II	IL-1β	0.35**	0.24^{NS}
	IL-6	-	0.01^{NS}

Values expressed as correlation coefficient (r), *P<0.05, **P<0.01, ***P<0.001, P=NS (non-significant)

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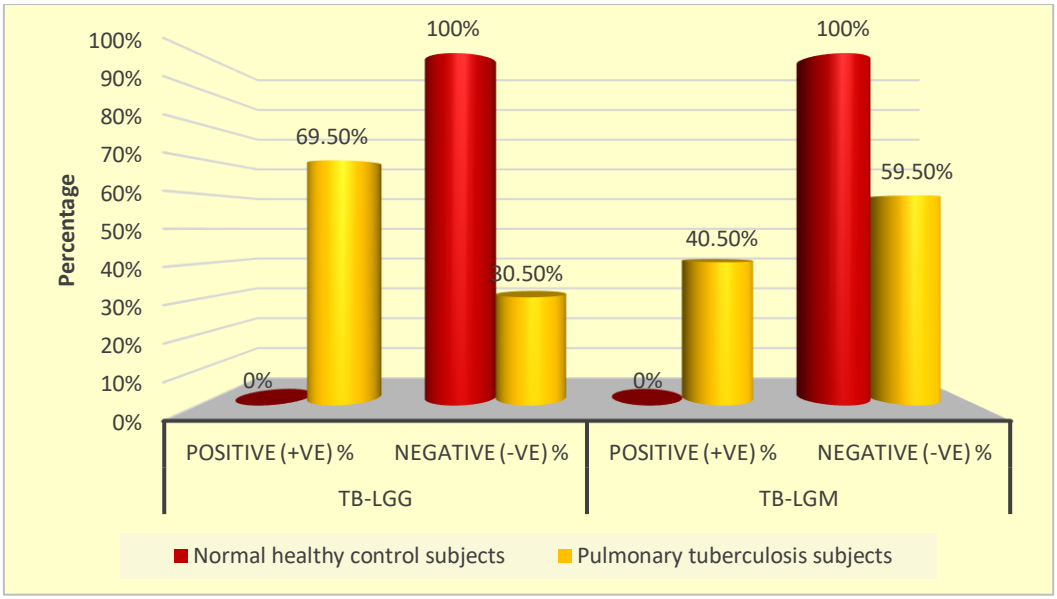

Figure-1: Status of TB-lgG and lgM in persons suffering from pulmonary tuberculosis and control subjects.

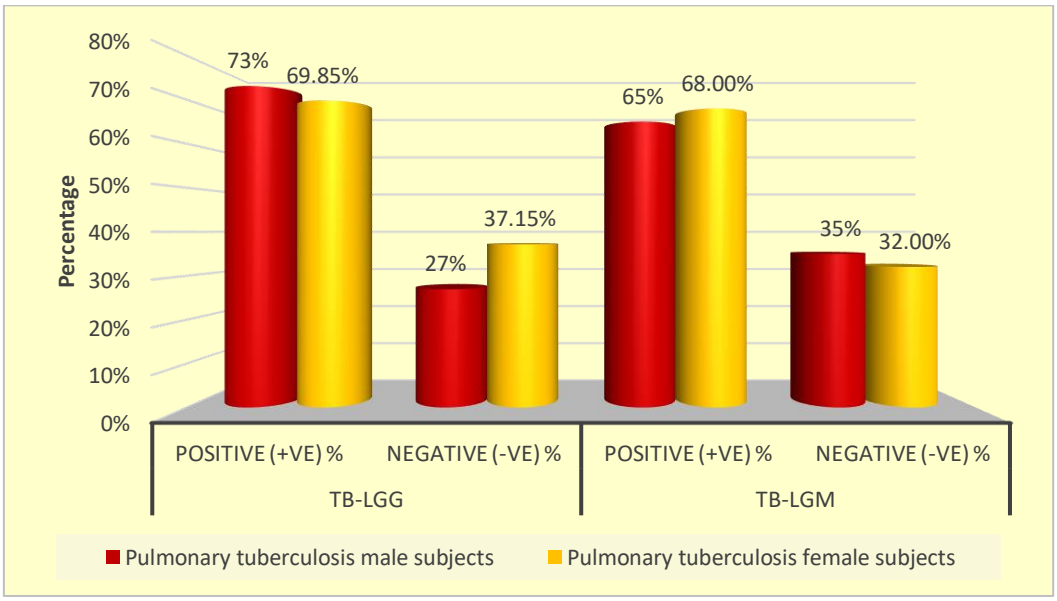

Figure-2: Status of TB-lgG and lgM in male and female subjects of pulmonary tuberculosis.

DISCUSSION

In the present study, a highly significant alteration was observed in males aged 20 to 60 years, while females in the same age bracket exhibited substantial changes in immunological parameters. These findings align with previous studies, suggesting that altered metabolism may contribute to the diverse immunological changes seen in pTB. [11,12]OloboJ et al. reported significant changes in IL-10 levels in both pTBpatients and healthy control groups. [13]

Our study found that serum levels of IL-1β, IL-6, and IL-10 significantly increased in both sexes of the age groups I and II. This aligns with the findings of Olobo J et al. [13], where the serum levels of these cytokines were notably elevated in patients with pulmonary tuberculosis compared to the control groups. Warwick-Davies J et al. [14] also showed TNF-aincrease in serum. cytokines like IL-1β, IL-10,and IL-6 also released in serum. [12] The females of the postmenopausal stage (age group II) are more prone topTB due to the increased release of immunological parameters.

Additionally, in a study by Farr et al. [15], cytokines were ranked for their specificity in diagnosing tuberculosis (TB), revealing IL-6 and IFN-γ as the most specific for pTB. Pro-inflammatory cytokines, including IL-6 and IL-8, are crucial in the immune response to MTB [16]. Tang et al. [17] noted elevated serum IL-6 and TNF-α in pTB patients compared to those with chronic obstructive pulmonary disease without pTB. Conversely, another study [18] found upregulated serum IL-6 and IL-8 in pTB patients but no change in TNF-α. Guzman-Beltran et al. [18] highlighted IL-6's role in driving early defence against

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MTB by promoting IFN-γ production by T helper-1 cells. Another study [19] also observed increased IL-6, alongside CRP and TNF-α, in pTB patients, indicating diverse inflammatory pathways in pTB.

Namaei M et al. [20] showed a significant upregulation of serum IL-10 in patients with normal spirometry and its downregulation with obstructive spirometry during tuberculosis treatment. IL-10, an anti-inflammatory cytokine, contributes to less inflammation-related lung damage in pulmonary infections. [21]This finding aligns with the idea that high IL-10 levels may help prevent tissue damage following infectious diseases. [22]

These findings showed a close association among various parameters in age-related pTB. We conducted correlation analyses between all immunological markers to explore these relationships further. Notably, a positive correlation was observed between IL-1β and IL-6. Additionally, elevated levels of IL-1β, IL-6, and IL-10 were identified in patients with pTB. IL-10, recognized as an anti-inflammatory cytokine, plays a role in macrophage inactivation by inhibiting IL-2 production, subsequently reducing IFN-γ production by T lymphocytes. [23] The anti-inflammatory activity of IL-10 aims to minimize tissue damage at the disease site by inhibiting the production of proinflammatory cytokines. [24]Furthermore, we observed elevated IL-10 levels in pTBpatients. The consistent levels of IL-10 and TNF-α imply a compensatory response, suggesting that to counteract TNF-α-induced tissue damage, patients may produce IL-10 cytokine. Olobo J et al. [13] reported elevated TNF-α, IL-10, and TGF-β levels in pleural fluid, with IL-10 exhibiting the highest concentration, followed by TGF-β and TNF-α. The effects induced by IFN-γ and TNF-α may indicate bacteriostasis rather than bacterial killing, raising the possibility that IL-10 also inhibits bacterial killing by macrophages rather than macrophages-mediated bacteriostasis.

Furthermore, Dlugovitzky et al. [11] demonstrated elevated serum IFN-γ levels in pTBpatients compared to control groups. Another study [12] also indicated increased cytokine levels in pTBpatients relative to control subjects. Pro-inflammatory cytokines, particularly interleukins (IL-1β, IL-6, IL-10), play a pivotal role in the pathogenesis of pTB. Our findings highlight elevated serum levels of these interleukins in pTBpatients, suggesting that assessing multiple cytokine levels may be valuable for evaluating TB disease activity and monitoring the clinical efficacy of antituberculosis treatment.

CONCLUSION

In conclusion, our study sheds light on the intricate dynamics of interleukins, specifically IL-1β, IL-6, and IL-10, in the context of pTB. The significant presence of TB-IgG and TB-IgM antibodies in pTB patients, along with pronounced increases in immunological parameters, emphasizes the pivotal role of these cytokines in the immune response against *Mycobacterium tuberculosis*. Noteworthy gender-specific variations in cytokine correlations suggest a nuanced immune landscape. Age-related differences further underscore the complexity of immune interactions in pTB. These findings have implications for targeted therapeutic interventions, emphasizing the need for personalized approaches considering both gender and age factors in managing pTB. Overall, our study contributes valuable insights towards understanding and addressing the multifaceted immune response in pTB. While our study provides valuable insights into the immune dynamics of pTB, it is crucial to acknowledge the limitation of the potential lack of generalizability inherent in a single-centric approach. To address this, we recommend future research to embrace multicentric studies, fostering a more comprehensive understanding by incorporating diverse research sites and populations.

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Source of funding: None

Consent: The authors have collected and preserved written participant consent per international or university standards.

Ethical approval: The author(s) has collected and preserved written ethical permission per international or university standards.

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