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ROLE OF CYTOKINES CHANGES IN THE DEVELOPMENT OF PULMONARY TUBERCULOSIS

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ABSTRACT

Introduction: Mycobacterium tuberculosis (MTB) remains a global health threat, demanding attention due to its wide geographic distribution. Immunity to MTB relies on Th1-cell activity, particularly cytokines such as Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α) and Transforming Growth Factor-beta (TGF-β) play crucial roles in protective immunity against MTB. This study delves into the impact of cytokine changes on immune responses and disease progression in the development of pulmonary tuberculosis (pTB).

Methodology: This three-year study involved 300 participants, stratified into 100 healthy controls and 200 with pTB. Blood samples were collected, and serum cytokine levels (TNF-α, IFN-γ, TGF-β) were analyzed using ELISA kits.

Results: In the pTB group, significant increases (P<0.001) in TNF-α, IFN-γ, and TGF-β indicated infection and inflammation. Males with pTB exhibited higher cytokine levels than controls, a pattern mirrored in females (P<0.001). Among males aged 20-40 with pTB, all parameters increased significantly. Similarly, females aged 20-40 with pTB showed significant elevations. In the 41-60 age group, both genders with pTB had significantly increased levels (P<0.001). Correlation analysis revealed a significant correlation among immunological parameters, emphasizing the impact of age and gender on cytokine changes in pTB.

Conclusion: These cytokines are valuable markers for assessing disease activity and inflammation in pulmonary tuberculosis (pTB).

KEYWORDS: *Mycobacterium tuberculosis*, Strains, Immune response, T lymphocytes, Cytokines

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INTRODUCTION

Mycobacterium tuberculosis (MTB) poses a critical global health threat, with approximately one-third of the world's population infected. [1] In 2021, the Global Tuberculosis Report highlighted that tuberculosis (TB) caused 1.6 million deaths globally. [1] In 2019, TB affected approximately 10 million people worldwide, ranking among the top ten causes of infectious disease-related mortality. [2] India, with 27% of the global 10 million cases, accounted for a substantial burden, including 25% of the estimated 1.6 million deaths. [3] In 2017, WHO estimated that 2.7 million individuals in India developed TB, resulting in over 400,000 deaths. [4] The National Family Health Survey (NFHS-4) recorded a self-reported TB incidence in India at approximately 304 per 100,000. [5] Clinical manifestations of tuberculosis encompass symptoms such as fever, anorexia, and a prolonged acute phase protein response, along with the recruitment of T lymphocytes to granulomas. [6] The interplay between T cells and infected macrophages is pivotal for protective immunity against MTB, involving key cytokines such as Tumor Necrosis Factor-alpha (TNF-α), Interferon-gamma (IFN-γ), and Transforming Growth Factor-beta (TGF-β). [7] Notably, TGF-β plays a crucial role in macrophage deactivation and suppressing T-cell responses to *MTB*. [8] Excessive TGF-β activity is observed in active pulmonary TB (pTB), and human mononuclear phagocytes infected or exposed to MTB show heightened TGF-β levels. [8] Insights from Dar et al. [9] indicate elevated C-reactive protein (CRP) and serum IFN-γ in active TB patients compared to healthy individuals, with CRP identified as a promising screening tool for TB. Additionally, a ranking of cytokines based on specificity revealed that IL-6, in conjunction with IFN-γ, exhibited the highest specificity for pTB. [10] TNF-α, playing a dual role in TB, contributes to disease pathophysiology and protective responses against *MTB*. It collaborates with IFN-γ to induce the formation of intermediates reactive to nitrogen and oxygen, mediating anti-TB activity in pTB patients. [11] However, excessive TNF-α production triggers oxidative stress, leading to tissue damage. Cytokines are found at elevated concentrations in more affected lungs during pTB, characterized by simultaneous immune depression and activation, necessitating acquired immune activation by CD4+ and CD8+ T-cells for infection control. [12] Thus, this study aims to explore the role of cytokine changes in the development of pulmonary tuberculosis.

MATERIAL AND METHODS

This study spanned a duration of three years and involved 300 participants aged between 20 and 60 years. The participants were stratified into 100 individuals designated as healthy controls and 200 individuals diagnosed with pulmonary tuberculosis. Prior to the commencement of the study, ethical clearance was obtained, and informed consent was secured from all participants. Participants were selected at the Medicine Outpatient Department (OPD) of Gandhi Medical College in Bhopal, Madhya Pradesh. Following the documentation of demographic information, 5 ml blood samples were obtained aseptically from the anticubital vein of individuals diagnosed with pTB for the purpose of immunological estimations. The collected blood was transferred to plain vials, allowing serum separation after clotting. The obtained supernatant serum was then subjected to centrifugation at 3000 rpm for 5 minutes. The Department of Microbiology analysed various serum cytokine levels using ELISA kit-based methods to assess immunological parameters. The investigated immunological parameters included Tumor Necrosis Factor-Alpha (TNF-α), Interferon-Gamma (IFN-γ) and Transforming Growth Factor-Beta (TGF-β). The specific ELISA kits and their corresponding catalogue numbers were employed for each analysis: TNF-α (Duoset ELISA, Dy210, USA), IFN-γ (8D OptEIA, 555138, USA) and TGF-β (Duoset ELISA Kit, Dy1679, USA). This comprehensive analysis of serum biomarkers aims to provide insights into the immunological profiles associated with pTB within the study population, with the chosen ELISA methodology ensuring accuracy and reliability in the measurements.

***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

Cytokine alterations were investigated in both a cohort of normal healthy control subjects (n=100) and individuals afflicted with pTB (n=200). The control group comprised 50 females and 50 males, while the pTB group comprised 130 males and 70 females. The control subjects were divided into 48 individuals in age group I and 52 in age group II. In contrast, the pTB subjects were distributed with 72 in age group I and 128 in age group II. (Figure-1 and 2) Table 1 illustrates the cytokine status in control and pTB subjects, revealing a significant increase in all immunological parameters: TNF-α, IFN-γ and TGF-β, in pTB subjects ($P<0.001$). This points to the presence of infection and inflammation in patients with pTB. Notably, males with pTB exhibited higher cytokine levels than controls ($P<0.001$), and a similar pattern was observed among female subjects. Furthermore, when analyzing cytokine changes in males aged 20-40 with pTB, all immunological parameters (TNF-α, IFN-γ, TGF-β) showed significant increases ($P<0.001$). Similarly, in females aged 20-40 with pTB, significant elevations were observed in TNF-α, IFN-γ, and TGF-β ($P<0.05$). In the age group of 41-60 years, both male and female patients with pTB exhibited significantly increased levels of TNF-α, IFN-γ, and TGF-β ($P<0.001$ and $P<0.01$, respectively), indicating the occurrence of pTB in this age group, particularly among females. (Table-2) Correlation analysis of cytokine changes in pTB subjects revealed significant correlations among immunological parameters. Moreover, when considering gender differences, TNF-α showed significant correlations ($P<0.001$) with IFN-γ and TGF-β in both male and female subjects. The analysis of cytokine changes in pTB subjects, stratified by age groups I and II, also demonstrated significant correlations ($P<0.01$). (Table-3)

DISCUSSION

Cytokines play an important role in regulating host immune response against intracellular pathogens, including *Mycobacterium tuberculosis*, by controlling the proliferation, differentiation, and effector functions of antigen-specific immune cells. Further, through autocrine and paracrine mechanism, cytokines regulate the production and the biological effects of one another, thus augmenting or diminishing beneficiary or detrimental host responses towards infectious agents. The interaction of T cells with infected macrophages is central to protective immunity against MTB and depends on the interplay of cytokines produced by each cell. [13]

Our study involved individuals diagnosed with pTB, with diagnosis based on clinical and radiological assessments by physicians. Some cases within this cohort presented concurrent conditions such as hypertension, diabetes, and coronary heart disease due to ageing. Therefore, we examined immunological parameters associated with the onset of pTB. Our observations indicated significant changes in immunological parameters among individuals of both genders aged 20 to 60, potentially attributed to the presence of pTB. These alterations may be linked to lung inflammation in individuals diagnosed with pTB.

We observed that in males of the age group 20 to 60 years, highly significant changes were present. Similarly, in females of this age group, significant changes in immunological parameters were noted. These observations aligned with the findings of D. Dlugovitzky et al. [14] and others [15], who reported similar findings, suggesting that altered metabolism might explain the diversity of immunological changes in pTB. Olobo J et al. [16] also reported significant changes in TNF-α, TGF-β, and IL-10 in pTB patients and healthy control groups.

All cytokines participated in the formation of granulomas in pTB. Evidence suggested that TNF-α was necessary at the beginning of the inflammatory process to limit the multiplication of mycobacteria. [16,17] Previous studies had shown higher serum levels of TNF-α in pTB patients than in control subjects. [18] Furthermore, an increased TNF-α level had been reported in the early stages of TB, extending to plasma from contacts of pTB patients suspected of being in the early stage of TB infection. [16]

Our results agreed with the above reports that serum TNF-α levels were elevated in pTB patients compared to controls. The study by Dlugovitzky et al. [19] demonstrated that the serum levels of IFN-γ were increased in pTB patients. In vitro evidence showed that TB patients with progressive disease failed to generate IFN-γ in response to stimulation with mycobacterial antigens. [20,21]

Other studies have shown that the evaluated serum IFN-γ levels in pTB patients increased relative to control subjects. [22,23] According to our results and others, IFN-γ is an important cytokine in TH1-mediated cellular immunity. T lymphocyte activation, following mycobacterial antigen recognition on antigen-presenting cells, induced various responses in CD4+ TH1 cells, including cytotoxic activity against infected macrophages to produce TNF-α and 1,25-dihydroxyvitamin D, both of which facilitated mycobacterial inhibition. [24] Mycobacterial products induced the production of TGF-β by monocytes and dendritic cells. TGF-β was produced in excess during pTB and expressed at the disease site. [25] It suppressed cell-mediated immunity; in T cells, it inhibited proliferation, IFN-γ production, and proinflammatory cytokine production, and it was known to counteract TNF-α production by monocytes in pTB. [26,27]

Our study found that the serum levels of TNF-α, IFN-γ, and TGF-β were significantly increased in both sexes of age groups I and II. This was consistent with the study of Olobo J et al. [16], who reported that these cytokines were significantly higher in pTB patients compared to control groups. Warwick-Davies J et al. [28] also showed increased serum TNF-α. Not only was TNF-α released in the serum, but along with this, other cytokines like TGF-β, IFN-γ, and IL-6 were also released in the serum. Females in the postmenopausal stage (age group II) were more prone to pTB due to the increased release of immunological parameters, i.e., TNF-α, IL-6, and IL-10. Estrogen, known for its cardioprotective and antioxidant properties in normal females, protects against the adverse effects of fat metabolism by preventing the oxidation of LDL. Hypercholesterolemia and hypertriglyceridemia also change to normal levels. Besides this, the ability of estrogen to target T cells, suppressing their production of TNF-α, is a key mechanism by which estrogen maintains the cytokine changes. [29]

These results prompted us to consider whether all these parameters were closely related to each other in age-related pTB. Therefore, we conducted correlation studies between all immunological markers. We observed a positive correlation of TNF-α, which was significantly correlated ($P<0.001$), with IFN-γ, and IFN-γ, which was significantly correlated ($P<0.001$), with TGF-β. TGF-β was significantly correlated ($P<0.001$). This was consistent with the study of Andrade Junior et al. [30]; they reported a positive correlation of TNF-α and other immunological parameters. TNF-α and IL-1β, associated with soluble TNF-alpha-receptor secretion imbalance, could correlate with pulmonary cavities in patients with pTB. [31] However, there were few studies in the medical literature about the correlation between serum TNF-α and other immunological parameters; further studies are necessary to

understand this phenomenon. We also found that TNF-α, IFN-γ, and TGF-β levels were elevated in pTB patients.

TNF-α was involved in the disease's pathophysiology and the immune response against MTB and other mycobacterial infections. [32] Its role was complex, and it was accepted that it acted in synergy with IFN-γ, inducing the formation of intermediates reactive to nitrogen and other oxygen, mediating the anti-TB activity of macrophages. [28,33] However, it has also been mentioned that TNF-α may be involved in the destruction of pulmonary tissue. The IL-10 cytokine had been considered an anti-inflammatory cytokine, acting in the inactivation of macrophages by inhibiting the production of IL-12 and consequently reducing the production of IFN-γ by T lymphocytes. [34] The IL-10 activity minimized the tissue impairment that occurred at the disease site by inhibiting the production of proinflammatory cytokines. [35]

Our study identified elevated levels of IL-10 in pTB patients, with consistent IL-10 and TNF-α levels, suggesting a compensatory mechanism to counter TNF-α-induced tissue damage. Olobo et al. [16] demonstrated heightened TNF-α, IL-10, and TGF-β in pleural fluid, with IL-10 levels surpassing TGF-β and TNF-α. The effects of IFN-γ and TNF-α may have signified bacteriostasis rather than killing, proposing IL-10's role in inhibiting macrophage bacterial killing while preserving bacteriostasis. Dlugovitzky et al. [14] highlighted elevated serum IFN-γ in pulmonary TB. Deveci et al. [15] and other studies reported increased cytokines in pulmonary TB, indicating immunological complexities. TNF-α and TGF-β, produced in excess during TB, influenced mycobacterial multiplication and granuloma formation. TNF-α's multiple roles and its involvement in host-mediated destruction emphasized pulmonary TB as an immunological disease. [24] This underscored the impact of age and socioeconomic conditions on immunological parameters in pulmonary TB.

CONCLUSION

The pathogenesis of pTB is notably influenced by proinflammatory cytokines. Our findings demonstrate elevated levels of anti-inflammatory cytokines (TNF-α, TGF-β, IFN-γ) in the serum of pTB patients. Measuring these cytokines in serum holds promise for assessing TB disease activity and monitoring the clinical efficacy of antituberculous treatment. Cumulatively, the outcomes of this study support the potential use of these cytokines as markers for gauging disease activity and inflammation in pTB, contributing to a better understanding of the immunological dynamics associated with the disease. Despite these insights, it's important to acknowledge the limitation of relying solely on serum cytokine levels, as they may not fully capture the intricate immune responses within the pulmonary microenvironment. For future studies, we recommend incorporating longitudinal assessments of cytokine levels and considering local immune responses in lung tissues to provide a more comprehensive understanding of pTB dynamics. Additionally, a larger and more diverse study population would enhance the generalizability of findings across different demographic groups.

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ETHICAL APPROVAL: As per international or university standards, the author(s) has collected and preserved written ethical permission.

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TABLES AND FIGURES

TABLE-1: Cytokine levels in control and pulmonary tuberculosis subjects stratified by gender.

Parameters			Cytokines		
			TNF-a (pg/ml)	**IFN-y (pg/ml)**	**TGF-β (pg/ml)**
Study Groups	**Control subject (n=100)**	**Mean ±SD**	1.24±0.20	1.30±0.21	1.30±0.21
	Pulmonary tuberculosis subjects (n=200)		4.01±0.89***	4.90±0.90***	7.50±1.10***
Male	**Control subject (n=100)**		1.57±0.26	1.47±0.22	1.35±0.21
	Pulmonary tuberculosis male subjects (n=130)		4.04±0.94***	4.98±0.98***	7.55±1.57***
Female	**Control subject (n=50)**		1.14±0.12	1.19±0.13	1.21±0.19
	Pulmonary tuberculosis female subjects (n=70)		3.15±0.45**	3.70±0.56**	4.21±0.78**

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

TABLE-2: Cytokine levels in control and pulmonary tuberculosis subjects stratified by gender and age groups.

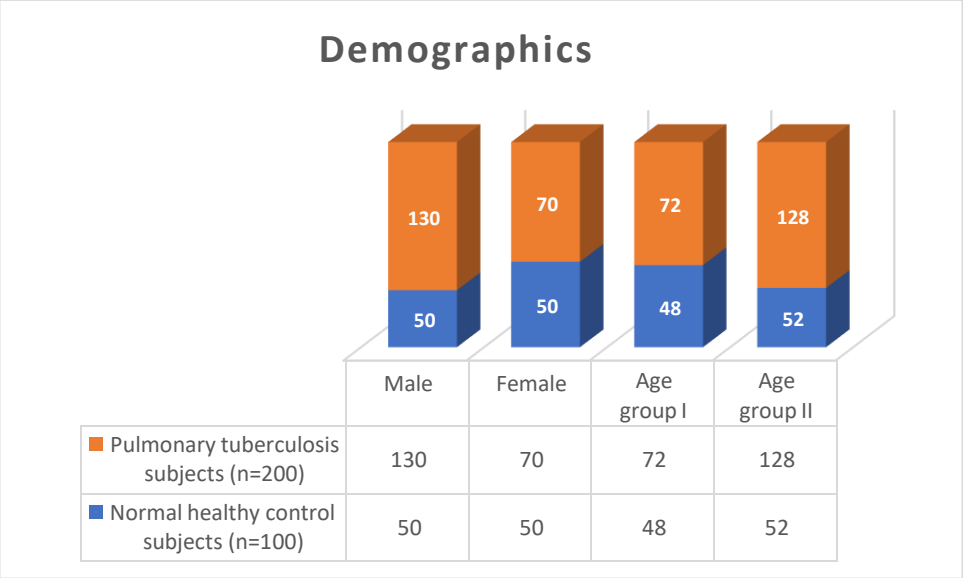
Parameters			Cytokines		
			TNF-a (pg/ml)	IFN-y (pg/ml)	TGF-β (pg/ml)
Male	Control subject age group I (n=22)	Mean ± SD	1.35±0.23	1.34±0.21	1.27±0.19
	Pulmonary tuberculosis subjects age group I (n=40)		3.54±0.79**	4.01±0.85**	6.75±1.27**
	Control male subject age group II (n=28)		1.65±0.29	1.54±0.25	1.42±0.24
	Pulmonary tuberculosis male subjects age groups II (n=90)		4.67±1.10***	5.10±1.14***	8.12±1.87***
Female	Control female subject age group I (n=26)		1.10±0.10	1.12±0.11	1.15±0.14
	Pulmonary tuberculosis female subjects age group I (n=32)		2.98±0.35**	3.01±0.45**	3.98±0.51**
	Control female subject age group II (n=22)		1.25±0.16	1.20±0.13	1.29±0.19
	Pulmonary tuberculosis female subjects age groups II (n=40)		4.15±0.51***	4.14±0.61***	5.10±0.81***

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

TABLE-3: Correlation analysis of cytokine levels in pulmonary tuberculosis subjects stratified by gender and age groups.

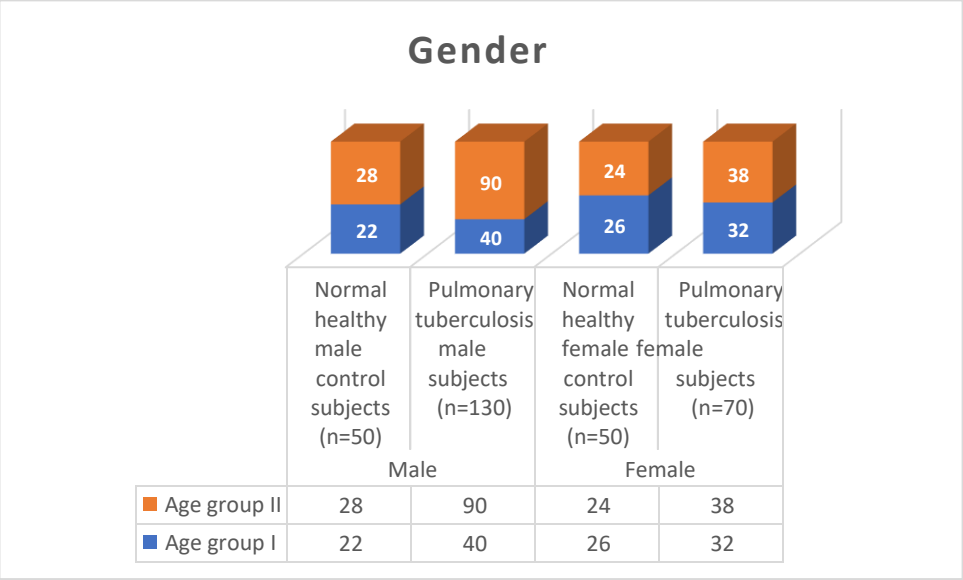
Study groups	Variables	IFN-γ	TGF-β
Pulmonary tuberculosis	TNF-α	0.94***	0.86***
	IFN-γ	-	0.71***
	TGF-β	-	-
Pulmonary tuberculosis male subjects	TNF-α	0.91***	0.84***
	IFN-γ	-	0.24*
	TGF-β	-	-
Pulmonary tuberculosis female subjects	TNF-α	0.36***	0.22*
	IFN-γ	-	0.31***
	TGF-β	-	-
Pulmonary tuberculosis male subjects of age group I	TNF-α	0.34**	0.36**
	IFN-γ	-	0.40**
	TGF-β	-	-
Pulmonary tuberculosis female subjects of age group I	TNF-α	0.33*	0.19^{NS}
	IFN-γ	-	0.71***
	TGF-β	-	-
Pulmonary tuberculosis male subjects of age group II	TNF-α	0.93***	0.36**
	IFN-γ	-	0.27**
	TGF-β	-	-
Pulmonary tuberculosis female subjects of age group II	TNF-α	0.32*	0.40**
	IFN-γ	-	0.39**
	TGF-β	-	-

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



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

FIGURE-1: Demographic distribution of subjects.



	Male		Female	
	Normal healthy male control subjects (n=50)	Pulmonary tuberculosis male subjects (n=130)	Normal healthy female control subjects (n=50)	Pulmonary tuberculosis female subjects (n=70)
Age group II	28	90	24	38
Age group I	22	40	26	32

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