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Original Article

Internal Medicine Section

Role of Biomarkers (Interleukins (IL)-2, 5,6,16,17,1β) in Saliva and Serum for Diagnosis of Pulmonary Tuberculosis and for Monitoring Response to Intensive Phase Treatment

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ABSTRACT

Introduction: Majority of the currently used diagnostic tests for Pulmonary Tuberculosis (PTB) have variability in sensitivity and specificity in diagnosing PTB. Therefore, evaluating new biomarkers in easily obtainable samples like serum and saliva can contribute to the diagnosis of PTB.

Aim: To evaluate the role of Interleukins(IL)- 2,5,6,16,17,1β in the diagnosis of PTB by comparing their levels in Other Respiratory Diseases (ORD) group and monitoring the response to treatment in PTB group.

Materials and Methods: This prospective observational study was conducted between January 2021 to May 2022 in a tertiary care hospital in New Delhi, India. A total of 80 cases were taken of which 40 cases of PTB and 40 cases having diagnosis of ORD were studied. IL-2,5,6,16,17,1β levels were measured in saliva and serum using Enzyme Linked Immune Sorbent Assay (ELISA) kits. These IL were also measured in diagnosed PTB patients in serum and saliva after two months of treatment. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 16.0. Kruskal Wallis H Test, Wilcoxon signed-rank sum test, Chi-square test and Fisher's exact test were applied at appropriate areas and a p-value <0.05 was taken as significant.

Results: On comparing the levels of IL between serum and saliva in PTB group at baseline, IL-2, IL-17 levels were higher in serum while IL-5, IL-1β levels were higher in saliva with these results being statistically significant (p-value <0.001). However, after two months of treatment, the levels of IL-2, IL-16 decreased significantly in both serum and saliva whereas IL-17 level decreased significantly only in serum after treatment. None of the IL showed significant difference in levels between serum and saliva in PTB and ORD groups.

Conclusion: The diagnostic role of IL in PTB could not be established whereas IL-2, IL-16 and IL-17 can be used for monitoring response to treatment as the levels decreased significantly with treatment.

Keywords: Antitubercular treatment, Cytokines, Other respiratory diseases

INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by *Mycobacterium tuberculosis* and is a major cause of morbidity and mortality worldwide [1]. The major reasons for the global TB epidemic were delay or failure in diagnosis [2]. Therefore, need for more accurate, rapid and cost-effective methods for the early diagnosis of TB are required. Majority of the currently used diagnostic tests have several shortcomings. Smear microscopy, the most widely available diagnostic test has poor sensitivity [3]. Sputum culture, the gold standard has a long turnaround time (upto 42 days) [4], whereas the recently developed GeneXpert, although rapid, is expensive which hamper its use in resource poor countries [5]. Therefore, evaluating new biomarkers (IL) in easily obtainable samples like serum and saliva can contribute to the diagnosis of PTB especially in people who have non productive cough or unable to expectorate.

A study by Phalane KG et al., compared the levels of biomarkers like IL 2,5,6 and 17 in serum and saliva. The IL 2,5,17 was more abundantly expressed in saliva in all participants whereas IL-6 showed no difference in expression between serum and saliva. IL-17 levels in saliva were higher in TB patients (18.9 pg/mL) compared to controls (12.6 pg/mL) [6]. The response of these IL to treatment was not studied.

A study by Jacobs R et al., also showed that some of the biomarkers detected in saliva (IL16, 17 and 1ß) showed diagnostic potential for PTB. Investigation was done into the usefulness of using host biomarkers in monitoring of treatment response and levels of some markers were found to change at the end of treatment (IL-17A). IL-17 levels were significantly higher in TB patients (13.8 pg/mL) compared to ORD patients (6.1 pg/mL) [7]. The results of IL-17 were similar in both the studies. There is no study available on these biomarkers from Indian population. Also, all of them have not been assessed in serum and saliva either for diagnosis or for monitoring response to treatment.

Thus, in this study, authors estimated the IL-2,5,6,16,17,1β levels in both serum and saliva in PTB group and ORD group separately to evaluate their role in diagnosis of PTB and their response to treatment in PTB was assessed.

MATERIALS AND METHODS

This prospective observational study was conducted in a Tertiary care hospital, New Delhi, India from January 2021 to May 2022. Ethical clearance was obtained from the Institutional Ethics Committee (IEC), ABVIMS and Dr. RML Hospital, New Delhi. (Approval number–TP(MD/MS)(124/2020)/IEC/ABVIMS/RMLH/390). Informed consent was taken from all participants.

Inclusion criteria: The study included cases of >18 years with signs and symptoms suggestive of PTB like cough for atleast two weeks with or without expectoration, fever, night sweats, unintentional weight loss, chest pain, hemoptysis, fatigue, malaise and anorexia [8].

Age >18 years with symptoms of fever, cough with or without expectoration, breathlessness lasting for atleast two weeks who were clinicoradiologically and microbiologically negative for PTB were considered in the ORD group.

Exclusion criteria: Pregnant and lactating women, patients already on Antitubercular Treatment (ATT) at the time of enrollment, patients who were on quinolones or aminoglycoside antibiotics, patients having concomitant chronic inflammatory and autoimmune disorders like rheumatoid arthritis, inflammatory bowel disease, psoriasis, ankylosing spondylitis, multiple sclerosis, sarcoidosis, systemic lupus erythematosus, patients with acute fever or inflammatory condition and patients having malignancy were excluded from the study.

Sample size calculation: In the study by Phalane KG et al., IL-6 has been shown to be significantly different in patients with PTB from patients having ORD in both saliva and serum [6]. At a cut-off level of >27.54, IL-6 had specificity of 96.3%. Using these values and at 95% level of confidence and 80% power and with prevalence of TB being 40%, the calculation of sample size for present study is as follows:

$$n=\frac{(Z_{\alpha-1/2})^2\times Specificity\times(1-specificity)}{(Precision)^2\times(1-prevalance)}$$

Where, $Z_{\alpha-1/2}=1.96$

Specificity=96.3%

Precision=5%

$$n=\frac{(1.96)^2\times0.963\times(0.037)}{(0.05)^2\times(0.6)}$$

$$=\frac{0.1368}{0.0015}=91.2$$

Study Procedure

After obtaining a valid consent, those participants who met the inclusion criteria were subjected to a detailed history and clinical examination, routine blood investigations, sputum microscopy and culture examination and chest X-Ray. Other tests like arterial blood gas analysis, ultrasound abdomen, contrast enhanced Computed Tomography (CT) chest, Fine Needle Aspiration Cytology (FNAC) from any enlarged lymph node, Cerebro-Spinal Fluid (CSF) analysis, Pleural Fluid (PF) analysis and Ascitic Fluid (AF) analysis were done, if required.

After obtaining results of these investigations, a participant was diagnosed as:

- PTB (Sputum positive or Sputum negative radiologically confirmed) [9] or
- ORD (having co-morbid lung illnesses like chronic obstructive pulmonary disease, asthma, interstitial lung disease, past history of TB).

Out of 55 participants diagnosed as PTB, 15 were lost to follow-up and of 45 participants diagnosed as ORD, 5 were excluded as they were found to have underlying TB later. IL-2,5,6,16,17,1β levels in saliva and serum were measured in participants of both the groups at the time of diagnosis.

The IL was also measured in diagnosed pulmonary TB participants in serum and saliva after two months of treatment.

For collecting saliva samples, participants were asked to fast for atleast one hour and unstimulated saliva samples were collected in a sterile container. It was then transported to the laboratory as per the instructions provided in the kit. IL was measured by ELISA. Kits used are mentioned below.

IL-2: Fine Test, Wuhan Fine Biotech Co., Ltd., (Wuhan, China)

IL-5: Shanghai Coon Koon Biotech Co., Ltd., (Shanghai, China)

Il-6: Bio-Detect (California, United States of America)

Il-16: Shanghai Coon Koon Biotech Co., Ltd., (Shanghaai, China)

IL-17: Diaclone, Weldon Biotech (I) Pvt., Ltd., (Delhi, India)

IL-1β: Fine Test, Wuhan Fine Biotech Co., Ltd., (Wuhan, China)

STATISTICAL ANALYSIS

The analysis was done on SPSS software (version 16, IBM Inc. Chicago). All categorical variables were mentioned as frequency (%). All quantitative variables were mentioned as mean±Standard Deviation (SD). Kruskal Wallis H Test was used to compare the IL levels between the two groups (participants with PTB and participants with ORD). For comparison between baseline and post two months treatment values of serum and saliva IL, Wilcoxon signed-rank sum test was used. The p-value <0.05 was taken as significant.

RESULTS

Eighty participants (40 with active PTB and 40 with ORD) were available for analysis. The mean age was higher in the ORD group (Mean±SD=49.83±10.06 years) compared to PTB group (Mean±SD=38.40±14.86 years) and 75% of the participants were males (60 participants).

The IL-2 and 17 levels were significantly higher in serum compared to saliva whereas IL-5 and 1β levels were significantly higher in saliva compared to serum in PTB group at the time of diagnosis (p<0.001) [Table/Fig-1].

None of the IL showed significant difference in levels between serum and saliva in two groups. However, IL-17 levels were nearly significantly different between two groups in both serum and saliva with higher level in serum in PTB group and higher level in saliva in ORD group [Table/Fig-2].

Interleukin	Serum (pg/mL)	Saliva (pg/mL)	p-value
IL-2	55.43±7.68	17.8±12.14	<0.001
IL-5	75.75±19.93	110.06±18.55	<0.001
IL-6	14.46±14.56	9.78±3.70	0.472
IL-16	1253.91±447.60	1030.94±310.29	0.008
IL-17	11.70±6.40	3.65±0.75	<0.001
IL-1β	12.44±32.22	82.67±104.2	<0.001

[Table/Fig-1]: Comparison of Interleukin (IL) levels (pg/mL) between serum and saliva in PTB group at the time of diagnosis.
All values are expressed in mean±standard deviation. IL: Interleukin

The levels of IL-2, 16 and 17 showed a significant fall in serum with treatment whereas the levels of IL-2, and 16 showed a significant fall in saliva with treatment (p-value <0.05) [Table/Fig-3].

	Serum (pg/mL)			Saliva (pg/mL)		
Interleukin	PTB group	ORD group	p-value	PTB group	ORD group	p-value
IL-2	55.43±77.68	39.04±27.66	0.348	17.80±12.14	16.36±6.67	0.931
IL-5	75.75±19.93	95.28±84.67	0.422	110.06±18.55	110.21±12.12	0.769
IL-6	14.46±14.56	12.28±42.11	0.722	9.78±3.70	10.53±5.53	0.758
IL-16	1253.91±447.6	1382.62±519.45	0.519	1030.94±310.29	1009.84±278.85	0.63
IL-17	11.7±6.4	10.76±14.42	0.08	3.65±0.75	3.75±0.70	0.054
IL-1β	12.44±32.22	8.95±19.48	0.541	82.67±104.2	75.77±97.72	0.832

[Table/Fig-2]: Comparison of Interleukin (IL) levels (pg/mL) in serum and saliva between PTB group at the time of diagnosis and ORD group.
All values are expressed in mean±standard deviation. IL: Interleukin; PTB: Pulmonary tuberculosis; ORD: Other respiratory diseases

Interleukin	Serum (pg/mL)			Saliva (pg/mL)		
	Diagnosis	Follow-up	p-value	Diagnosis	Follow-up	p-value
IL-2	55.43±77.68	11.41±22.34	<0.001	17.80±12.14	10.06±5.94	<0.001
IL-5	75.75±19.93	90.61±12.45	<0.001	110.06±18.55	110.93±14.41	0.856
IL-6	14.46±14.56	14.41±8.94	0.354	9.78±3.70	14.78±9.49	0.01
IL-16	1253.91±447.6	913.66±116.21	<0.001	1030.94±310.29	850.77±133.95	<0.002
IL-17	11.7±6.4	9.03±3.35	0.003	3.65±0.75	3.62±1.52	0.657
IL-1β	12.44±32.22	29.07±39.09	0.181	82.67±104.2	58.59±43.53	0.788

[Table/Fig-3]: Comparison of IL levels (pg/mL) in serum and saliva in PTB group at the time of diagnosis and after two months of treatment (follow-up).
All values are expressed in mean±standard deviation. IL: Interleukin; PTB: Pulmonary tuberculosis

DISCUSSION

The present study shows that IL-2,5,6,16,17 and 1β were not significantly different between PTB patients and ORD patients and hence, they have no role in diagnosing PTB. However, after two months of intensive phase treatment, the levels of IL-2 and IL-16 decreased significantly in both serum and saliva whereas, IL-17 level decreased significantly only in serum. IL-2, IL-16 and IL-17 levels monitoring may be used to assess response to treatment in PTB patients especially in those who are unable to produce sputum or had sputum negative PTB.

Role of IL in serum for diagnostic purposes of PTB has been studied in past also. [Table/Fig-4] shows summary of such studies conducted [6,10-12].

Interleukin	Study	Place of study	TB cases	ORD cases or healthy controls	p-value
IL-2	Turgut T et al., 2006 [10]	Turkey	164±58.91 pg/mL n=30	79.20±14.81 pg/mL n=15 (healthy controls)	<0.001
IL-5	Namuganga AR et al., 2017 [11]	Uganda	3.6 pg/mL n=39	1.6 pg/mL n=39 (latent TB infection and healthy controls)	<0.001
IL-6	Phalane KG et al., 2013 [6]	South Africa	11.5 pg/mL n=11	0 pg/mL n=27 (Non TB cases)	0.01
IL-17	Hafid ES and Ismael MK 2021 [12]	Iraq	43.06±3.64 pg/mL n=50	41.009±0.009 pg/mL n=40 (healthy controls)	
IL-2	Present study	India	n=40 55.43±77.68 pg/mL	n=40 39.04±27.66 pg/mL	0.348
IL-5			75.75±19.93 pg/mL	95.28±84.67 pg/mL	0.422
IL-6			14.46±14.56 pg/mL	12.28±42.11 pg/mL	0.722
IL-17			11.7±6.4 pg/mL	10.76±14.42 pg/mL	0.08

[Table/Fig-4]: Studies showing the diagnostic role of Interleukins (IL) in serum in Pulmonary Tuberculosis (PTB) [6,10-12].

For IL-2,6 and 17, the results in present study were similar to the previous studies [6,10,12]. The result about IL-5 as observed in present study was not similar to the previous study [11]. IL-5 is a strong proinflammatory cytokine, exerting multiple effects on eosinophil maturation, activation, survival, migration from bloodstream and recruitment to airways causing pulmonary inflammation and thereby, causing underlying allergic airway diseases [13]. The ORD group in the present study as mentioned earlier had co-morbid lung illnesses like chronic obstructive pulmonary disease, asthma, and past history of PTB. Hence, IL-5 could be elevated in serum due to this reason. There are no previous studies showing the diagnostic role of IL-16 and IL-1β in serum in PTB.

Unstimulated saliva is an easily obtainable sample as compared to serum to assess IL levels.

A brief description of studies analysing interleukins in saliva for diagnosing PTB is presented in [Table/Fig-5] [6,7,11].

Interleukin	Study	Place of study	TB cases	ORD cases or healthy controls	p-value
IL-5	Namuganga AR et al., 2017 [11]	Uganda	1.6 pg/mL n=39	1.6 pg/mL n=39 latent TB infection and healthy controls)	0.52
IL-6	Namuganga AR et al., 2017 [11]	Uganda	5.11 n=39	4.8 n=39 latent TB infection and healthy controls	0.019
IL-16	Jacobs R et al., 2016 [7]	South Africa	20.01 pg/mL n=22	56.1 pg/mL n=33 (ORD cases)	0.016
IL-17	Phalane KG et al., 2013 [6]	South Africa	18.9 pg/mL n=11	12.6 pg/mL n=27 (Non TB cases)	0.085
IL-1β	Jacobs R et al., 2016 [7]	South Africa	16.9 pg/mL n=22	36.4 pg/mL n=33 (ORD cases)	0.027
IL-5	Present study	India	n=40 110.06±18.55 pg/mL	n=40 110.21±12.12 pg/mL	0.769
IL-6			9.78±10.53 pg/mL	10.53±5.53 pg/mL	0.758
IL-16			1030.94±310.29 pg/mL	1009.84±278.85 pg/mL	0.63
IL-17			3.65±0.75 pg/mL	3.75±0.70 pg/mL	0.054
IL-1β			82.67±104.2 pg/mL	75.77±97.72 pg/mL	0.832

[Table/Fig-5]: Studies showing the diagnostic role of Interleukins in saliva in Pulmonary Tuberculosis (PTB) [6,7,11].

The results for IL-5,6,17 and 1β in present study were comparable to the previous studies [6,7,11]. However, the difference observed was not significant. Result for IL-16 was different from the previous study. IL-16 was initially called a lymphocyte chemoattractant factor, because of its ability to attract CD4 T cells. IL-16 can increase the synthesis of proinflammatory cytokines, including IL-1, IL-6 and TNF-α [14]. This cytokine is considered to be both proinflammatory and immunoregulatory with an important role in the recruitment and activation of immune cells at the site of injury either eliminating the infecting organism or resulting in granuloma formation. Also, as stated by Namuganga AR et al., immunological responses differ among people in different regions [11]. These could be the reasons for higher IL-16 levels observed in saliva in present study PTB patients.

Monitoring response to TB is an important aspect in TB management. For PTB, response is assessed by sputum examination for detecting acid fast bacilli. However, after treatment many patients have scanty or no sputum examination. Also, monitoring patients who had sputum negative PTB cannot be done with sputum examination. Hence, interleukins in serum and saliva have been studied previously as markers to assess response to treatment of TB. The studies showing the change in levels of interleukins in serum with treatment are enlisted below in [Table/Fig-6] [15-18].

Interleukin	Study	Place of study	Diagnosis	Follow-up	p-value
IL-2	Berktas M et al., 2004 [15]	Turkey	164.5 pg/mL n=18	92.11 pg/mL n=18	<0.05
	Al-Khafaji JK et al., 2015 [16]	Iraq	169.429±69.139 pg/mL n=60	96.997±27.113 pg/mL n=60	0.003
IL-17	Xu L et al., 2016 [17]	China	Higher n=20	Lower n=20	<0.001
IL-1β	Anusiem CA and Okonkwo PO, 2017 [18]	Nigeria	30.2±2.0 pg/mL n=42	21.8±1.1 pg/mL n=42	<0.05
	Present study	India	n=40	n=40	
IL-2			55.43±77.68 pg/mL	11.41±22.34 pg/mL	<0.001
IL-17			11.7±6.4 pg/mL	9.03±3.35 pg/mL	<0.001
IL-10			12.44±32.22 pg/mL	29.07±39.09 pg/mL	0.11

[Table/Fig-6: Studies showing the change in the levels of Interleukins in serum with treatment [15-18].

The results for IL-2 and IL-17 in present study were similar to the previous studies [15-17]. The dissimilarity observed in results for IL-1β could be due to different races (African and Asian) of the study population. There are no previous studies showing the response of IL-5, 6 and16 either in serum or saliva with treatment.

Regarding interleukins in saliva to assess response to treatment, Jacobs R et al., showed that the mean level of IL-17 in saliva at baseline was 13.4 pg/mL and at follow-up was 8.2 pg/mL [7]. This was consistent with the present study results. For other interleukins, there are no studies investigating the change in levels of those biomarkers in saliva with treatment in PTB.

Limitation(s)

The main limitation of present study was the small sample size. Hence, future studies may be conducted in larger population so as to obtain any clinical significance, if at all it exists, between interleukin levels of patients with PTB and ORD for the purpose of diagnosis. In this study, ORD group had patients who had other concomitant diseases which could have altered the levels of interleukins. In future studies, ORD group may be taken as patients having only lower respiratory tract infection and having no other concomitant illness. Present study had not included patients with extra PTB. Future studies can enroll PTB and extra PTB and study the interleukins for diagnostic purpose as well as for monitoring response to treatment.

CONCLUSION(S)

On comparing the levels of interleukins between serum and saliva in PTB group at baseline, IL-2, IL-17 levels were higher in serum while IL-5, IL-1β levels were higher in saliva with these results being statistically significant with p-value <0.001. There was no statistically significant difference in the level of interleukins between PTB group and ORD group either in serum or saliva at the time of diagnosis. However, on follow-up, the levels of IL-2, IL-16 decreased significantly in both serum and saliva after two months of treatment whereas IL-17 level decreased significantly only in serum after treatment.

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