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Research Article

Evaluation of the Feasibility of using Urine IP-10 as a Biomarker to Assess the Treatment Response to the Pharmacotherapy of Active Pulmonary Tuberculosis in the Intensive and Continuation Phase

Padmaja Sukumar[1], Ruckmani Arunachalam[2], Meenakshi Narasimhan[3]

[1]Postgraduate, [2]Former Professor and Head, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu, Tamil Nadu, India.
[3]Professor and Head, Department of Respiratory Medicine, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu, Tamil Nadu, India.
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I N F O

Corresponding Author:
Ruckmani Arunachalam, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu, Tamil Nadu, India.
E-mail Id:
chetpharmrp@gmail.com
Orcid Id:
https://orcid.org/0000-0002-4581-9787
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A B S T R A C T

Introduction: Tuberculosis is well known for its chronicity, treatment failures, and drug resistance. Interferon-gamma Inducible Protein 10 (INF IP-10) has been reported to be relatively specific for assessing the severity of tuberculosis, and it can be easily estimated in both urine and blood.

Objective: To determine whether urinary IP-10 levels can be used as a biomarker for monitoring treatment response in patients with active Pulmonary Tuberculosis (PTb).

Materials and Method: 40 participants were enrolled. Urine samples were collected at diagnosis, at the end of 1st, 2nd & 6 months. Sputum smear and culture were done at diagnosis, end of 2nd and 6th month. IP-10 levels were estimated and correlated with treatment response.

Results: All the patients were positive for Mycobacterium Tuberculosis (Mtb) at baseline. At the end of 2nd and 6 months, all of them became smear and culture-negative. The mean urine IP-10 values at diagnosis, end of 1st, 2nd and 6th month were 10.76 ± 2.76, 15.37 ± 3.09, 21.83 ± 4.10 and 8.38 ± 2.46 pg/dl. IP-10 levels increased following intensive therapy and decreased significantly towards the end of treatment. The mean values of IP-10 at baseline, at the end of 2nd and 6th month were correlated with mean scores of clinical symptoms at respective time points. Pearson's linear correlation was done which showed that IP-10 values and clinical symptoms did not correlate with each other with p=0.836.

Conclusion: Increase in IP-10 level during the intensive therapy indicates the response to treatment and bacterial clearance. Hence urinary IP-10 can be considered as biomarker for monitoring treatment response in PTb patients.

Keywords: Pulmonary Tuberculosis, Urine IP-10, Biomarker

Introduction

WHO 2021 Tuberculosis (TB) report shows that globally ten million people were infected by TB.[1] Moreover, there is a concern for an increasing rate of disease relapse/ reinfection, after the completion of tuberculosis treatment. Also, there is a raising trend of drug-resistant TB (multidrug/ extensive M/ XDR) around the world. Treating drug-resistant TB is even more challenging and complicated. The current treatment of tuberculosis involves a combination of antimicrobials. A longer duration of treatment is necessary to eradicate the heterogenous population of mycobacteria.[2] Response to treatment is assessed at present by standard methods like sputum conversion and clinical outcome. Treatment failure can be due to many reasons such as poor compliance to treatment, comorbidities, and the development of drug resistance. The early assessment of the overall response to anti-TB treatment is mandatory for treating the patient effectively and helping the clinicians adopt alternative therapy.

In this regard, several biomarkers have been reported to be of use in diagnosing and assessing the severity of tuberculosis and some of them include Mtb cell wall Lipoarabinomannan (LAM), Tumour Necrosis Factor (TNF) alpha, Interleukin (IL)-1b, IL-2, IL-6, IL-23, Inducible Protein (IP)-10 and others.[3] Among these biomarkers, IP-10 which is an Interferon-gamma (IFN – ɣ) inducible protein 10, has clinical relevance in TB and it has been detected in the bronchoalveolar lavage of active TB patients.[4] It is also known as CXCL (CXC chemokine (C-X-C motif) ligand) 10, a member of the cytokine family. It is a tiny protein that interacts with CXCR 3 to mediate immunological responses by activating and attracting leucocytes as well as T cells, monocytes, eosinophils and natural killer cells at the site of inflammation.[5] IP-10 is secreted by a variety of cells such as astrocytes,[6] monocytes,[7] neutrophils,[8] endothelial cells,[9] keratinocytes,[10] fibroblasts,[11] mesenchymal cells,[12] hepatocytes,[13] and dendritic cells.[14] Many studies have evaluated the role of IP-10 as a biomarker for pulmonary tuberculosis.[4,15,19]

Though its presence and importance in tuberculosis have been evaluated, its role as a prognostic marker has not been studied in detail. Hence this present study was conducted to estimate the IP-10 levels in urine during the course of anti-tubercular drug therapy and after completion of the treatment in patients with active pulmonary tuberculosis and correlate these levels with clinical and laboratory outcomes in order to evaluate its use as a biomarker for assessing the patient response to treatment.

Materials and Methods

This was a prospective pre-post single-arm study. A total of 40 newly diagnosed pulmonary tuberculosis patients, aged 18 to 70 years, who were positive for sputum AFB were enrolled in the study. Pregnant and lactating women, patients with other comorbid conditions like hypertension, diabetes mellitus, hepatic disease, renal disease, and HIV-positive patients were excluded. The study was initiated after getting approval from the Institutional Human Ethics Committee. The study was conducted in Chettinad Hospital and Research Institute, Chengalpattu, Tamilnadu for 1 year and 4 months from April 2021 to July 2022. After taking written informed consent from the patients, their demographic details were recorded. Spot urine sample of approximately 10 ml was collected from the subjects at the time of diagnosis, during the treatment (after 1st and 2nd months of treatment), and after the completion of six months of treatment. Sputum smear examination and bacteriological culture were done before the start of therapy, and after 2 and 6 months of ATT. Clinical symptoms were also recorded before the start of therapy and monitored after 2 and 6 months of ATT. Urine IP-10 was estimated using an ELISA kit (BT Lab Pvt Ltd) according to the manufacturer's protocol. To analyse the improvement or worsening of clinical symptoms with treatment, each symptom was given a score of 1, if it is present and 0, if not.[20] As we included 7 symptoms, each patient had a score out of 7, during each assessment i.e., at baseline, and end of 2 and 6 months. The patients were given standard anti-TB chemotherapy according to the national guidelines.

Sample Size Calculation and Statistics

The sample size was calculated using n Mater 2.0 as 40 participants, based on the probability of observing the difference in the study group before and after intervention as 85%, with 80% power, 95% confidence interval, and 10% attrition rate. The quantitative variables, IP-10, and score of clinical symptoms at baseline and end of 2 and 6 months were summarised as mean ± SD and compared using repeated measures analysis of variance (ANOVA) and Friedman's test respectively. P value < 0.05 was considered to be significant. The analysis was done using Graphpad Instat version 3.1 software.

Results

Demographic Details

In this study, 40 pulmonary TB patients were recruited. Among them, 31 were male and 9 were female. The mean age of the participants was 47 (± 14.8) years.

IP-10

The IP-10 levels were estimated at baseline and end of 1, 2, and 6 months and it was observed that there was a significant increase at the end of 1st and 2nd months as compared to the baseline, but at the end of the 6th month, the IP-10 levels significantly reduced, compared to the 2 months as well as baseline Table 1.

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Table 1.Urine IP-10 Values

Para-meter	Baseline (pg/ml)	End of 1st Month (pg/ml)	End of 2nd Month (pg/ml)	End of 6th Month (pg/ml)	P-value
IP-10	10.76 ± 2.76	15.37 ± 3.09	21.83 ± 4.10	8.38 ± 2.46	< 0.0001*

Statistics: Values expressed as mean ± SD, Within-group analysis: Repeated measures ANOVA, *Statistically significant

Clinical Symptoms

Among the baseline, the clinical symptom of cough with expectoration (75%) was the commonest, followed by loss of weight (70%), fatigue (65%), fever (55%), decreased appetite (25%), exertional breathlessness (12.5%), and chest pain (10%) Figure 1.

The mean clinical score of the participants was compared at baseline and at the end of the 2nd and 6th month. The mean scores significantly decreased at the end of the 2nd and 6th month as compared to baseline Table 2.

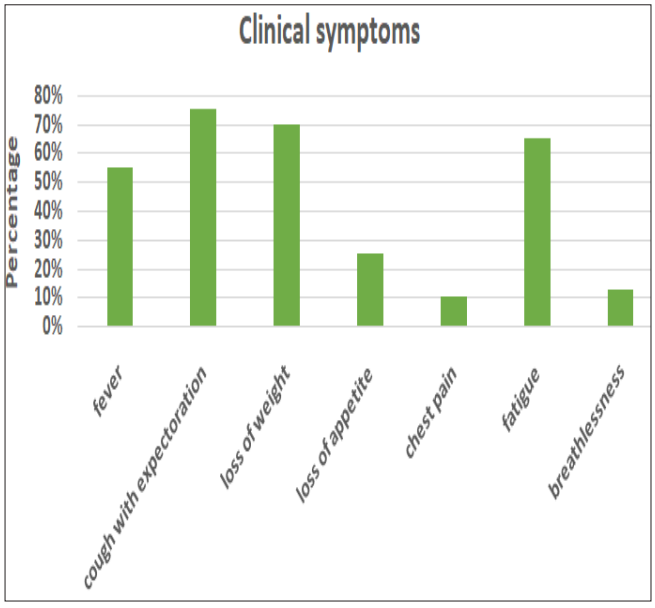

Figure 1.Clinical Symptoms Presentation at Baseline

Table 2.Mean Score of Clinical Symptoms

Parameters	Baseline	End of 2nd Month	End of 6th Month	P-value
Mean Score of Clinical Symptoms	3 ± 1.01	0.375 ± 0.54	0.05 ± 0.22	< 0.0001*

Statistics: Values expressed as mean ± SD, Within-group analysis: Friedman test *Statistically significant

Clinical Symptoms vs IP-10

The mean values of urine IP-10 at baseline, at the end of 2nd month and 6th month, were correlated with the mean scores of the clinical symptoms at the respective time points. Pearson's linear correlation was done and it was observed that IP-10 values and clinical symptoms did not correlate with each other (r = -0.2536) with p-value=0.836.

IP-10 vs Sputum Smear and Culture

Association between urine IP-10 and sputum smear as well as sputum culture results was not analysed, since all the patients turned out to be sputum negative and culture negative at the 2nd and 6th month of treatment.

Discussion

This study was done with the primary objective of finding out the usefulness of IP-10 as a prognostic urinary biomarker in newly diagnosed active pulmonary tuberculosis patients on treatment with ATT. New biomarkers as predictive factors to assess treatment response in tuberculosis are needed due to the limitation of using sputum conversion as an index of treatment response. For the identification of TB bacilli, there should be at least 10,000 bacilli/ml of sputum and the test is limited due to its poor sensitivity. The chance of getting a sputum-positive result is only less than 10% if the concentration of the bacilli falls below the cut-off value.

A sputum culture can be relatively sensitive but time-consuming and requires a Biosafety Level 3 facility. Collection of sputum from children is not easy as in adults, whereas, a urine sample can be easily collected from children and elders and the biomarker IP-10 in urine can be easily measured using an ELISA kit without any waiting time. Hence it can be used as a simple and quick way of assessing and monitoring the treatment response of tuberculosis patients.

The correlation between the IP-10 and the mean clinical symptom score values showed that IP-10 values and clinical symptoms did not correlate with each other.

The mean baseline IP-10 level was 10.76 pg/ml before the start of antitubercular treatment. After initiating intensive phase treatment for the period of 2 months, the mean level of IP-10 had increased to 21.83 pg/ml which was comparable to the study done by Kim et al.[19] where the value reached 19.17 pg/ml at the end of 2nd month from the baseline (7.89 pg/ml). Though the reason for the increase in IP-10 levels at the end of 2nd month is not clear, it may be postulated that it could be due to the initial intensive therapy that aggressively kills the TB bacilli. The bacterial clearance thus occurred causes sputum conversion to negativity at the end of 2 months denoting the response to treatment.

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Once the intensive phase is completed, continuation phase treatment is initiated and monitored. In this phase, as the lysis of bacteria is complete, there was no further rise in IP-10 after six months, but the value declined below the basal level. At baseline, IP-10 was 10.76 pg/ml whereas, at the end of six months, it was 8.38 pg/ml. Similar to our study, Kim et al.[19] also showed a declining tendency of levels of IP-10 from the diagnosis to the completion of treatment. The IP-10 level at the end of treatment in their study was 5.47 pg/ml. So, the rise in IP-10 level at 2 months indicates the lysis of TB bacilli and therefore a positive response to ATT.

Canas et al.[21] also observed a decreasing level of urine IP-10 levels after antitubercular therapy which was compared to the levels at the baseline. They evaluated the presence of potential immune biomarkers for pulmonary TB in the urine samples of TB patients. IP-10 was detected consistently and it was also higher when compared with healthy subjects. They also observed lower IP-10 levels in patients who were cured of TB. Azzurri et al.[22] studied the use of INF-ɣ IP-10 for monitoring the inflammation and progression of the disease in pulmonary tuberculosis patients and observed that levels of IP-10 were reduced significantly at the end of treatment. They also observed that levels of IP-10 consistently remained elevated in untreated patients.

Another study done by Saini et al.[23] showed a mild increase in IP-10 levels initially from the diagnosis till the completion of 2 months of treatment in newly diagnosed pulmonary tuberculosis patients. The mean IP-10 level at baseline was 30.04 pg/ml and at the end of 2nd month, it was 54 pg/ml. In our study, we had a significant increase in IP-10 levels from the diagnosis to the end of 2nd month.

Limitations

The limitations of the present study include the sample size and exclusion of other forms of tuberculosis (smear-negative and extrapulmonary tuberculosis). Further studies with a larger population and in different categories of tuberculosis would give more precise information on the use of IP-10 as a biomarker in TB.

Conclusion

Based on the observations made in our study and the earlier studies, it can be concluded that IP-10 can be used as a biomarker not only for diagnosis but also to assess the prognosis in terms of response to treatment.

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Conflict of Interest: None

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