ORIGINAL ARTICLE

Diagnostic value of inducible protein-10 in pulmonary tuberculosis

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KEYWORDS
IP-10;
Pulmonary TB;
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Abstract There is increased need for alternative biomarkers in diagnosis, prognosis and follow up of patients with pulmonary tuberculosis and for differentiation between active and latent tuberculosis.

The aim of this work is to evaluate the efficacy of inducible protein-10 (IP-10) as a biomarker in the diagnosis of pulmonary tuberculosis as well as to elucidate its ability in distinguishing between active and latent tuberculosis.

This study was carried out on 20 apparently healthy subjects (group I), 20 active pulmonary tuberculosis (TB) patients (group II) and 20 latent TB patients (group III). They were matched in age and sex. Group II were sub-classified into three subgroups according to the radiological extent of the pulmonary lesion into: (Minimal, moderately advanced and far advanced lesions).

Blood samples were obtained and the determination of serum IP-10 levels by enzyme linked immunosorbent assay methods were done (Sandwich) ELISA.

Tuberculin skin test (TST) was significantly higher in group II and III compared to group I and it was significantly higher in group II compared to group III.

Serum IP-10 level was significantly higher in group II and III as compared with group I and it was significantly higher in group II compared to group III.

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Tuberculin skin test (TST) was significantly higher in group II and III compared to group I and it was significantly higher in group II compared to group III.

Serum IP-10 level was significantly higher in group II and III as compared with group I and it was also, significantly higher in far advanced lesions and moderately advanced lesions than minimal lesions.

Significant positive correlations were found between serum IP-10 level and both TST and blood lymphocyte%.

IP-10 showed sensitivity 88.9%, specificity 100% and accuracy 95.5% with positive predictive value 100% and negative predictive value – 75% in diagnosis of active pulmonary and latent tuberculosis.

It was concluded that IP = 10 could be used as a diagnostic biomarker in the diagnosis of active pulmonary and latent tuberculosis and it correlates well with disease severity.

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Introduction

Despite the global effort to reduce the burden of tuberculosis (TB), TB is the highest infectious cause of mortality and morbidity worldwide, with 1.7 million deaths and 9.4 million incident cases in 2009 alone. Efforts to reduce the TB burden are linked to the development of rapid diagnostic tests for infection with *Mycobacterium tuberculosis*. The interferon-g (IFN-γ) release assay (IGRA), recently developed immunodiagnostic test for TB, is available [1].

Compared with the tuberculin skin test (TST), the IGRA is less influenced by the bacilli Calmette–Guérin (BCG) vaccine and environmental mycobacterial exposure [2]. However, its sensitivity is suboptimal in immunocompromised patients, and its ability to discriminate between active TB and latent TB infection (LTBI) is questionable [3,4].

The sensitivity of the IGRA can be enhanced by using alternative or additional biomarkers. IP-10 is produced primarily by monocytes/macrophages and has a role in trafficking of Th1 lymphocytes to inflamed foci through an interaction with a CXC chemokine receptor. High levels of IP-10 were found in the pleural effusion and lung tuberculosis granuloma. IP-10 expression following stimulation with *M. tuberculosis*-specific antigens is reported to be a promising biomarker with high sensitivity for the immune-diagnosis of TB infection [5,6] (see Fig. 2).

In contrast to IFN-γ, IP-10 expression in response to TB-specific antigen was not influenced by the ability to respond to mitogens or by the CD4 cell number in HIV-infected patients [7]. However, there have been discordant results regarding whether IP-10 can distinguish between active TB and LTBI. Plasma levels of IP-10 were higher in active TB than in LTBI and showed a reduction at the end of *M. tuberculosis* treatment. In addition, baseline plasma IP-10 and CFP-10-stimulated IP-10 levels were significantly higher in active TB than in LTBI in patients with rheumatoid arthritis [8]. Conversely, TB-specific antigen-stimulated IP-10 could not distinguish between active TB and LTBI in children diagnosed by IGRA [9].

Subjects and methods

This study had been carried out on 60 subjects admitted in the El-Mahala Chest Hospital, and attending the outpatient clinic of the Chest Department of Tanta University Hospitals, the duration of the study was 18 months. Subjects were classified into three groups (see Table 1).

Group I: Included 20 healthy volunteers with negative tuberculin test, they didn’t have any history of contact with active pulmonary tuberculosis patients and they were free of tuberculosis symptoms.

Group II: Included 20 active tuberculous patients with positive sputum examination for acid fast bacilli by Ziehl–Neelsen stain and positive tuberculin test.

Group III: Included 20 latent tuberculous patients with a history of contact with active pulmonary tuberculosis cases...
for more than one month, normal Chest X-ray, negative spu-
tum examination for acid fast bacilli by Ziehl–Neelsen stain
and positive tuberculin test.

The protocol of the study was approved by the ethics com-
mittee in the Tanta Faculty of Medicine and written informed
consent was taken from all subjects in the study (see Table 3).

Exclusion criteria:

- Patients with other co-morbid pulmonary diseases, e.g. COPD, bronchial asthma, pneumonia and respiratory
  failure.
- Patients with other medical co-morbid diseases, e.g. D.M., heart failure and renal failure.
- Patients with autoimmune disorders including systemic lupus erythematosus, primary biliary cirrhosis and atopic
  dermatitis.
- Patients with HIV infection, lymphoma, leukemia, patients who had received anti tuberculous therapy for more than
  2 weeks and patients who received immunosuppressive ther-
  apy within 3 months of enrollment.

All subjects in this study were subjected to the following:

1. Thorough history taking and complete physical
   examination.
2. Calculation of body mass index.
3. Routine laboratory investigations including complete blood
   picture, ESR, fasting and postprandial blood glucose levels,
   blood urea, serum creatinine levels.
4. Sputum examination for acid fast bacilli on 3 successive
   days by Ziehl–Neelsen staining.
5. Plain X-ray and CT for some cases for the exclusion of
   other pulmonary co-morbidity and confirming diagnosis &
   extent of the lesions.

6. Tuberculin skin test.
7. Blood samples withdrawn and examined for inducible
   protein-10 using the ELISA technique (OmniKine™
   Human IP-10 ELISA Kit).

Results

This study had been carried out on 60 subjects admitted in El-
Mahala Chest Hospital, and attending the outpatient clinic of
the Chest Department of Tanta University Hospitals, the
study extended from June 2013 to September 2014 (see
Table 4).

The subjects were classified into three groups:

Group I: Included 20 healthy volunteers. They were 8 males
and 12 females, their ages ranged from (23 to 60) years with
a mean value of 42.35 ± 11.06 years.

Group II: Included 20 active tuberculous patients. They
were 15 males and 5 females, their ages ranged from (14
to 73) years with a mean value of 44.0 ± 15.94 years.

Group II was reclassified into three subgroups according
to the extent of the lesion in the CXR.

Minimal lesion: included 13 patients (10 males and 3
females).


<table>
<thead>
<tr>
<th>Table 1</th>
<th>Laboratory data of the three studied groups.</th>
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<tbody>
<tr>
<td></td>
<td>Group I</td>
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<tr>
<td>Hemoglobin (gm %)</td>
<td>12.5 ± 1.22</td>
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<tr>
<td>Total leukocytic count (TLC × 10³)</td>
<td>10.95 ± 0.945</td>
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<tr>
<td>Lymphocytes %</td>
<td>26.0 ± 7.53</td>
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<tr>
<td>ESR mm</td>
<td>4.9 ± 1.74</td>
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* Significance compared to group I & II.

<table>
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<tr>
<th>Table 2</th>
<th>Mean value ± SD and statistical comparison of TST diameter in the three studied groups.</th>
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<tbody>
<tr>
<td>TST diameter</td>
<td>G I</td>
</tr>
<tr>
<td>Range</td>
<td>2.0–4.0 mm</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.75 ± 0.638 mm</td>
</tr>
<tr>
<td>F test</td>
<td>266.689</td>
</tr>
<tr>
<td>P value</td>
<td>0.001*</td>
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<tr>
<td>Scheffe test</td>
<td>G I and G II</td>
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<td>0.001*</td>
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</table>

* Significance.
Moderately advanced lesion: included 3 patients (2 females and one male).

Far advanced lesion: included 4 males.

Group III: Included 20 latent tuberculous patients. They were 9 males and 11 females, their ages ranged from 21 to 74 years with a mean value of 47.45 ± 14.17 years (see Fig. 3).

The mean value ± SD of TST in the three studied groups were (2.75 ± 0.638) mm in group I, (12.25 ± 1.74) mm in group II and (11.9 ± 0.788) mm in group III. It was significantly higher in group II and group III as compared with group I. No statistical significant difference seen between group II and III (Table 2).

Serum inducible protein-10 concentration in pg/ml in the three studied groups in table (Fig. 1):

The mean values of serum IP-10 concentration in the three studied groups were 72.87 ± 18.17 pg/ml in group I, 515.5 ± 378.99 pg/ml in group II and 286.5 ± 135.10 pg/ml in group III. One way analysis of variance showed a statistical significant difference (F = 18.123, p < 0.001). Pairwise multiple comparisons (Scheffe test) showed that serum IP-10 was significantly higher in group II and group III compared to group I and it was significantly higher in group II compared to group III (p < 0.05) (see Fig. 4).

Discussion

Tuberculosis (TB) is a major cause of death around the world, with most of the 1.5 million deaths per year attributable to the disease occurring in developing countries. One of the most important and common complaints of tuberculous patients which also affects the immune status is weight loss. Some cytokines promote inflammation and are called proinflammatory cytokines (tumor necrosis factor-Alfa (TNF-α), interleukin (IL)-1, IL-6, and IL-8), whereas other cytokines suppress the activity of proinflammatory cytokines and are called anti-inflammatory cytokines (IL-4, IL-10, and IL-13)(121). Proinflammatory cytokines are prime candidates as causative agents of the metabolic changes that eventually result in tuberculosis-associated weight loss. TNF-α has some harmful effects, such as acute-phase pathophysiologic events including fever and tissue necrosis. It also plays a protective role against mycobacterial infection [10].

IP-10 is produced primarily by monocytes/macrophages and has a role in chemo-attraction of Th1 lymphocytes to inflamed foci through an interaction with a CXC chemokine receptor. High levels of IP-10 were found in the pleural effusion and lung tuberculosis granuloma of TB patients. Previous studies have reported that IP-10 expression following stimulation with M. tuberculosis-specific antigens is a promising biomarker with high sensitivity for the immunodiagnosis of TB infection [11].

In this study, Hemoglobin level (gm%) was significantly lowered in group II as compared to group I and group III and this was in accordance with Atomsa et al.,[12] their study showed that mean RBC counts, Hgb, MCV, MCH and MCHC values were significantly lower than the corresponding control group for both males and females.

Other similar studies have reported lower hemoglobin levels among TB patients. However, a study conducted on adult patients diagnosed with TB at the Seoul National University Hospital, Korea among 880 patients, anemia was identified in 281 patients (31.9%) at the time of diagnosis which was lower than the present study. It also showed that normocytic normochromic anemia was the most common and identified in 202 (71.9%) patients and followed by microcytic hypochromic anemia 26 (9.1%). On the other hand, a study conducted in India reported normocytic normochromic anemia as the most common abnormality observed in all cases, groups and subgroups. The variation might be due to the difference in

<table>
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<tr>
<th>ROC curve</th>
<th>Sens.</th>
<th>Spec.</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
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<tr>
<td>Cutoff</td>
<td>88.9</td>
<td>100.0</td>
<td>100.0</td>
<td>75.0</td>
<td>95.5%</td>
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* Specify the IP-10 cutoff value in TB.

![Figure 3](image-url) Correlation between serum IP-10 and TST diameter in the studied groups.
the stage of the disease during diagnosis, geographic, nutrition and other cultural differences that may directly or indirectly be related to anemia [13].

In this study, total leukocytic counts were within the normal range in the three studied groups with no statistical significant difference. Blood Lymphocytes % was significantly higher in group II compared with group I and group III. This was in accordance with Gambón-Deza et al., [14] their study has shown peripheral blood lymphocytosis produced during the initial weeks of *M. tuberculosis* infection. Since *M. tuberculosis* bacilli grow inside phagocytic vacuoles, the bacterial antigens must be presented by class II major histocompatibility complex molecules, and, therefore, the response must be conveyed by CD4 T cells. Other studies showed an increase in the numbers of CD8 T lymphocytes in recent infections, while these cells were also present in the pleural fluids of the active tuberculosis patients. These CD8 lymphocytes may correspond to those directed against self-heat shock protein that may have been produced during the infectious process.

Erythrocyte sedimentation rate was significantly higher in group II as compared with group I and group III. Previous studies have documented an elevated ESR level in majority of patients which decreased significantly in those with sputum conversion. It was in accordance with the study done by Al-Marri and Kirkpatrick [15] which found that children with symptomatic tuberculosis had significantly higher ESR values than asymptomatic children with tuberculosis and likewise children with positive culture for tuberculosis had significantly higher ESR values than children with negative tuberculosis cultures. Nevertheless, there was a large range of individual values with considerable overlap, making it difficult to see how individual patient values could be of any utility in either diagnosing or excluding tuberculosis [16].

Inducible protein-10 (IP-10) level was significantly higher in tuberculous and latent groups as compared with the control group and significantly higher in active pulmonary tuberculosis compared to latent tuberculosis. So, serum IP-10 can differentiate between active, latent TB and normal cases. Hong et al., [1] evaluated inducible protein 10 (IP-10) as a diagnostic biomarker for specific tuberculosis (TB) infection and evaluated the ability of IP-10 to distinguish between active TB and latent TB infection (LTBI). Their study was carried out on 46 patients with active pulmonary TB, 22 participants with LTBI, and 32 non-TB controls were enrolled separately. IP-10 was measured in serum and in supernatants from whole blood stimulated with TB-specific antigens. The results showed

![Figure 4](a and b): Sensitivity and specificity of IP-10 in the diagnosis of TB.
that TB antigen-dependent IP-10 secretion was significantly increased in the active TB patients and LTBI subjects compared with controls, but did not differ significantly between the active TB patients and LTBI subjects. Serum IP-10 levels were higher in active TB than in LTBI [1].

The present work was in accordance with Azab et al., [17]. Their study was investigating the utility of IP-10 in both blood and bronchoalveolar lavage (BAL) in the diagnosis of TB infection in clinically suspected patients. The study was carried out on thirty patients with clinical and/or radiological suspicion of pulmonary tuberculosis and negative sputum smear for AFB with Z-N stain were included in the study. BAL and blood samples were sent for the estimation of the level of IP-10. The results showed that levels of IP-10 in both blood and BAL were significantly higher in TB patients.

Also the present study was in accordance with Syed Ahamed Kabeer [18] his study carried out on 277 subjects recruited during the study period. There were 100 healthy controls and 177 PTB patients. Blood was unavailable for 2 PTB patients. Tuberculin skin test results were unavailable for 6 healthy controls and 58 (32.8%) PTB patients. All the subjects were negative for HIV infection and the demographic profile of those subjects is given. All the healthy controls were negative for sputum smear microscopy and sputum culture. In PTB, sputum smear microscopy was positive in 151 (85.3%) subjects and sputum culture was positive in 162 (91.5%) subjects. All the sputum smear positive cases were positive for sputum culture. Levels of IP-10 in unstimulated plasma ranged from 0 to 6975 pg/ml (median 390 pg/ml) in healthy controls and 0–5973 pg/ml (median 898 pg/ml) in PTB patients respectively. TB specific antigens and mitogen stimulated significantly higher levels of IP-10 secretion in healthy controls as well as TB patients. Levels of IFN-γ were also significantly higher in TB antigens as well as mitogen stimulated samples than unstimulated samples of healthy controls and PTB subjects.

Jeong et al., [19] their study aimed to identify a parameter that helped to discriminate active TB and LTBI. This study was carried out on 33 active TB patients, 20 individuals with LTBI, and 26 non-TB controls. The results showed that IP-10 was more useful in discriminating active TB from LTBI and it was not in accordance with our present study because in their study other parameters like Quantiferon-TB Gold, IFN-γ and TNF-α and others were used with IP-10 to discriminate active TB and LTBI.

Other studies showed also elevated IP-10 level in pleural effusions as in Dheda et al., [20] their study investigated the comparative diagnostic utility of established (adenosine deaminase [ADA]), more recent (standardized nucleic-acid-amplification-test [NAAT]) and newer technologies (a standardized LAM mycobacterial antigen-detection assay and IP-10 levels) for the evaluation of pleural effusions in 78 consecutively recruited South African tuberculosis suspects. All consenting participants underwent pleural biopsy unless contra-indicated or refused. The reference standard comprised culture positivity for M. tuberculosis or histology suggestive of tuberculosis. Of the 74 evaluable subjects 48, 7 and 19 had definite, probable and non-TB, respectively. IP-10 levels were significantly higher in TB vs non-TB participants.

In the present study, there was a positive correlation between serum IP-10 and severity of TB in group II and it was in accordance with Tonby et al., [21] their study analyzed IP-10 levels in plasma directly and extracted from dry plasma spot (DPS) in parallel by ELISA from 34 clinically well characterized patients with TB disease before and throughout 24 weeks of effective anti-TB chemotherapy, they detected a significant decline of IP-10 levels in both plasma and DPS already after two weeks of therapy with good correlation between the tests. So IP-10 level decreases with a decrease in the severity of TB[132].

References


