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Diverse Pattern of Cytokine Production, their Functional Association and mRNA Expression in Tuberculosis Patients with Diabetes Mellitus and their Household Contacts

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Abstract

Cytokine genes are associated with their altered production and play a significant role in tuberculosis patients with diabetes mellitus and their household contacts. We aimed to study the influence of IL-1β and IL-6 gene polymorphisms on cytokine gene production and to evaluate their mRNA expression in *M.tbAg85A* stimulated culture supernatants of tuberculosis patients with diabetes mellitus, pulmonary tuberculosis patients, household contacts and diabetes individuals. Enzyme linked immunosorbent assay was used to estimate cytokine production in the culture supernatants and TRIZOL method for mRNA expression. Cytokine production was low in all the subjects when compared to the healthy controls and IL-6 levels increased with treatment. The CC genotype of IL-6 -174 G/C was associated with its low production in household contacts of tuberculosis patients with diabetes mellitus and pulmonary tuberculosis patients. The mRNA expression of IL-1β increased and that of IL-6 decreased in tuberculosis patients with diabetes mellitus, while that of IL-6 decreased with treatment in pulmonary tuberculosis patients; however, there was no significant difference in the household contacts. The cytokine levels were almost similar in patients and their household contacts at the time of diagnosis suggesting that even the household contacts might be infected thus aiding in protecting the high-risk individuals by their prior identification.

Keywords: Antigen 85A; Cytokine gene correlation; mRNA expression; Household contacts

Abbreviations: TBDM: Tuberculosis with Diabetes Mellitus; HHC: House-Hold Contacts; HC: Healthy Controls; IL: Interleukin; ROC: Receiver Operating Characteristic

Introduction

Tuberculosis (TB) an infectious disease caused by Mycobacterium tuberculosis (*M.tb*) is the leading cause of death worldwide [1]. Several co-morbidities, one of which is type 2 diabetes mellitus (DM) increased the risk of susceptibility to TB by threefold [2,3]. It is predicted that globally 15% of TB cases are projected to be attributable to DM thus becoming an increasingly important factor challenging TB control [4,5]. The immune response variation in DM patients against M.tb makes them more vulnerable to infection or progression towards active TB disease and thereby diminished treatment response [6,7]. Diabetes alters immunity to tuberculosis, leading to higher baseline mycobacterial burdens and longer times to culture conversion with treatment resulting in higher rate of relapse [8]. Nevertheless, the fundamental biological mechanisms remain largely unidentified [9,10].

The infection is exclusively transmitted from pulmonary TB patients through aerosol. Contact investigation among TB patients is a systematic evaluation to identify active disease or latent M.tb infection (LTBI) among household members of TB patients and to interrupt the disease transmission [11-14]. The risk of transmission is maximum when index case is sputum smear positive, in overcrowded living conditions, a higher density of bacilli in respiratory secretions and extent of lung fields involved [15,16]. Henceforth, those living in the same household are at higher risk than the usual contacts.

TB and DM patients have been associated with dysregulated cytokine responses towards M.tb. Pro-inflammatory cytokines are necessary for protection against *M.tb*, anti-inflammatory cytokines may counteract these effects. Inflammatory cytokines like IL-1β and IL-6 may play a major role in the pathogenesis of TB and DM that are produced upon stimulation of peripheral blood mononuclear cells (PBMC) with *M.tb* antigen 85A [17]. A major portion of the secreted proteins in *M.tb* is formed by the Ag85 complex, a 30- to 32-kDa family of three proteins (Ag85A, Ag85B and Ag85C) that is important in relation to their particular role in the interaction between the bacilli and the infected host. It induces strong T-cell proliferation and IFN-γ production in *M.tb* infected individuals.

IL-1β secreted by infected macrophages, in particular, is implicated in granuloma formation signaling and its deficiency leads to induced *M.tb* growth at the site of infection in mice [18]. The majority of TB patients with infiltrative lesions on chest radiographs also expressed significantly elevated IL-1β and TNF-α compared to the baseline values. The amount of IL-1β secreted to the microenvironment is regulated at the mRNA and protein level. It mediates a local inflammatory response conferring resistance to infection by regulating the expression of several other inflammatory genes [19]. The pro-inflammatory cytokine IL-1β response is strongly induced by *M.tb* and is encoded by the polymorphic genes. IL-1β +3954C>T and -511C>T polymorphisms and their correlation has been associated with TB. It is mostly involved in the early recruitment of inflammatory cells to *M.tb* or PPD stimulated granulomas and in establishing anti-mycobacterial adaptive immunity [20].

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IL-6 is a pleiotropic cytokine with a wide range of humoral and cellular immune effects relating to inflammation, host defense, tissue injury and is produced in response to several mediators like IL-1, IFN-γ and TNF. In mycobacterial infection, mice deficient with IL-6 exhibit an altered immune response and thereby increased susceptibility to *M.tb* infection when compared to the wild-type mice [21]. IL-6 expressed by a variety of cell lineages with diverse functions, contributes to the differentiation and activation of the cells leading to an immune response [22]. A common polymorphism in the IL-6 gene promoter -174G>C regulates transcription in response to lipopolysaccharides while that at -572G>C affects IL-6 gene transcription and its serum levels [23,24].

Hence, we designed the present study to understand the IL-1β and IL-6 cytokine production pattern, their functional association and mRNA expression in PBMC of tuberculosis patients with and without diabetes mellitus and their household contacts.

Materials and Methods

Study population

We enrolled 300 subjects including tuberculosis patients with diabetes mellitus (TBDM), their household contacts (TBDM HHC), pulmonary tuberculosis patients (PTB), their household contacts (PTB HHC), diabetes mellitus patients (DM) and healthy controls without clinical complaints (HC), 50 in each category, attending Bhagwan Mahavir Medical Research Center (BMMRC) under the public private mix directly observed treatment shortcourse program (PPM-DOTS). PTB diagnosis was confirmed by sputum smears or culture positivity for *M.tb* or by clinical-epidemiologic data compatible with active TB. Diabetes was confirmed based on the blood sugar levels. Body mass index (BMI) was analyzed in all the subjects. Tuberculin Skin Test (TST) was performed in all the subjects except in the DM and HC, wherein 5 TU purified protein derivative (PPD) was injected into the left forearm and the reading was noted after 48 h. An induration >10 mm was considered positive. Patients concurrent with other active granulomatous diseases or HIV were excluded. TBDM and PTB patients received six months treatment. For the evaluation of immunologic function, patients' samples were collected based on the anti-TB treatment timeline, defined as 0M: after diagnosis before start of treatment; 4M: with four months of treatment; 6M: with six months of treatment and 12M after six months of treatment completion. All the subjects agreed to participate in the study, after study clarification and written informed consent.

Enzyme linked immuno-sorbent assay (ELISA), genotyping and reverse transcriptase PCR (RT-PCR)

Stimulation assays on PBMCs by Ag85A was already discussed previously [25]. Culture supernatants were obtained from the *M.tb* stimulated PBMC of all the subjects and the cytokines present in these supernatants were analyzed by ELISA using the kits available from BD biosciences.

In the present study, four Single Nucleotide Polymorphisms (SNPs) were analyzed: IL-1β +3954C>T, IL-1β -511C>T, IL-6 -174G>C and IL-6 -572G>C. For genotyping, 2 ml blood was drawn, collected in EDTA coated tubes, DNA isolated by Flexigene DNA isolation kit, quantification and purity determined on a spectrophotometer (NanoDrop 2000 Thermo Fisher Scientific). Amplification of the genomic regions of interest was performed by PCR using 20-50 ng of DNA, recombinant Taq DNA polymerase, 0.2 mM of each dNTP (deoxy-nucleotide-adenine, guanine, thymine or cytosine-triphosphate), 0.3-1 mM concentration of each of the specific primers, appropriate buffer and ultrapure water. The primer sequences and PCR conditions were shown in Tables 1 and 2.

cDNA was synthesized from the mRNA isolated from the stimulated PBMCs and amplified using the mRNA primers (Table 3). The IL-1β, IL-6 and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was carried out using SYBR green in iCycler IQ multicolor real-time detection system (Bio-Rad). Amplifications and thermal-cycler parameters were followed according to the manufacturer's directions. The delta-delta method (i.e., comparative ct [Δct] method) was used for quantitative analysis of gene expression. The results were normalized to those for GAPDH in the same sample and were expressed as fold increase. Gene expressions were determined based on results of stimulated cells as a fold change compared with basal levels in unstimulated cells.

Statistical analysis

Comparisons between different genotypes in the control and patient

Gene Chromosome	SNP Property	PCR Primers	PCR Product size
IL-1β +3954C>T Chr 2	Intron	**Forward primer -**5′-GTT GTC ATC AGA CTT TGA CC-3′ **Reverse primer -**5′-TTC AGT TCA TAT GGA CCA GA-3′	Taq 1 349 bp,135 bp, 114 bp
IL-1β -511C>T Chr 2	Promoter	**Left primer -**5′-TGG CAT TGA TCT GGT TCA TC-3′, **Right primer -**5′-GTT TAG GAA TCT TCC CAC TT-3′	Ava 1 305 bp,190 bp,114 bp
IL-6 -174G>C Chr 7	Promoter	**Common primer -**5'-GAGCTTCTCTTTCGT TC-3' **Right primer -**5'-CTAGTTGTGTCTTGCC-3' **Left primer -**5'-CCCTAGTTGTGTCCTGCG-3'	ARMS 234 bp
IL-6 -572G>C Chr 7	Promoter	**Right primer -**5'-GGAGACGCCTTGAAGTAACTGC-3' **Left primer -**5'-GAGTTTCCTCTGACTCCATCGCAG-3'	Fok 1 163 bp

Table 1: Primer sequences of IL-1β and IL-6 cytokine genes.

Cytokine	Initial Denaturation	Annealing	Extension	Final Extension	No. Of cycles
IL-1β +3954C>T	95°C – 4 min 95°C – 30 s	59°C – 30 s	72°C – 30 s	72°C – 4 min	33
IL-1β -511C>T	94°C – 5 min 94°C – 1 min	55°C – 1 min	72°C – 1 min	72°C – 7 min	45
IL-6 -174G>C	95°C- 4 min 95°C- 30 s	60°C - 30 s	74°C-30 s	74°C – 7 min	35
IL-6 -572G>C	94°C – 4 min 95°C – 40 s	55°C – 40 s	72°C – 1 min	-	35

Table 2: PCR conditions of IL-1β and IL-6 cytokine genes.

groups were made using Mann-Whitney Test with two-tail value. For the analytical comparison between the time points of the treatment (0M, 4M, 6M and 12M), a Friedman test was used to verify which time point differed from the other, a Dunn's multiple comparisons test was applied as a post-test. Results were considered significant when $p<0.05$. Tests were performed using Graph Pad Prism version 5.00 for Windows, Graph Pad Software (San Diego, CA, USA). The IL-1β and IL-6 cytokine production in *M.tb* stimulated culture supernatants were studied in 50 cases of each TBDM, PTB, TBDM HHC, PTB HHC, DM and HC and were correlated with their cytokine gene polymorphisms. TBDM, PTB, TBDM HHC, PTB HHC, 25 each were followed at different intervals 0M, 4M, 6M and 12M for the IL-1β and IL-6 production and 5 cases were followed for their mRNA expression.

Results

Cytokine production and functional correlation

Cytokine production of IL-1β was significantly low in TBDM (52.78 ± 33.93, p=0.002), TBDM HHC (54.94 ± 34.92, p=0.012), PTB (58.18 ± 40.43, p=0.012), PTB HHC (54.38 ± 37.81, p=0.02) and DM (55.81 ± 40.87, p=0.02) when compared to HC (73.57 ± 30.91) pg/ml. Similarly IL-6 production was low in TBDM, TBDM HHC, PTB, PTB HHC and DM compared to HC (82.76 ± 29.92, 91.95 ± 34.51, 82.13 ± 28.23, 113.3 ± 73.5, 97.23 ± 43.46, 171.2 ± 40.19 pg/ml respectively with statistical significance of p=0.0001 in each category). Statistical significance was observed with IL-6 production in TBDM vs DM at p=0.044 and in PTB vs PTB HHC at p=0.005. Almost similar IL-1β was secreted by TBDM, PTB, TBDM HHC, PTB HHC during follow-up. High levels of IL-6 were secreted at 12M by TBDM (99.57 ± 34.91, p=0.02); at 4M (107.0 ± 35.68, p=0.0091) and 6M (103.8 ± 48.0, p=0.0034) by PTB when compared to that at 0M (Figures 1 and 2).

Results for the IL-1β +3954C>T SNP showed that TBDM and TBDM HHC with CC genotype, PTB HHC and DM with CT genotype presented low levels of IL-1β when compared to the HC with TT genotype. TBDM, PTB, HC with CT genotype; HHC and DM with TT genotype presented high levels of IL-1β. Statistical significance was observed with CC (58.3 ± 42.7) vs. TT (92.2 ± 25.3) at p=0.043 and with CT (46.5 ± 38.6) vs. TT (92.2 ± 25.3) at p=0.009 in DM. At -511C>T variant, TBDM, PTB HHC and HC with TT genotype; TBDM HHC with CT genotype and PTB and DM patients with CC genotype produced low levels of IL-1β. TBDM with CT genotype, HHC and HC with CC genotype, PTB and DM with TT genotype produce high levels of IL-1β. For IL-6 -174G>C variant, TBDM, TBDM HHC, DM with CC genotype, PTB, PTB HHC and HC with GG genotype produce low IL-6 levels. In HC, statistical significance was observed at p=0.03 with respect to CC vs. GG (186.7 ± 51.5 vs. 150.8 ± 36.0) genotype. TBDM, TBDM HHC and DM with GG genotype, PTB, PTB HHC and HC with CC genotype produce high levels of IL-6. At IL-6 -572 G>C, TBDM, HHC and DM with GC genotype, PTB and HC with CC genotype produce low levels of IL-6. While TBDM patients with CC genotype, TBDM, PTB, HHC, DM and HC with GG genotype produce high levels of IL-6 (Figures 3 and 4).

Upon Receiver Operating Characteristic (ROC) curve analysis, the sensitivity and specificity of IL-1β and IL-6 cytokine gene markers were computed and ROC curves were constructed in the TBDM, TBDM HHC, PTB, PTB HHC, DM vs HC where the Area under the curve (AUC) with statistical significance was determined at 95% confidence interval. The cut-off value is derived and the sensitivity, specificity determined to identify the individuals risk with respect to the cytokine gene marker. AUC was more for IL-6 (0.97, 0.944, 0.96, 0.75, 0.90) and that of IL-1β (0.68, 0.65, 0.60, 0.65, 0.63) was less in TBDM, TBDM HHC, PTB, PTB HHC and DM respectively when compared to HC indicating that the test was performed more accurately in IL-6 with statistical significance ($p<0.0005$). The cut-off value, sensitivity and specificity were the best for IL-6 that were 125.56 pg/ml, 90 and 4 in TBDM, 119.93 pg/ml, 92 and 24 in TBDM HHC, 13.19 pg/ml, 88 and 6

GENE	Primer sequence	Amplicon size
IL-1β	F- CCACAGACCTTCCAGGAGAATG R- GTGCAGTTCAGTGATCGTACAGG	148 bp
IL-6	F- TACCCCCAGGAGAAGATTCC R- TTTTCTGCCAGTGCCTCTTT	175 bp
GAPDH	F- GCCAATCAATGACCCCTTCATT R- TTGACGGTGCCATGGAATTT	320 bp

Table 3: mRNA primer sequences of IL-1β, IL-6 and GAPDH for expression analysis

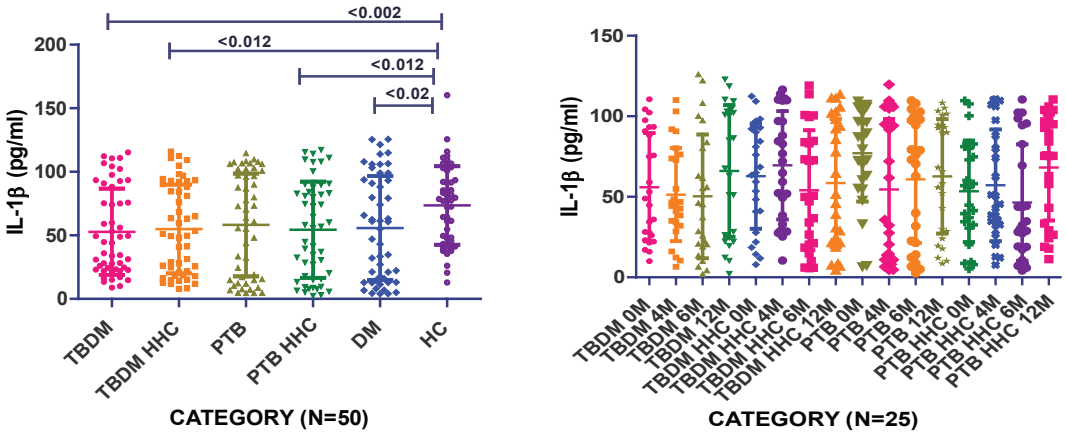


Figure 1: IL-1β levels in *M.tb* Ag85A stimulated culture supernatants of TBDM, PTB patients, their HHC, DM and HC and during follow-up of TBDM, PTB and their HHC.
Bars indicate the mean and SD for each group. Horizontal lines indicate a statistically significant difference between groups. Differences between the groups were analyzed by Mann-Whitney U test
*p=0.05 was considered statistically significant

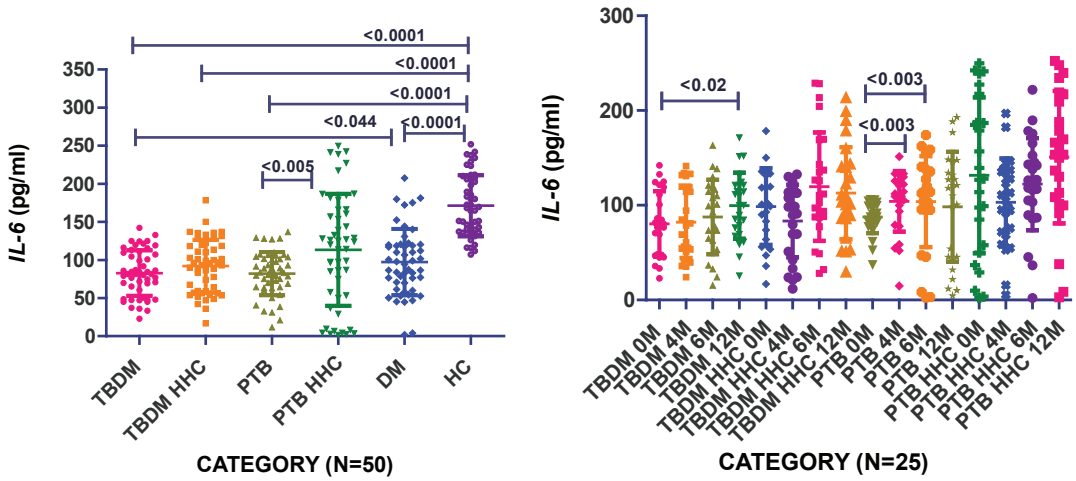
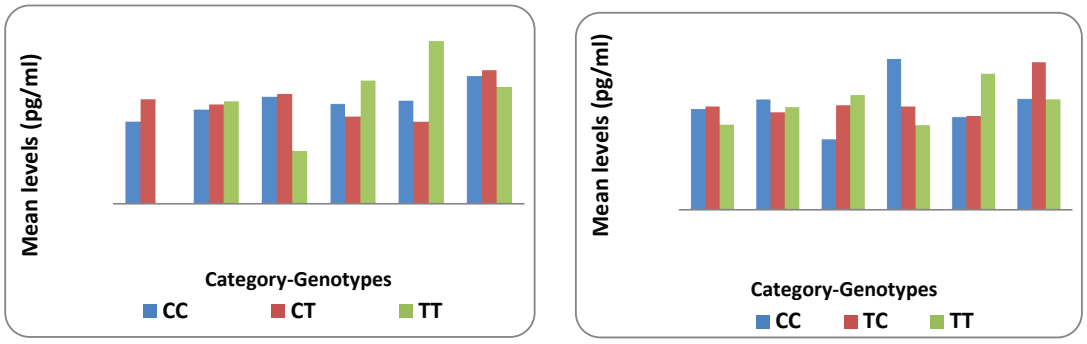
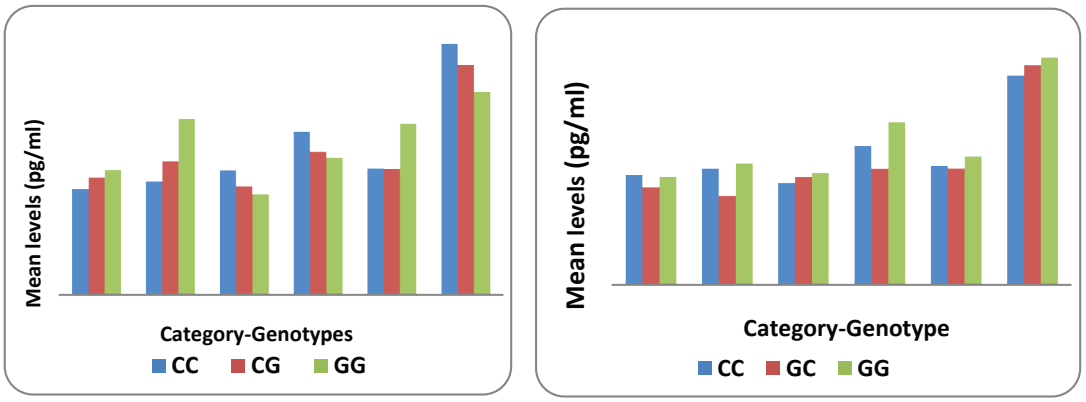


Figure 2: IL-6 levels in *M.tb* Ag85A stimulated culture supernatants of TBDM, PTB patients, their HHC, DM and HC and during follow-up of TBDM, PTB patients and their HHC.
Bars indicate the mean and SD for each group. Horizontal lines indicate a statistically significant difference between groups. Differences between the groups were analyzed by Mann-Whitney U test
*p=0.05 was considered statistically significant



TBDM: Tuberculosis with Diabetes Mellitus; HHC: Household Contacts; PTB: Pulmonary Tuberculosis; DM: Diabetes Mellitus; HC: Healthy Controls

Figure 3: IL-1β +3954 C>T and -511C>T genotypes related to its levels stimulated with *M.tb* Ag85A in TBDM, PTB patients, their HHC, DM and HC.



TBDM: Tuberculosis with Diabetes Mellitus; HHC: Household Contacts; PTB: Pulmonary Tuberculosis; DM: Diabetes Mellitus; HC: Healthy Controls

Figure 4: IL-6 -174 G>C and -572G>C genotypes related to its levels stimulated with *M.tb* Ag85A in TBDM, PTB patients, their HHC, DM and HC.

in PTB, 130.12 pg/ml, 88 and 38 in PTB HHC and 119.3 pg/ml, 92 and 26 in DM when compared to the HC. This indicates the importance of IL-6 cytokine gene for the risk analysis in HHC.

mRNA expressions

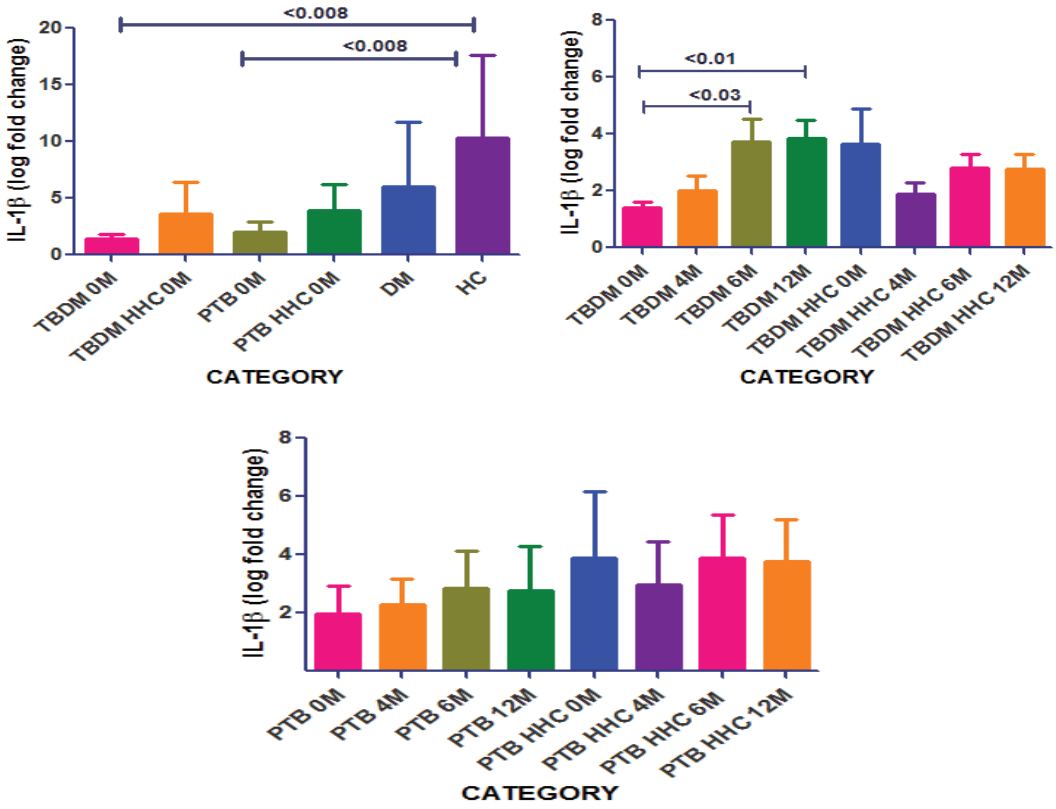
IL-1β mRNA expression was significantly low in TBDM (by 7 fold, $p<0.008$), PTB patients (5 fold, $p<0.008$), TBDM HHC (2.8 fold), PTB HHC (2.6 fold) and in DM (1.7 fold) when compared to HCs. During follow-up, statistically significant increase was observed at 6M vs. 0M ($p<0.03$) and 12M vs. 0M ($p<0.01$) by 2.6 fold in TBDM. Significant difference was not observed at 4M in TBDM and at different stages of their follow up in PTB, PTB HHC and TBDM HHC.

IL-6 mRNA expression was statistically high in TBDM (by 12 fold, $p<0.0009$) PTB (8.9 fold, $p<0.025$), and without significant difference in TBDM HHC (4.8 fold), PTB HHC (4 fold) and DM (2.7 fold) when compared to HC. During follow-up, significant decrease was observed in TBDM (6M vs. 0M by 6.2 fold; 12M vs. 0M by 8.7 fold at 0M ($p<0.008$) and PTB (6M vs. 0M by 2 fold, $p=0.038$; 12M vs. 0M by 2.6 fold, $p<0.037$). Statistically significant difference was not observed in TBDM HHC and PTB HHC during their follow up (Figures 5 and 6).

Discussion

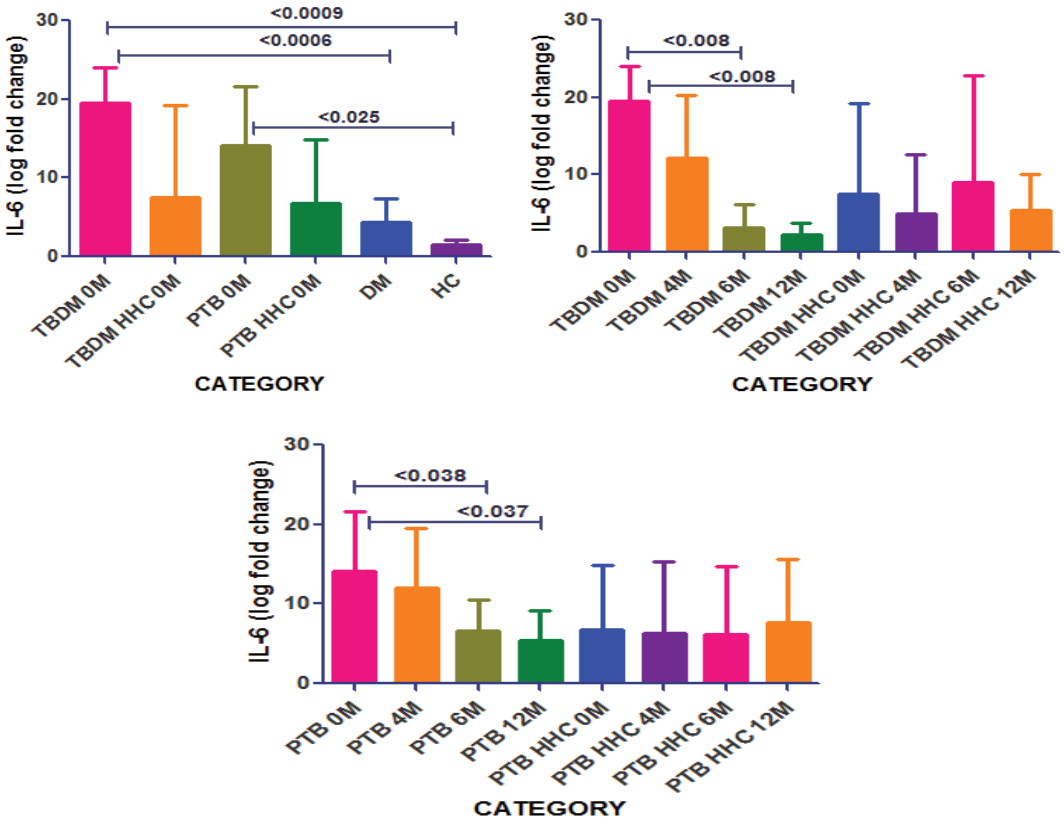
IL-1β production was low in TBDM compared to PTB without significant difference similar to a study in Japan where it was low in TBDM patients and was in contrast to our study with respect to DM patients where almost similar IL-1β production was observed in TBDM and DM patients [26]. Low T cell proliferation and cytokine production in DM patients may be due to the intake of oral anti-diabetic drugs [27]. It was low in PTB patients in contrast to a Taiwan study where it was high [28]. A significant difference was observed in TBDM, TBDM HHC, PTB HHC and DM when compared to HC. Significant difference was not seen in *M.tb* H37Rv stimulated culture supernatants of TBDM and PTB patients similar to an Indonesian study where there was no difference in their IL-1β secretion and significantly large difference was observed between PTB patients and HCs with high levels in PTB. TBDM patients after *M.tb* stimulation have shown higher IL-1β production similar to Indonesian study that may be due to severe hyperglycemia [29]. IL-1β secretion was found to be significantly high in TBDM compared to PTB patients similar to a study in Chennai [30]. There were no follow up studies with this cytokine secretion. The PTB patients with CC genotype of IL-1β +3954C>T and TC genotype of IL-1β -511C>T produce significantly low IL-1β when compared to the HCs. In comparison to our study, the two polymorphisms did not correlate with *M.tb* stimulated production of IL-1β to the same extent in the PTB patients [31]. However, there were no functional correlation studies in the TBDM, TBDM HHC, PTB HHC and the DM.

A significant difference in IL-1β expression was observed in TBDM and PTB patients, we were the first to work on mRNA expression in



0M-0 month follow-up, 4M-4th month follow-up, 6M-6th month follow-up, 12M-12th month follow-up, *p=0.05 was considered statistically significant

Figure 5: IL-1β mRNA expression in response to *M.tb* Ag85A stimulation in TBDM, PTB patients, their HHC, DM and HC and during follow-up in TBDM, PTB patients and their HHC.



0M-0 month follow-up, 4M-4thmonth follow-up, 6M-6thmonth follow-up, 12M-12thmonth follow-up, *p=0.05 was considered statistically significant

Figure 6: IL-6 mRNA expression in response to *M.tb* Ag85A stimulation in TBDM, PTB patients, their HHC and DM vs. HC and during follow-up in TBDM, PTB patients and their HHC.

TBDM and TBDM HHC. In this study, PTB patients and PTB HHC have not shown any significant difference during their follow up. IL-1β expression up-regulated in the alveolar macrophages of TB patients in a Chinese study and was significantly low in ESAT-6 stimulated PBMC of PTB patients when compared to the LTBI individuals, however statistical significance was not observed with respect to healthy controls [32,33]. IL-1β expression was low in the DM compared to HCs, which was contrary to a study where increased IL-1β mRNA was examined in the beta cells of DM patients with minimal or no expression [34].

The TBDM and their HHC have shown significantly low IL-6 production when compared to HCs without significant difference between TBDM and HCs similar to a Chennai study [30]. There were no reports in TBDM HHC to support our results. Its secretion was low in PTB when compared to HC in contrast to an Indonesian study where it was comparatively high [29]. A study by Jo et al. [34] and Ludmila et al. [5] reported that there was no statistical difference in the IL-6 production on stimulation with Ag85B and PPD in PTB patients and HCs and PTB HHC have not shown any variation from the HCs [35,36]. Our results were similar to a study where significantly higher levels were induced in PTB HHC when compared to PTB patients on proteomic analysis identification of immune-reactive T cell antigens [37]. IL-6 production significantly increased in plasma from active PTB patients and highly secreted after *M.tb* stimulation in central African population and specifically increased in pleural effusions in two different studies of Chinese population in contrast to our study [38]. This early response in the lungs might elicit initial restriction of mycobacterial growth. Decreased IL-6 expression may highlight its protective role in PTB patients. Low IL-6 production was observed in DM patients similar to Brazilian study; its elevation was reported in human adipocyte supernatants of both humans and Rabbits in USA and in individuals with Crohn's disease [39-41]. The CC genotype was considered as the high producer genotype in the PTB patients which was distinct from a study where IL-6 levels did not differ significantly between the -174G>C genotypes in the PTB patients and the HCs [42]. The G allele or GG genotype in the IL-6 gene promoter region (-174), was associated with high IL-6 production in TB patients which may have promoted TB by inhibiting production of other cytokines like TNF and IL-1β which was dissimilar to our study where GG genotype was associated with low IL-6 production in PTB patients [43,44]. Selvaraj et al., has not reported any significant association between the genotypes of IL-6 -174G>C polymorphisms and production both in PTB and HCs unlike our study population [45]. Studies have not been reported in TBDM, TBDM HHC, PTB HHC and DM. There were no studies on the functional association of IL-6 -572G>C polymorphisms and its production.

IL-6 mRNA expression was high in TBDM patients when compared to HCs and decreased with treatment, however there were no studies in TBDM and TBDM HHC. To our knowledge, this was the first study on

IL-6 mRNA expression in TBDM, TBDM HHC in response to *M.tb* Ag85A. IL-6 mRNA expression was significantly high in PTB patients when compared to HC which was in contrast to a Korean study [35] where it was similar in PTB patients and in HCs. Distinct results were reported in a study which has shown low IL-6 expression in the ESAT-6 stimulated PBMCs of PTB patients when compared to the LTBI individuals without any difference in the HCs [33]. IL-6 expression was low in DM patients compared to the HCs dissimilar to other studies where it was elevated in DM patients [46]. Increased IL-6 mRNA was even observed in DM with induced oxidative stress and in the PBMC of metabolic syndrome patients and in T2DM patients [47-49]. TBDM patients responded to treatment at a similar pace in our study subjects which was in contrast to a retrospective study where it was found that TBDM patients who were on metformin, an anti-diabetic drug had fewer pulmonary cavities and significantly better survival. Metformin inhibited the intracellular growth of *M. tuberculosis*, restricted disease immunopathology and enhanced the efficacy of conventional anti-TB drugs in mice.

Conclusion

The mean cytokine levels were almost similar in the patients and household contacts without significant difference during their follow up. This might be due to the latent infection developed by the household contacts but due to their anti- inflammatory response, they might have not developed the disease. Our results were similar to certain studies, even discriminating results were reported which could be due to the different proliferation time of the different antigens used in different studies. The pro-inflammatory cytokine gene expression correlates with protective immunity against TB. Significantly low cytokine production and longer duration for treatment response was observed in the TBDM compared to PTB who were cured at an early stage. Presence of diabetes might be one of the reasons for the treatment delay in the TBDM. The IL-6 cytokine gene was considered as a candidate gene and the most potential biomarker based on the ROC curve analysis with the highest sensitivity and specificity, hence may be used in the diagnostics for the analysis of risk in the household contacts. However, higher levels of pro inflammatory cytokine mRNA expression post infection signify a risk factor for developing active TB. Thus, our data suggests that the mechanisms that control the excessive inflammatory responses during *M.tb* infection may have a critical role in the immunopathology of TB.

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