Correlation of cytokine gene variants and mean serum cytokine levels in pulmonary and lymph node tuberculosis in north Indians
7. Introduction

Tuberculosis is a highly infectious disease and a major public health problem globally. India has almost 30% of the global burden of tuberculosis (TB). One person dies of the disease every minute. The average prevalence of all forms of tuberculosis in India is estimated to be five per thousand. The prevalence of smear positive cases is 2.27 per thousand and the average annual incidence of smear positive cases is 84 per 1,00,000 annually (Chakraborty, 2004). The severity of infection with *Mycobacterium tuberculosis* (*Mtb*) reflects the balance between the bacillus and host defence mechanism in which the quality of host defence determines the outcome. Immune response to TB is regulated by a complex set of interactions between the antigen-presenting cells, lymphocyte (T CD4+ and TCD8+), macrophages, monocytes and a vast array of immune mediators, the cytokines (Ladel et al., 1995). Effective production of cytokines in response to the bacilli would result in the immune clearance of the *Mycobacterium* from the host cells.

During *Mtb* infection, Th1-type cytokines have been shown to be essential for protective immunity which is secreted after 3 weeks of infection activating the macrophages to exert an anti-microbicidal action leading to formation of granuloma (Henderson et al., 1997; Pieters and Garfield, 2002; Young et al., 2002). IFN-γ is one of the most important cytokines involved in macrophage activation (Schultz and Kleinschmidt, 1983), stimulating antitumor and anti-microbicidal activities as well as expression of MHC-II (Pace et al., 1983; Basham and Merigan, 1983; Nathan et al., 1983; Torrico et al., 1991).

TNF-α is a potent pro-inflammatory and immunoregulatory cytokine that plays a key role in the initiation, regulation, and perpetuation of the inflammatory response (Orme and Cooper, 1999). TNF-α is also required for induction of apoptosis in response to mycobacterial infection (Keane et al., 1997).

IL-12, a heterodimeric pro-inflammatory cytokine produced by activated macrophages, monocytes, B-lymphocytes and dendritic cells is the principal Th1 response inducing cytokine (Abbas et al., 1996). This cytokine has been found to be important for sustaining a sufficient number of memory/effector Th1 cells to mediate long-term protection to intracellular pathogen.

IL-10 is an anti-inflammatory cytokine produced by T lymphocytes (Barnes et al., 1993) and macrophages after phagocytosis of *M. tuberculosis* (Shaw et al., 2000). IL-10 antagonizes the proinflammatory cytokine response by downregulation of production of IFN-γ, TNF-α and IL-12.
IL-4, an anti-inflammatory cytokine has been implicated to down-regulate IFN-\(\gamma\), and thus have a deleterious effect on TB patients (Powrie and Coffman, 1993; Lucey et al., 1996). It also promotes the induction of Th2 cells (Abbas et al., 1996). Owing to such vital role these cytokines was selected for the present study.

Although there are reports on ex-vivo cytokine production in response to mycobacterial antigens and their correlation with variant genotypes (Danis et al., 1995; Wilkinson et al., 1999; Ansari et al., 2009; Akahoshi et al., 2003; Lopez-Maderuelo et al., 2003; Yilmaz et al., 2005) few have studied the serum cytokine levels and its implications in the context of genotypes of the patients (Hurme et al., 1998; Vallinoto et al., 2010). Though there are some recent reports from Southern Indian population (Vidyarani et al., 2006; Selvaraj et al., 2008) such data on north Indian population is lacking. Moreover, there exists a significant difference in gene pools of both the populations as indicated by IGVC (Indian Genome Variation Consortium, 2008). The present study was therefore undertaken to examine the association between SNP genotype and serum cytokine levels in patients and healthy controls in north Indian population. Here we investigated the hypothesis that in a given (north Indian) population varying genotypes of the related cytokine genes namely IFN\(\gamma\), TNF\(\alpha\), IL4, IL10 IL12, IL6, IL8, IL2, IL1B, IL1RA, IL18 in patients with pulmonary tuberculosis (PTB) and lymph node TB may account for variable serum cytokines levels and contribute to the pathogenesis.

Understanding of the genetic make up of the patients in this context may have a futuristic impact on better disease countering strategies. Our results, albeit based on a limited data, reveal significant bearing of the genotype variant of the cytokine genes on the corresponding serum cytokine levels in the tuberculosis patients from north India. The cytokine genes considered here play an important role in the pathogenesis and mounting of protective immunity against Mtb. Among the cytokine genes studied the variant of the TNF\(\alpha\) gene at rs3093662, the IL12 gene at rs3213094 and rs3212220 and the IL10 gene at rs3024498 did show a strong indication to be of relevance to immunity to tuberculosis. To our knowledge this is the first report from this region relating genotypes and serum cytokine levels and contributes to the picture of genotype bias in the context of tuberculosis in north-Indians.
7.1 Results:

7.1.1 Correlation between serum cytokine levels and pulmonary tuberculosis.

7.1.1.1 *IFNG* polymorphisms and serum IFN-\(\gamma\) levels

Differential serum IFN-\(\gamma\) level could not be significantly correlated to variant genotypes among PTB cases although at rs2430561 a trend was seen with TT genotype accounting for high serum IFN-\(\gamma\) level followed by TA genotype and AA accounting for the low serum IFN-\(\gamma\). Among HC also the AA genotype was found to be the lowest serum IFN-\(\gamma\) producer and the TA genotype being the highest serum IFN-\(\gamma\) producer. (figure 7.1.1.1A). For variant rs2069718 among PTB cases CC genotype was associated with lowest serum IFN-\(\gamma\) level with TT being higher serum IFN-\(\gamma\) producer followed by TC genotype. Interestingly CC genotype was associated with lowest serum IFN-\(\gamma\) levels even among HC preceded by TT and TC genotypes respectively (figure 7.1.1.1B).

7.1.1.2 *TNFA* polymorphisms and serum TNF-\(\alpha\) levels

Within the HC group when compared among the genotypes for the rs3093662 the AA genotypes were high TNF-\(\alpha\) producers and GG genotypes were significantly low (p < 0.05) producers. PTB cases did not show any significant variation in the serum TNF-\(\alpha\) levels among the genotypes but a trend was observed with AA genotype being the higher producer followed by GA genotype and GG being the low producer of serum TNF-\(\alpha\) (figure 7.1.1.2).
7.1.1.3 IL4 gene polymorphism and serum IL-4 levels

The variant rs2243266 studied for the first time in TB did not show any significant association of different genotypes with serum IL-4 levels in either PTB cases or HC. Nonetheless, GA genotype showed high serum IL-4 levels closely followed by GG and AA genotypes respectively in PTB cases and HC (figure 7.1.1.3).

7.1.1.4 IL10 gene polymorphisms and serum IL-10 levels

Analysis of correlation between serum cytokine level and genotypes showed that GA genotype of variant rs3024498 demonstrate significantly high serum levels (p < 0.05) as compared to GG among PTB cases. No such association was observed for HC but GA genotype was higher producer of serum IL-10 followed by AA while GG genotype shows low IL-10 production similar to PTB cases. (Figure 7.1.1.4A) The IL10 gene variants at rs3024496 and rs3024490 did not show any association with the levels of serum IL-10; however a trend was clearly evident from the data obtained. Among PTB cases CC genotype accounted for high serum IL-10 levels followed by TT genotype with CT genotype showing lower serum IL-10 level for rs3024496. Interestingly CT genotype was the
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higher producer of serum IL-10 among HC showing a reverse trend as compared to PTB cases (Figure 7.1.4B). For variant at rs3024490 among PTB cases, the GT genotype accounted for higher serum levels followed by TT genotype and GG being lowest producer. In HC TT genotype accounted for least serum IL-10 levels preceded by GG and GT genotype respectively (Figure 7.1.4C).

![Graph A: rs3024498 IL-10 levels](image1)

![Graph B: rs3024496 IL-10 levels](image2)

![Graph C: rs3024490 IL-10 levels](image3)

FIGURES 7.1.4 Analysis of IL10 variants A. rs3024498, B. rs3024496 and C. rs3024490 and serum IL-10 levels in PTB cases and HC. ELISA was used to evaluate the serum cytokine levels and genotyping was by Sequenom platform. ELISA results are expressed as Mean with SEM and shown on a log Y axis expressed in picograms/ml. Significant comparisons are shown by dashed bar between compared groups. The numbers in parentheses indicate the sample size for each genotype. PTB: Pulmonary tuberculosis; HC: Healthy controls.

7.1.5 IL12 gene polymorphisms and serum IL-12 levels

Varying genotypes among the subjects accounted for differential serum IL-12 levels for variants rs3213094 and rs3212220 of the IL12 gene. Among the PTB cases at rs3213094 significantly decreased serum IL-12 level (p < 0.05) was seen for GG genotype as compared to AA genotype. For the same variant the serum IL-12 levels did not vary significantly among genotypes of HC (Figure 7.1.5A). For rs3212220 among PTB cases TT genotype showed significantly higher serum IL-12 level when compared to either GT (p < 0.05) or GG (p < 0.01) genotype. However the serum IL-12 levels did not vary significantly according to genotypes among HC (Figure 7.1.5B). For rs2853694 among PTB cases AA genotype showed trend towards higher serum IL-12 level in contrast to a reverse trend observed in HC where AA accounted for lowest
FIGURES 7.1.1.5. Analysis of *IL12* variants A. rs3213094, B. rs3212220, C. rs2853694, D. rs318216, E. rs730690 and serum IL-12 levels in PTB cases and HC. ELISA was used to evaluate the serum cytokine levels and genotyping was by Sequenom platform. ELISA results are expressed as mean with SEM and shown on a log Y axis expressed in picograms/ml. Significant comparisons are shown by dashed bar between compared groups. The numbers in parentheses indicate the sample size for each genotype. PTB: Pulmonary tuberculosis; HC: Healthy controls. Two variants depicted by A and B showed significant variation in serum IL-12 level with varying genotypes among PTB cases. Others C, D, E and F did not show any significant association but a trend was evident from the data obtained.
serum IL-12. Other genotypes were not found to be significantly differing in cytokine levels in either PTB cases or HC for this variant (Figure 7.1.1.5C). For variant rs3181216, among PTB cases the TT genotype recorded the highest serum IL-12 level followed by TA and AA genotype respectively. Among HC, a reverse trend was observed. The AA genotypes were higher producers as compared to TA and TT, in that order (Figure 7.1.1.5D). The variant at rs730690 failed to give any significant association between varying serum IL-12 level with varying genotypes among both PTB cases and HC; but a trend was observed among PTB cases with AA genotype accounting for high serum IL-12 levels followed by GA and GG genotype respectively (Figure 7.1.1.5E).

7.1.1.6 IL8 gene polymorphisms and serum IL-8 levels

Analysis of serum IL-18 levels and the corresponding genotypes revealed that mean serum levels of IL-8 could not significantly correlate with the genotype.

7.1.1.7 IL6 gene polymorphisms and serum IL-6 levels

For IL6 the studied variants i.e. rs2069849 and rs154606 did not significantly correlate with differing serum cytokine levels. For rs2069849 among PTB cases CC genotype accounted for higher serum as compared to GC and a similar trend was observed for HCs. GT genotype accounted for higher serum levels as compared to GG genotype for rs154606 among PTB cases, while the serum IL-6 levels among genotypes of HCs recorded a reverse trend with GG accounting for higher serum levels as compared to both GT and TT genotypes.

7.1.1.8 IL18 gene polymorphisms and serum IL-18 levels

Among the five variants studied for IL18 gene none could correlate significantly with the varying serum levels among the genotype. One interesting observation was that the loci rs5744256 and rs1834481 which were earlier shown to be in LD (section 5.2.1.1, figure 5.2.1.1) showed similar serum cytokine profile varying with the genotypes extending the genetic phenomena to the protein level also meaning that in addition of showing same allelic pattern as is expected in LD the different genotyped showed similar levels of serum IL-18 (e.g. the minor homozygous allele showed same allele frequencies and showed same serum cytokine levels). Interestingly rs5744292 which is not LD with previously mentioned variant mimicked the same profile as the variants in LD.
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The serum IL-18 levels did not vary significantly among the genotypes of the two other studied variants rs3882891 and rs795467.

7.1.1.9 IL1RA gene polymorphisms and serum IL-1Ra levels

Among the eight variants studied for IL1RA gene none could correlate significantly with the varying serum levels among the genotypes. Two pairs of loci namely rs3181052, rs4252001 and rs9005, rs315951 which were earlier shown to be in LD showed a duplicate serum cytokine profile varying with the genotypes extending the genetic phenomena to the protein level also.

The serum IL-1Ra levels did not vary significantly among the genotypes of the other studied variants rs380092, rs3213448, rs419598 and rs1794068 and did not significantly correlate with the mean serum IL-1Ra levels. No significant trend was observed.

7.1.1.10 IL1B gene polymorphisms and serum IL-1β levels

For the studied variants of the IL1B gene rs1143639 and rs1143643 the mean serum cytokine levels did not vary significantly with the genotypes among the cases as well as controls.

![Figure 7.1.1.11. Analysis of TNFB variant rs909253 and serum TNF-β levels among PTB cases and HC. ELISA was used to evaluate the serum cytokine levels and genotyping by Sequenom platform. ELISA results are expressed as mean with SEM and shown on a linear axis expressed in picograms/ml. Significant comparisons are shown by dashed bar between compared groups. The numbers in parentheses indicate the sample size for each genotype. PTB: Pulmonary tuberculosis; HC: Healthy controls.](image)

7.1.1.11 TNFB gene polymorphisms and serum TNF-β levels

Among the five studied variants passing all the exclusion criteria only one variant could be significantly correlated with serum TNF-β levels and pulmonary TB among healthy controls.

The CC genotype of the variant rs909253 correlated with higher TNF-β levels as compared to the CT genotype. CC genotype was higher producer among PTB cases also but the difference between the levels of CC and other genotypes was not significant (figure 7.1.1.11).
The genotypes of other variants viz. rs2239704, rs2229094, rs746868 and rs1041981 could not significantly correlate with serum TNF-β levels.

**7.1.12 IL2 gene polymorphisms and serum IL-2 levels**
Detected Serum IL-2 levels were very basal and did not differ between various groups in the study and could not be correlated with the corresponding genotypes.

**7.1.2 Correlation between serum cytokine levels and lymph node tuberculosis**

**7.1.2.1 IFNG polymorphisms and serum IFN-γ levels**
Differential serum IFN-γ level could not be significantly correlated to variant genotypes among LNTB cases although at rs2430561 a trend was seen with TA genotype accounting for high serum IFN-γ level followed by TA genotype and AA accounting for the low serum IFN-γ. A trend was also seen for rs1861493 where GG genotype was low serum IFN-γ producer as compared to AG or AA with AG accounting for highest serum levels, another loci rs1861494 which is in perfect LD with rs1861493 showed exactly the same profile with CC being low producers as compared to TT or CT genotype.

For variant rs2069718 among LNTB cases the mean serum level difference was not statistically significant among the genotype groups.

**7.1.2.2. TNFA polymorphisms and serum TNF-α levels**
The 32 samples for which genotypes were available picked for the analysis were monomorphic (although the loci was in HWE after considering 50 samples) and could not be analyzed for difference in serum levels.

**7.1.2.3 IL4 gene polymorphism and serum IL-4 levels**
The mean serum level of IL-4 among LNTB cases were very basal and was not considered for this comparison.

**7.1.2.4 IL10 gene polymorphisms and serum IL-10 levels**
Analysis of correlation between serum cytokine level and genotypes showed that the among the variants genotypes of rs3024498, rs3024490 and rs1878672 the serum level difference was not
7.1.2.5 *IL12* gene polymorphisms and serum IL-12 levels

Varying genotypes among the subjects did not account for differential serum IL-12 levels for variants rs3213094, rs3212220, rs3213096 and rs2853694 of the *IL12* gene. The serum IL-12 levels did not vary significantly according to genotypes.

7.1.2.6 *IL18* gene polymorphisms and serum IL-18 levels

Analysis of serum IL-18 levels and the corresponding genotypes revealed that mean serum levels of CC genotype of rs3882891 among LNTB cases was significantly lower when compared to CA or AA genotype with AA accounting for highest serum IL-18 levels (figure 7.1.2.6).

For the other studied variants viz. rs5744292, rs5744256 and rs1834481 a trend was seen. The AA genotype of rs5744292 was higher serum producer as compared to both GA and GG genotype. Similar differing serum level was observed for rs5744256 with TT correlating with higher levels compared to TC or CC genotype.
7.1.2.7 *IL6* gene polymorphisms and serum IL-6 levels

For *IL6* the studied variants i.e. rs2069849, rs1548216 and rs154606 did not significantly correlate with differing serum cytokine levels. For rs2069849 among LNTB cases CC genotype accounted for higher serum IL-6 levels as compared to CT genotype. GG genotype accounted for higher serum levels as compared to GT or TT genotype for rs154606 among LNTB cases, while the serum IL-6 levels among genotypes of rs1548216 was higher for GG genotype as compared to GC genotype.

7.1.2.8 *IL8* gene polymorphisms and serum IL-8 levels

The only variant rs2227538 could not be significantly correlated with varying serum cytokine levels.

7.1.2.9 *IL1RA* gene polymorphisms and serum IL-1Ra levels

A total of eight genetic variants of *IL1RA* gene namely rs3181052, rs4252001, rs380092, rs3213448, rs419598, rs1794068, rs9005 and rs315951 were studied for correlation with the serum IL-1Ra levels with corresponding genotypes among the variants. The mean serum IL-1Ra level did not significantly differ among the different genotypes of the *IL1RA* variants studied. Among two loci of the *IL1RA* gene namely rs3181052, rs4252001, which are in linkage disequilibrium with each other meaning that these variants can act as proxy of each other, the distribution of serum cytokine levels among the different genotypes was also same for the two variants. No significant trend was observed.

7.1.2.10 *IL1B* gene polymorphisms and serum IL-1β levels

For the studied variants of the *IL1B* gene rs1143639 and rs1143643 the mean serum IL-1β levels did not vary significantly with the genotypes among the cases as well as controls. However a trend was observed with serum levels of GG genotype of rs1143639 being higher serum producers than AG or AA genotypes. Similarly for rs1143643 AA genotype correlated with high mean serum IL-1β as compared to CA or CC genotype.

7.1.2.11 *TNFB* gene polymorphisms and serum TNF-β levels

Among the five studied variants passing all the exclusion criteria none of the variant could be significantly correlated with serum TNF-β levels and gene variants.

The genotypes of studied variants viz. rs2239704, rs2229094, rs746868, rs909253 and rs1041981
could not significantly correlate with serum TNF-β levels. No trend was observed.

### 7.1.2.12 IL2 gene polymorphisms and serum IL-2 levels

Detected Serum IL-2 levels were very basal and were not differing between various groups in the study and so could not be correlated with the corresponding genotypes.

### 7.2 Discussion

Cytokines as key mediators of immune response play a central role in homeostasis and any variation may account for a less effective immune response affecting the outcome of tuberculosis in humans (Flynn and Chan, 2001). In the present chapter we investigated the serum cytokine levels in active pulmonary tuberculosis and LNTB patients and the possible effect of gene variants of the corresponding cytokine genes on serum cytokine levels. On the basis of our results we were able to define a certain higher or lower producer phenotypes.

When analyzing a genetic dataset a surmounting challenge is to determine whether the samples are from a random homogeneous population or there is any evidence of a structured population which is genetically heterogeneous. Admixed or structured population check should be included in all genetic analysis (Devlin and Roeder, 1999) as structured population can induce high false positive results (Heiman et al., 2004) and give confounding genetic associations (Choudhry et al., 2006). In our study we used a principal component analysis as illustrated by Price et al., (2006) and found that the study population formed a homogenous group when related genetically thereby reducing chances of false positive results.

A recent study relating plasma IFN-γ levels and the IFNG gene promoter variant rs2430561 in Brazilian subjects (Vallinoto et al., 2010) showed that TT genotype accounted for higher plasma IFN-γ as compared to AT or AA genotype with AA accounting for the lowest plasma IFN-γ level. Although not statistically significant we also found a similar trend in our study with TT genotype showing higher serum IFN-γ levels as compared to AT and AA; with AA genotype showing the lowest serum IFN-γ level. Studies from south India (Vidyarani et al., 2006; Selvaraj et al., 2008) for the IFNG +874 polymorphisms also could not find any significant correlation between the IFNG variant genotypes and mycobacterial antigen induced IFN-γ levels. The other IFNG variant rs2069718 studied here has been implicated in a case-control association study in the west-African population for tuberculosis (Cooke et al., 2006). The polymorphism is adjacent to an octamer transcription factor 1 (OCT-1)
binding site and alters a TAAA transcription motif that might be clinically relevant. However, that study did not explore any association of the cytokine level and variants. For the same variant in our study we observe that the genotypes had no bearing on the serum IFN-\(\gamma\) level.

The TNFA variant rs3093662 included in the present study, although studied in relation to the outcome of sepsis (Wufrel et al., 2008) and type 1 diabetes in south Croatian population (Boraska et al., 2009) has not been reported so far in pulmonary tuberculosis. Our results indicate that this locus may be important in protection against tuberculosis as serum TNF-\(\alpha\) level for AA genotype was significantly high as compared to GG genotype among HC (p < 0.05). AA genotype being higher producer of TNF-\(\alpha\) might enjoy certain degree of tolerance to tubercle bacilli. PTB cases did not show any significant variation in the serum TNF-\(\alpha\) levels although GG genotype was certainly lower producer of TNF-\(\alpha\).

IL-10 is an anti-inflammatory cytokine which suppresses Th1 response and has macrophage deactivation property. Out of the three variants studied here, namely rs3024498, rs3024496 and rs3024490 only rs3024498 showed significant correlation between genotype and the serum IL-10 level. The GA genotype showed higher serum IL-10 levels (p < 0.05) than GG among PTB cases. This observation suggests a role for GA genotype in determining outcome of tuberculosis as PTB cases as this genotype might have a suppressed Th1 response against Mtb infection. Although both AA and GA genotype had high serum IL-10 levels, the difference between GA and GG was statistically significant (p < 0.05). Notably this variant has not been studied in tuberculosis so far; only implicated in obesity and colorectal cancer (Tsilidis et al., 2009). The variant rs3024490 has also not been studied in relation to tuberculosis till date. Present in 3’UTR of the IL10 gene the locus has been subject to study in asthma (Bosse et al., 2009). Variant rs3024496 also present in 3’ UTR of the IL10 gene has been studied in relation to tuberculosis in a case-control association study in the Korean population (Dooshin et al., 2005) and very recently also in South Africans (Möller et al., 2010). In our study we did not find any association of disease with these variants.

IL-12 is an important cytokine in mediating protective immunity to tuberculosis and its variants have been reported in many studies including from south India (Selvaraj et al., 2008), north India (Morahan et al., 2007) and Honkong Chinese population (Tso et al., 2004). However, these studies did not explore any association with serum IL-12 level. Among five variants of the IL12 gene studied here only two were observed to show correlation with serum IL-12 level. These two are novel variants studied for the first time in tuberculosis patients. Among the PTB patients studied here the GG
genotype of variant rs3213094 showed significantly low IL-12 level in comparison to AA genotype (p < 0.05). Although the patients in each genotype group was less, the patients for GG genotype were more (n = 15) as compared to AA genotype (n = 5). Interestingly even among healthy controls the GG genotype individuals were more (n = 19) than AA genotype (n = 3). It is possible that AA genotype of north Indians would mount a better immune response than GG genotype and the GG genotype individuals being more in number add to TB susceptible population. This point needs to be probed further in a larger study group. As for rs3212220, only five PTB patients showing high serum IL-12 level were of TT genotype whereas seventeen patients were of GG genotype that showed lowest serum IL-12 level. Among the HC also the GG genotype individuals showed low serum IL-12 level. This observation further suggests that in north Indian population these two variants may play a role in immune response to TB. Our observation is further strengthened by a recent report from South Africa which appeared while this study was going on in our laboratory (Möller et al., 2010) in which the variant rs3212220 contributed to a haplotype and has been shown to be relevant to tuberculosis. In addition when an attempt was made to find out genotype profile of individual patients considering rs3213094 and rs3212220 we found three common combinations of probable predictor genotypes for low serum IL-12 level which are GA-GT (n=12), GA-GG (n =10) and GG-GG (n= 9) of both the variants respectively. Incidentally both the variants are in strong linkage disequilibrium (LD) r²=1 and contribute to common multiallelic haplotypes (data not shown) further accentuating the importance of these loci in influencing serum IL-12 in patients. The genotypes of HC did not show any significant association hence not analyzed for predictors.

The rsIDs in the following genes namely IL1B, IL1RA, IL2, IL8 and IL6 could not correlate significantly with the varying serum cytokine levels. However a trend was evident for most of them and is discussed in the relevant section in results.

TNF-β, a pro-inflammatory cytokine, which functions in conjunction with TNF-α for the control of tuberculosis (Kaufmann, 2002) was found not to be significantly correlating with varying serum cytokine levels except for CT genotype of rs909253 which was significantly elevated as compared to CC genotype. This could imply that persons with CT genotype of rs909253 might have a degree of tolerance owing to an effective immune response as compared to CC genotype. The variant rs909253 had not been studied in tuberculosis but extensively correlated with myocardial infarction risk (Clark et al., 2006) and risk of gastric cancer (Lu et al., 2012) to name a few.
In chapter 6 we have characterized the serum cytokine profile of lymph node tuberculosis patients for the first time from north India. In the present chapter we correlated the serum cytokine levels of lymph node TB cases with genotypic variants of the corresponding cytokine genes. For lymph node tuberculosis only serum IL-18 levels correlated with varying genotypes with AA genotype (highest number of cases) of rs3882891 being highest serum IL-18 producers as compared to CC genotype. This variant of the IL18 gene has not been studied in relation to tuberculosis susceptibility but has been studied for the risk of myocardial infarction (Koch et al., 2011).

The rest of the genetic variants in all other studied genes did not correlate significantly with varying serum levels. However, a trend was seen for some of these as mentioned in the results of respective genes. Previous studies from India have sought to investigate the ex-vivo cytokine production after stimulation by mycobacterial antigen (Vidyarani et al., 2006; Selvaraj et al., 2008) but none have to our knowledge looked into the serum cytokine levels and associated gene variants. The serum cytokine level correlation with corresponding gene variants has been considered in only a handful of studies and analyzed mostly at single polymorphic locus (Vallinoto et al., 2010; Hurme et al., 1998). Thus to our knowledge this is the first study to have implicated the serum cytokine levels variation with their gene variants in a large number of cytokine genes.

The results presented here indicate that single nucleotide polymorphisms in the cytokine genes play a role in alteration of the levels of the corresponding cytokines in the serum in patients with active pulmonary tuberculosis. Many variants included in the study are novel loci which have been included in a tuberculosis polymorphism study for the first time and includes variants of the TNFA gene at rs3094662, the IL10 gene at rs3024498, rs3024490, the IL12 gene at rs3213094, rs2853694, rs3181216, rs730690, the IL4 gene at rs2243266 and TNFB gene at rs909253. These loci need to be further probed for relative risk for developing tuberculosis.

For LNTB, IL18 gene variant at rs3882891 significantly correlated with varying serum cytokine levels. The variants in the TNFA gene, the IL12 gene, the IL10, the IL12 and TNFB gene in case of pulmonary tuberculosis and IL18 gene for LNTB may have a role in varying serum cytokine levels in the north-Indian population for that may have a bearing on the genetic proneness or resistance to tuberculosis in this population group.