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RESEARCH ARTICLE

THE -*159C*/T POLYMORPHISM IN THE CD14 GENE AND PULMONARY TUBERCULOSIS

***Protiti Bose, Prabha Desikan, Ram P. Punde, Akanksha Shrivastava, Vikramsingh Gujar, Lalit Kumar and Manoj Pandey**

Bhopal Memorial Hospital and Research Centre, Karond, Raisen Bypass Road, Bhopal (MP) 462038 India

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ABSTRACT

Background: CD14 is expressed principally by cells of monocyte/macrophage lineage and plays a pivotal role in the innate immunity to intracellular infections. Recent research findings have revealed an association between the CD14 gene promoter polymorphism and several major infectious diseases. The purpose of the present study is to confirm the association between *CD14-159C/T* polymorphism (rs2569190) and pulmonary tuberculosis. Furthermore, association of various serum based biomarkers would be helpful in understanding better susceptibility to the disease state.

Methods: This study included a tertiary hospital based age and sex matched 12 patients with pulmonary tuberculosis and 15 subjects with normal lung functioning. These cases were recruited from January 2014 to April 2014. Subjects were included in the pulmonary tuberculosis group on the basis of smear positive for acid fast bacilli. DNA extraction was done by isopropanol precipitation method and amplification of *CD14* gene was done by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP).

Results: It was found that *CT* heterozygotes were more susceptible to pulmonary tuberculosis (OR=5.6, 95% CI=1.01 - 30.91, p = 0.05) whereas *TT* homozygotes were found to be less susceptible to pulmonary tuberculosis in Central Indian Cohort. Elevated level of IgE (182.5ng/ml) were found in *CT* genotype. Slight elevated N-GAL level were found in cases with *CT* genotype 5.31 ng/ml when compared with control's level. It was found that *CT* genotype had high bacterial load of $10.4x10^3$ copies/µl) whereas *TT* has bacterial load of $48.39x10^2$ copies/ µl and *CC* has very less bacterial load. Serum sCD14 estimation displayed somewhat high values (741 pg/ml) in cases with *TT* genotype. The results of our study on sCD14 level in TB

Conclusion: An association between the *C-159-T* polymorphism of the *CD14* gene and presence of tuberculosis in the Central Indian population was evident as the risk of TB was greater in participants with heterozygous *CT* genotype than in those with homozygous *CC* genotype. It was also seen that heterozygous *CT* genotype was found to be more susceptible for pulmonary tuberculosis whereas *TT* genotype was found to be less susceptible for TB and homozygous *CC* genotype was found as protective role against TB, which was evident from elevated cytokines in CT heterozygotes.

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INTRODUCTION

It is well known that TB susceptibility is determined not only by the infectious agent and environmental factors but also by the host genetic factor (Comstock, 1978; Sorensen, 1988; Bellamy, 2003). Several candidate genes have been examined to determine the probable TB 'susceptibility factor'. Cluster of differentiation 14 (CD14) is one of the candidate gene related to TB development (Rosas-Taraco,2007). The CD14 gene is localized on chromosome 5q31.1 and is a 55-kDa glycosyl phosphatidylinositol anchored glycoprotein expressed on the surface of immune cells such as monocytes, macrophages (Alavi-Naini, 2012).

****Corresponding author: Protiti Bose,***
Bhopal Memorial Hospital and Research Centre, Karond, Raisen Bypass Road, Bhopal (MP) 462038 India.

It is a membrane recognition factor involved in the binding of bacterial components or inhaled endotoxin, which can causes significant lung inflammation and activate immune pathways (Anas, 2010). CD14 play an important role in mediating clearance of pathogens and apoptotic cells from the lungs, as it is expressed primarily on the surface of monocytes, macrophages and neutrophil. CD14 exists in both membrane-bound (mCD14) and soluble forms (sCD14). Soluble CD14 (sCD14) is present in the circulation or body fluids, and its level increases in serum plasma during inflammation and other infectious diseases (Ayaslioglu, 2005; Juffermans, 1998). CD14 does not have an intracellular signaling domain and requires interaction with a TLR for cell activation. The best example of such an interaction is the binding of lipopolysaccharide (LPS) from Gram-negative bacteria by CD14 and the transfer of LPS from CD14 to TLR4.

Aside from phagocytosis, recognition of M. tuberculosis is an essential step in an effective host response. Lipoarabinomannan (LAM) is the major mycobacterial cell wall component which appears to resemble that of gram-negative bacterial lipopolysaccharide (LPS) (Zhang, 1993). Several circulating factors and receptors are involved in the immune recognition. Plasma LPS-binding protein enhances macrophage responses to LPS and LAM by transferring these microbial products to the cell surface receptor CD14 (Fenton, 1998). Similarly, soluble CD14 confers responsiveness to both LAM and LPS in CD14-negative cells (Yu, 1998). Concentrations of CD14 and LPS-binding protein in serum were elevated in patients with active tuberculosis (Juffermans, 1998). CD14, as an LRR containing pattern recognition receptor, has an important role in the host defense against different pathogens, because it can recognize not only LPS but other conserved structural molecules originating from Gram-negative and gram-positive bacteria and yeasts. Such molecules are peptidoglycan, lipoteichoic acid, mycobacterial lipoarabinomannam or polyuronic acid from Pseudomonas.

Polymorphism of *CD14* Gene : The latest community based transversal study on tuberculosis (TB) prevalence in tribal and non- tribal populations of 11 districts of Madhya Pradesh reported that the situation of TB in the tribes of this region was not very much different from that in the non tribal populations of the country (Bhat, 2009). In the central Indian state of Madhya Pradesh, it accounts for about one fourth of the total state populations. TB remains a leading cause of mortality worldwide, while the pathogenesis of TB has not been well understood.

Gene variants may transform protein production, function and individual's sensitivity to disease, thus play a role in pathogenesis of TB. CD14 is one of the extracellular adaptor protein that is required for recognition of *M. tuberculosis* and it is important for bring out lung inflammatory response during *M. tuberculosis* infection (Wang *et. al.,* 2013). CD14 was characterized as a receptor for bacterial endotoxin (LPS) in 1990, before the discovery and characterization of TLR and it can be known as the first described pattern-recognition receptor (Wright, 1995). The protein was first identified as a differentiation marker on the surface of monocytes and macrophages and was designated as CD14. The genomic DNA of human CD14 was cloned in 1988 and the gene was later mapped to chromo some 5q23–31 (Levan, 2005). CD14 is expressed pre-dominantly on monocytes, macrophages and neutrophils, but at lower levels also on epithelial cells, endothelial cells and fibroblasts. In addition to being expressed as a glycosylphosphatidyl (GPI)-anchored membrane protein, CD14 is also expressed in a soluble form (sCD14) (Wright, 1995).

Recent studies suggest that host genetic factors play critical roles in the pathogenesis and outcome of TB, especially genetic polymorphisms in the immune response to *Mycobacterium tuberculosis* may explain individual differences in both susceptibility to disease and the course of infection (Wang *et al.*, 2013). In the proximal promoter region of the *CD14* gene a functionally important polymorphism *C-159-T* has been described. In various respiratory diseases the commonly occurring *C-159-T* polymorphism of the *CD14* gene promoter has been shown to be an important genetic modifier of airway disease. The T allele of this SNP is associated with increased production of *CD14*, and persons with *TT* genotype would be more responsive to endotoxin. The *C-159-T* single nucleotide polymorphism (SNP) in the *CD14* gene promoter is reported to affect expression of CD14, with *TT* homozygous persons having higher CD14 expression (Baldini, 2002; Zambelli-Weiner, 2005). A common single-nucleotide polymorphism (SNP) is found at position -159 in the CD14 promoter, where a C-T change occurs. This polymorphism, C>T identified on the -159 position of promoter region of *CD14* gene, is found to be linked with increased transcriptional activity that affects expression level of CD14. The T allele showed enhanced transcriptional activity in those cells where the inhibitory transcription factor Sp3 had lower concentration (like monocytes), while in Sp3-rich hepatocytes the transcriptional activity of the T and C allele showed no difference (LeVan, 2001). CD14 expression, soluble sCD14 concentration, polymorphism of the *CD14* gene and alterations in the expression and function of TLRs showed association with tuberculosis. The inflammatory response to inhaled endotoxin begins when LPS binds to either the soluble or membrane-bound version of lipopolysaccharide-binding protein (LBP). This LPS-LBP complex then interacts with a cell surface CD14 protein which, together with an accessory protein called MD2, connects the LPS-LBP complex to the trans-membrane protein toll-like receptor 4 (TLR-4) on lung macrophages (Liebers, 2008). This leads to the inflammation and infection which can be measured by the increased levels of some proteins, immunoglobulins, proinflammatory or anti inflammatory mediators. These mediators act as biomarkers.

Soluble CD14: CD14 induced activation of macrophages results in the release of pro-inflammatory cytokines. These inflammatory cytokines lead to the production of acute phase reactants. Soluble CD14 (sCD14) is produced by enzymatically cleaved membrane CD14 (mCD14), mediated mainly by phospholipase C, and via secretion of CD14 (Ulevitch, 1995). Soluble CD14 or serum CD14 **(**sCD14**)** is believed to be an acute-phase protein that increases in several inflammatory processes. The increase in sCD14 was suggested to be associated with the accumulation of neutrophils at inflammatory sites (Lee, 2003). In the case of pulmonary tuberculosis increased level of sCD14 has been reported in TB patients (Rosas-Taraco, 2007).

Human Neutrophil Gelatinase - Associated Lipocalin (N-GAL): In respiratory mycobacterial infection, alveolar macrophages are the major targets of invasion. However, several evidences indicate that mycobacteria also interact with epithelial cells in the respiratory tract and invade these cells. Epithelial cells in the lungs play a role during mycobacterial infection by producing antimicrobial mediators (Aoki *et al*., 2004). Human Neutrophil Gelatinase Associated Lipocalin (N-GAL) also known as Lipocalin 2 (Lcn2), siderocalin or uterocalin is a member of lipocalin family of proteins that bind to small hydrophobic molecules and is produced by epithelial cells and macrophages (Kjeldsen *et al*., 2000). Upon activation human neutrophils secrete the content of these specific granules. N-GAL has microbicidal properties and plays a role in regulation of inflammation. Measurement of N-GAL is an experimental tool to understand the pathogenesis of tuberculosis. In injured epithelia, N-GAL expression is induced and consequently NGAL concentrations are elevated acute bacterial infections (Eagan, 2010).

Immunoglobulin E (IgE) : IgE is an antibody subclass found in the lungs, skin, and mucous membranes implicated in airway

inflammation and allergic reactions. IgE is one of the five classes of immunoglobulin that serve as a receptor for allergens, mast cells, parasitic antigens and produced by B-lymphocytes. The levels of circulating Ig-E are extremely low compared to other immunoglobulins (Aschalew *et. al.,* 2012).
The purpose of the present study is to confirm the association between *CD14-159C/T* polymorphism with pulmonary tuberculosis. This polymorphism is likely to be important in patients with pulmonary tuberculosis as polymorphisms have already been identified that are associated with both hypo- and hyper-immune responsiveness. Furthermore, association of various serum based biomarkers would be helpful in understanding better susceptibility to the disease state.

Methods

Our study was conducted on the Central Indian Population of Bhopal city. People suffering from Pulmonary Tuberculosis from Bhopal based tertiary care hospital were examined. This pilot study enrolled 12 patients with pulmonary tuberculosis (smear positive for Acid Fast Bacilli) and 15 subjects with evidence of normal lung functioning. The Institutional Ethical Committee approved the study. Cases and Control subjects were provided with all the information related to the study and informed consent forms were obtained from them.

A pulmonary TB (PTB) case was defined as an individual whose sputum was positive for AFB by Ziehl–Neelsen (ZN) microscopy and/or growth of **M. tuberculosis** by culture examination. These specimens were processed for culture on Lowenstein-Jensen's medium and mycobacterial growth was identified. The BacT/ALERT 3D system (BioMérieux Inc., North Carolina, USA) was used for the culture based detection. Samples used for the study were blood and sputum. Demographic characteristics, spirometric data, Body Mass Index (BMI), medical history, smoking status and other medical problems were taken by interviewing and from their medical file records (as much information as was possible was gathered). Inclusion criteria for cases, subjects with pulmonary tuberculosis cases, with at least two sputum smear positive for acid fast bacillus (SSP-AFB); Exclusion criteria were: subjects with extra-pulmonary TB; Control subjects with normal lung functioning and no episodes of PTB nor extra PTB infection.

Sample Processing: Four millilitres blood from both, cases of tuberculosis and control subjects was collected in EDTA vacutainers (BD Biosciences, USA). Blood was processed by centrifugation (Bench top centrifuge, REMI, India) separating the plasma. DNA was extracted from the whole blood using DNA isolation by Isopropyl alcohol precipitation method.

DNA Isolation: DNA was isolated from blood by phenol chloroform method described by Sambrook and Russel, 2001. Genomic DNA was amplified by Polymerase Chain Reaction. DNA quantification by Smartspec UV spectrophotometer (BioRad, USA) was performed to check the purity of DNA.

Polymerase chain reaction-Restriction fragment length polymorphism: The DNA was denatured at 96ºC for 3 minutes and temperature cycling was set at 96°C for 40 seconds for denaturation,61ºC for 40 seconds for annealing, 72°C for 50 seconds for extension, then step 2 for 38 cycles and 72ºC for 10 minutes for final extension. PCR as carried out in Thermocycler (PTC-200, MJ Research, USA) in a total volume of 25 µl of PCR mixture was prepared which contains 100ng/µl genomic DNA,10X PCR (Invitrogen, USA),50mM $MgCl_2$ (Invitrogen, USA), 10mM) deoxynucleotide triphosphate dNTPs (GeNei, U.S.A.),5U Taq DNA polymerase (Merck, U.S.A.) and 5pM of each primers (GeNei, USA) - 5'GTGCCAACAGATGAGGTTCAC-3'and 5'- GCCTCTGA CAGTTTATGTAATC- 3'. The amplified samples were subjected to 2% Agarose gel electrophoresis to obtain a band of -497bp in all PCR products and gel was visualized with ethidium bromide staining. The obtained and of CD14 promoter was digested by commercially available *AvaII endonucleases* (Thermo Scientific, Lithuania, USA), for detection of SNPs in CD14.

Quantitative detection by Real Time PCR

Real time assay was performed in the extracted DNA with the help of Fluorescence resonance energy transfer FRET probes using LC PCR 2.0. Absolute quantification of bacterial load was performed by using Light cycler software 4.1 with appropriate quantitative standards (MTBC positive controls) and by following the guidelines for the quantitative analysis on the Light Cycler 2.0 instrument. Quantitative bacterial load means the total amount of bacterial DNA present in the given sample expressed in copies/µl.

Total N-GAL levels: Total N-GAL levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioporto Diagnostics, Denmark). Absorbance of the wells were read at 450nm in a microplate reader (ELISA reader, BioRad, India).

Human Ig-E level: Total serum IgE levels were measured using a commercially available ELISA kit (Immunology Consultants Laboratory, Inc. Portland) following the manufacturer's instructions. Absorbance were read at 450nm in a microplate reader (ELISA reader, BioRad, India).

Serum CD14 levels: Serum sCD14 levels were measured using a commercially available ELISA kit supplied by RayBio® US. Finally absorbance of the wells were read at 450nm immediately in a microplate reader (ELISA reader, BioRad, India).

Statistical Analysis: Statistical Package for EndNote was used for statistical analysis. The quantitative results were expressed as means ± standard deviation (SD). Differences were considered to be significant when the **p**-value was ≤ 0.05.The relative risk associated with rare alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI). We calculated the ORs and corresponding 95% CI values, to evaluate the association between *CD14 2159 CT* polymorphism and TB risk.

RESULTS

This study included 12 patients with tuberculosis and 15 people as healthy volunteers with normal lung functioning. The mean age of the patients was 44 ± 18 and of controls was 48 ± 59. The cases and controls did not differ significantly by sex, age and ethnic characteristics. Genotyping was done in all study population and healthy controls. The screening of the promoter region of the CD14 gene (*rs2569190*) revealed the presence of single nucleotide polymorphism (SNP), a C-to-T transition. To genotype the subjects for *C-159-T* polymorphism a restriction fragment assay was performed.

The risk of pulmonary tuberculosis was greater in individuals with the CT genotype than in CC and TT genotype (Odds Ratio=5.6, 95% CI =1.0147 - 30.905, *P* = 0.05) of the Central Indian Population. A significant difference was observed when the genotypes of cases and controls were compared. Genotypic distribution of study population between cases, with tuberculosis was found to be, CC=17%, CT=59%, TT=25%, whereas in controls CC=27%, CT=20%, TT=54%. CT heterozygotes had high mycobacterial load (p=0.05) as compared to TT and CC homozygotes (Table 1).

Table 1. Distribution of various *CD14* gene (rs2569190) genotypes in the study population among cases with *MTB* infection

Genotypes	Control Subjects	Test subjects	p value	odds ratio	95% confidence interval
CC	27%	17%	0.66	0.55	0.08 - 3.68
CT	20%	59%	0.05	5.6	1.01 - 30.90
TT	54%	25%	0.24	0.2917	0.05 - 1.53

Plasma levels of N-GAL: Mean plasma concentrations of N-GAL were 4.82 ng/ml in the TB patient's group and 3.73ng/ml in the control group, demonstrating significantly raised plasma levels of N-GAL in the TB patient's group (Fig.1). TB is an inflammatory disease of the lung and enhanced levels of NGAL in TB patients showed the level of inflammation.

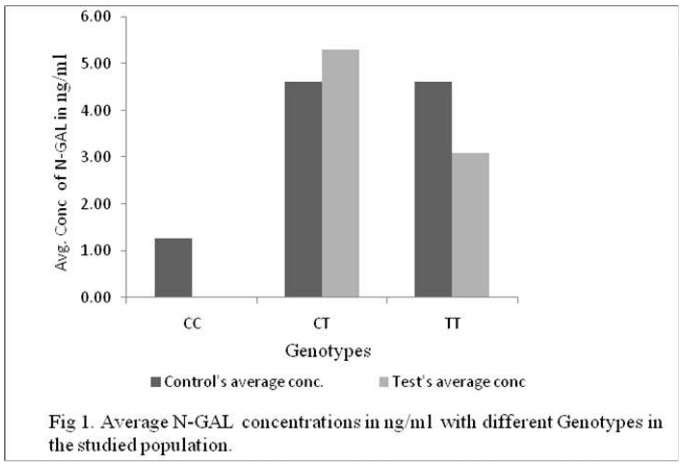

Fig 1. Average N-GAL concentrations in ng/ml with different Genotypes in the studied population.

Levels of Ig-E in plasma: The findings of the plasma concentrations of IgE were 157.1 ng/ml in the tuberculosis patients and 144.5 ng/ml in the in normal healthy volunteers. There was an elevation in the levels of Ig-E when compared to normal healthy volunteers (Fig 2).

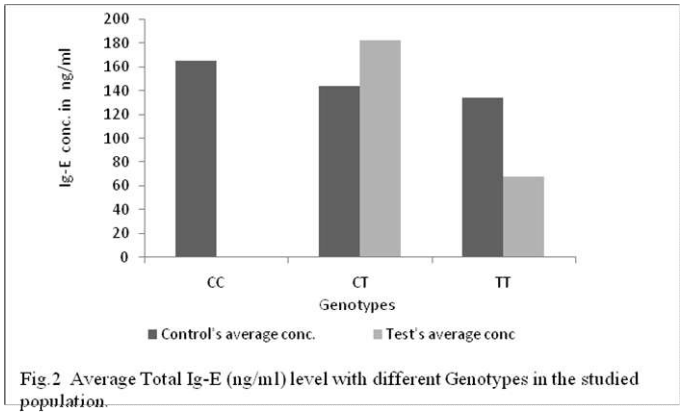

Fig.2 Average Total Ig-E (ng/ml) level with different Genotypes in the studied population.

Levels of sCD14: The levels of sCD14 were higher in patients with TB than those in the control subjects (cases, 706.78 ng/ml and controls, 609.30 ng/ml). Mean sCD14 levels were significantly higher in the TB patients than in the healthy controls. Serum sCD14 estimation displayed somewhat high values (741 pg/ml) in cases with *TT* genotype, whereas serum IgE and N-GAL showed slight elevated level in cases with *CT* genotype (182.5ng/ml and 5.31 ng/ml respectively) when compared with control levels (Fig 3).

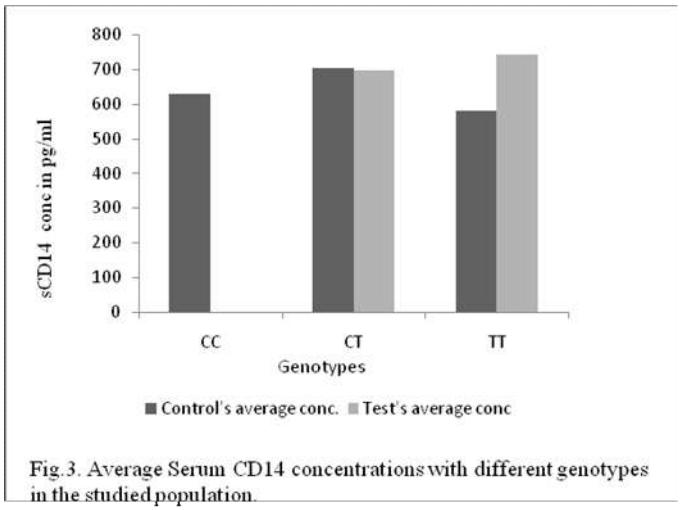

Fig.3. Average Serum CD14 concentrations with different genotypes in the studied population.

The Total Bacterial Load In LC PCR Positive Samples: It was found that CT genotype had high bacterial load (10.42 $x10^3$ copies/µl) whereas *TT* has bacterial load of $48.39x10^2$ copies/ µl and *CC* has very less bacterial load. It indicates the high bacterial infection among heterozygous *CT* genotypes as compared to homozygote *TT* and *CC* genotype (Fig 4).

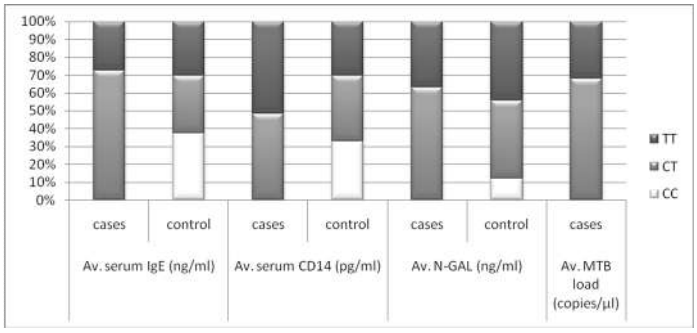

Fig. 4. Association of different genotypes and screened Biomarkers and MTB load in the Central Indian population

DISCUSSION

Our results demonstrate an association (p=0.05) between the *C-159-T* polymorphism of the CD14 gene alongwith the presence of tuberculosis in Central Indian population. It was found that *CT* heterozygotes greater incidence of pulmonary tuberculosis (OR, 5.6; 95% CI=1.01- 30.905, p = 0.05) whereas *TT* homozygotes were found with less incidence of pulmonary tuberculosis. The frequency of *CT* genotype was higher in TB patients than in healthy controls. In addition, the risk of TB was greater in participants with heterozygous *CT* genotype than in those with homozygous *CC* genotype. It was evident that heterozygous *CT* genotype was found to be more susceptible for pulmonary tuberculosis in studied Central Indian population; whereas *TT* genotype was found to be less susceptible for TB and homozygous *CC* genotype was found as protective role against TB. There are a few studies investigating the role of *CD14-159C/T* (*rs2569190*)

polymorphism in tuberculosis. Similar to our study, a study conducted by Alavi-Naini (2012) on Iranian population indicated that the presence of *CD14-159TT* which might be a risk factor for the development of pulmonary TB and that elevated serum concentrations of sCD14 could be a good index for TB development. They demonstrate a statistically significant association between *C-159-T* polymorphism of the CD14 gene and TB in Iranian population groups. The frequency of *TT* genotypes was higher in the TB patients than in the healthy controls. In addition, the risk of TB was 3.5-fold greater in participants with the *TT* genotype than in those with the *CC* genotype. Kang and coworkers (2009) reported that the *-159TT* genotype of the CD14 gene is associated with the development of tuberculosis in the Korean population. They showed that the frequency of the *-159TT* genotype was higher in TB patients than in healthy controls, with an OR of 1.6 in the *TT* genotype. In 1999, Baldini and co-workers, reported of CD14 polymorphism and susceptibility to infectious disease. In their studies on Hispanic and non-Hispanic white population a polymorphism at position *159(C/T)* in the promoter region of the human CD14 gene was reported. Similar study done (Chao *et al*., 2005) on correlation between *C-159-T* polymorphism and diseases such as liver cirrhosis, acute pancreatitis also reports of the same. Apart from this other studies show that *C-159-T* polymorphism influences the binding of the SP1 and SP3 transcription factors that regulate the surface expression of CD14 (LeVan *et al*., 2001). This polymorphism could therefore be a genetic factor for inter individual differences in susceptibility to infectious disease.

Furthermore, study conducted by Rosas-Taraco and colleagues (2007) on a Mexican population demonstrated that the frequency of the *TT* genotype was higher in TB patients than in controls (35.6% vs. 12.3%), and that the risk of TB was 2.26-fold greater in those with the *TT* genotype. Whereas, genotypic distribution of our study population in patients with tuberculosis is (*CC*=17%, *CT*=59%, *TT*=25%) and controls (*CC*=27%, *CT*=20%, *TT*= 54%). Our study demonstrates that frequency of *CT* genotype was higher in TB patients as compared to healthy controls. Therefore, the risk of TB was greater in participants with *CT* genotype than in those with *TT* and *CC* genotype. Furthermore, they observed higher levels of sCD14 in TB patients and declining patterns after anti-tuberculosis treatment; however they did not report any association between sCD14 level and *C-159-T* polymorphism genotypes. sCD14 found in normal human serum and is increased in several conditions characterized by local or systemic activation of monocytes/macrophages. Although the function of sCD14 in human disease is not yet clarified, a potential pathogenic role has been proposed for several infectious diseases. There is some evidence to suggest that the *C-159-T* polymorphism was associated with circulating sCD14 levels (Baldini *et al*., 1999), but Levan and associates (2001) reported no correlation between sCD14 levels. It is also known that the serum sCD14 concentration is higher in individuals homozygous for the *T/T* than in the two other genotypes(*C/C* and *C/T*).

Thus there is suggestive evidence that an association between serum sCD14 level and *159C/T* genotype only after infection by *M. tuberculosis*. Very few studies have been published on the association between *C-159-T* polymorphism, sCD14 and TB. Our study provided the data which shows elevated levels of sCD14 in the patients with pulmonary TB compared with control subjects (706.78 ng/ml and 609.30 ng/ml respectively). Serum sCD14 estimation displayed somewhat high values (741 pg/ml) in cases with *TT* genotype. The results of our study on sCD14 level in TB patients is supported by studies on Colombian population (Pacheo *et al*., 2004) as well as population of Netherlands (Juffermans *et al*., 1998) reporting of high levels of sCD14 during active TB. In a prospective study done by Adams and colleagues (1999) in the Western Cape Province of South Africa, where tuberculosis and intestinal-parasite infection are common. In this study it was found that Ig-E levels were higher in the patients with active TB and Ig-E concentrations decreased after successful treatment of tuberculosis. Similar results were found by Ohrui and colleagues (2000) on Japanian population. Specific Ig-E levels were elevated in both tuberculosis and leprosy patients, suggesting that this antibody response is towards the shared mycobacterial antigens (Yong *et al*., 1989). Another study by Rohini and colleagues (2012) on a south Indian population reported that sIgE in tuberculosis patients were compared with normal healthy volunteers and were found to be increased significantly (p<0.001). There was a significant elevation in the levels of IgM and IgE when compared to normal healthy volunteers of Chennai cohort. Our study also signifies the elevated levels of IgE in patients with tuberculosis as compared to controls. Mean plasma concentrations of Ig-E were 157.11 ng/ml in the tuberculosis patients and 144.55 ng/ml in the in normal healthy volunteers. Elevated level of IgE (182.5ng/ml) were found in *CT* genotype of Bhopal cohort.

In our study it was found that mean plasma concentrations of NGAL were 4.82 ng/ml in the TB patient's group and 3.73 ng/ml in the control group, demonstrating significantly raised plasma levels of NGAL in the TB patient's group. Our study showed slight elevated level in cases with *CT* genotype 5.31 ng/ml when compared with control's level. TB is an inflammatory disease of the lung and enhanced levels of NGAL in TB patients showed the level of inflammation. Additionally, we quantified the MTB DNA of 12 TB patients by Real Time PCR (qPCR). Our all samples were found to be MTB positive when the samples were tested on qPCR. It was found that *CT* genotype had high bacterial load (10.4 $x10^3$ copies/µl) whereas, *TT* has bacterial load of 48.4 $x10^2$ copies/ µl and *CC* has very less bacterial load. It indicates the high bacterial infection among heterozygous *CT* genotypes as compared to homozygote *TT* and *CC* genotype. Furthermore, the study by Alavi-Naini (2012) on Iranian population reported of high TB patients in heterozygous CT genotype. Ayaslioglu and co-workers (2013) did not find any association between CD14-*159C/T* population and tuberculosis in Turkish population.

Limitations of the Study: The study has few limitations. First, our finding is based on small sample size observations; we cannot, therefore, exclude the possibility of confounding variables that may be associated with each of the exposures. Secondly, we compared PTB patients with non TB patients and frequency of CD14-*159C/T* polymorphism (*rs2569190*) in each group was analyzed. Our control group may not truly represent to the whole local population as far as prevalence of PTB in family history nor their socioeconomic status .

Conclusion

Our data indicate that the presence of CD14-*159CT* might be a risk factor for the development of pulmonary TB in central Indian cohort. Elevated levels of biomarkers IgE, N-GAL,

sCD14 in *CT* genotype were found in patients whose sputum samples had a high mycobacterial load. It may be possible to explore the utility of these biomarkers in TB patients as prognostic indicators. Our results are based on relatively small number of subject; further investigations using a larger sample size are needed to demonstrate the role of the CD14-*159C/T* gene polymorphism in susceptibility to tuberculosis disease. Further elucidation of the molecular pathways that explain this association may provide important new insights.

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