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CD14 Gene (-159 C>T) Polymorphism and its Surface Expression on Monocytes in Pulmonary Tuberculosis Patients

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Abstract

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BACKGROUND: Tuberculosis (TB) is an infectious disease that affects millions of people around the world. The innate immune response against TB starts by interaction of several receptors on monocytes with mycobacterium tuberculosis (MTB). CD14 is one of these receptors present on the monocytes which facilitate the entry of MTB into the cell. Certain polymorphisms in CD14 gene, for example, CD14 (-159 C>T) in the promotor region have suggested susceptibility of TB.

AIM: This study was designed to determine and compare CD14 (-159 C>T) gene polymorphism and its surface expression in pulmonary TB patients (before and during anti-TB treatment) and healthy controls.

MATERIALS AND METHODS: The study population comprised three groups (pulmonary TB patients before treatment, pulmonary TB patients during treatment, and healthy controls) whereas 53 blood samples were collected from each group. The percentage of monocytes and CD14 mean fluorescence intensity (MFI) was measured by flow cytometry whereas polymerase chain reaction-restriction fragment length polymorphism was used to determine gene polymorphism.

RESULTS: CD14 MFI was significantly high in healthy controls than in TB patients (432 as compared to 193 and 365, $p < 0.0001$). There was no significant difference in CD14 single nucleotide polymorphism allele frequencies or genotypes between TB patients and healthy controls.

CONCLUSION: CD14 gene (-159 C>T) polymorphism was not associated with pulmonary TB disease in a sample of Pakistani population and surface expression of CD14 receptor on peripheral blood monocytes decreases with active TB disease and during treatment.

Introduction

Tuberculosis (TB) is a bacterial infectious disease caused by an acid-fast bacillus; i.e., mycobacterium tuberculosis (MTB). Approximately one-third of the world's population is infected with MTB; however, a relatively small proportion (5–10%) of infected individuals develops TB disease during their lifetime [1]. Mortality rate of TB has reached up to 1.5–2 million deaths per year; hence, it became second high mortality rate disease after HIV/AIDS [2]. More than half of the world's TB cases (54%) had been reported in Asia. Pakistan ranks fifth among high TB burden countries where its mortality rate is 26 cases per 100,000 individuals [3].

The host defense mechanisms start with the recognition of MTB by phagocytic cells which bear germ-line encoded pattern recognition receptors (PRR) on their surface [4], [5]. CD14 is one of these receptors encoded by human CD14 gene located on chromosome 5q31.3 [6].

CD14 is a multifunctional glycoprotein antigen that is mainly expressed on monocytes and serves as the marker of identification. It contributes to receptor

mediated uptake of non-opsonized MTB through recognizing mycobacterial cell wall components, such as lipoarabinomannan, lipoteichoic acid, and lipoproteins [7]. Activated macrophages upregulate some of their PRRs expression, while CD14 expression is decreased in active TB infection [8].

The blood signatures are promising marker of TB which not only correlates with the magnitude of radiographic findings of the disease but it is also moderated on effective treatment [9]. Variations in the percentage of circulating monocytes and their subsets during MTB infection have already been documented [10], [11].

Host's genetic factors also influence the extent of immune response and hence determine the fate of MTB infection as clinical or sub-clinical [12]. Single nucleotide polymorphism (SNP) of the CD14 gene (-159C>T) at position rs2569190 is close to the recognition site of Sp1 transcription factor, and mutant allele T genotype is associated with the increased expression of CD14 [13], [14]. The studies have suggested that CD14-159TT genotype (rs2569190) could be a risk factor for pulmonary TB [15], [16], [17], [18]. Therefore, this study was designed to determine CD14 gene (-159 C>T) polymorphism and surface expression of CD14

receptor on monocytes before and during treatment of pulmonary TB patients in a local population.

Materials and Methods

It was a cross-sectional comparative study conducted during April 2017–April 2018 in the Department of Immunology University of Health Sciences (UHS) Lahore Pakistan after approval of Ethical Review Committee of UHS and Gulab Devi Hospital Lahore.

Study subjects

A total of 159 subjects were recruited from the Gulab Devi Chest Hospital Lahore and they were made into three groups of 53 individuals in each, i.e., pulmonary TB patients before anti-TB treatment, during treatment, and healthy individuals as controls. Pulmonary TB patients were diagnosed on the basis of sputum smear microscopy and chest X-ray. Age, gender, and ethnicity matched unrelated individuals having neither history nor current signs and symptoms of TB infection were recruited as healthy controls. After obtaining a written informed consent 3 ml of venous blood of all the subjects was drawn in EDTA tube.

Flow cytometric analysis for mCD14

The peripheral blood leukocytes were isolated using lyse-wash method from freshly collected EDTA blood. Monocytes were stained using monoclonal anti-human CD14-FITC and CD45-PerCP antibodies for 10 min at 4°C. The final cell suspension was prepared using 500 µl of ×1 sheath fluid. Processed samples were acquired and analyzed on FACSCalibur (BD, USA) using software CellQuest. At least 50,000 events were analyzed and percentage of CD45⁺CD14⁺ monocytes and CD14 mean fluorescence intensity (MFI) was determined.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for SNP analysis of CD14 gene

Genomic DNA was extracted using phenol chloroform method from the whole blood [19]. PCR was carried out using 100 ng/uL of extracted DNA and specific set of primers (Forward 5'-TAGATTCTCTGGGATATAAGG-3' and Reverse 5'-CTGACAGTTTATGTAATCCTG-3'). DNA was initially denatured at 94°C for 5 min. The cycling conditions were set as denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s for

35 cycles followed by the final extension at 72°C for 5 min. The amplified PCR product was digested with restriction endonuclease, *Eco47I* (10 units/uL) followed by electrophoresis using 2% agarose gel to identify different genotypes. The band size of 357 bp indicated CC genotype, 357–217–140 bp indicated CT heterozygous genotype, and 217–140 bp showed TT genotype [20].

Statistical analysis

The data were entered and analyzed using SPSS 20.0. Mean ± SD was calculated for quantitative variables whereas frequencies were calculated for qualitative variables. Data were analyzed using Pearson's Chi-Square test, one-way ANOVA and *post hoc* Tukey's test. Polymorphism analysis and allele frequencies of *CD14* (–159 C>T) were compared among the groups using Chi-square test. Odds ratios (OR) and confidence intervals (CIs) were calculated using logistic regression. $p \leq 0.05$ was considered as statistically significant.

Results

Demographic data of study subjects

Demographic data of 106 pulmonary TB patients and 53 healthy controls are shown in Table 1. Mean age was compared between groups using independent sample t-test (mean difference = 7.1). A positive correlation of history of smoking and household contact was detected with TB (21.6% as compared to 7.4%, $p = 0.01$, OR, 95% CI = 1.3 and 21.6% as compared to 9.4%, $p = 0.02$, OR, 95% CI = 1.3, respectively).

Table 1: Demographic data of the study subjects

Variables	Pulmonary TB cases (n=106)	Healthy controls (n=53)	p-value	Odds ratio (95% CI)
Age (years)				
Mean±SD	39.14±16.2	32.06±11.5		
Gender				
Male n (%)	44 (41.5)	30 (56.6)		
Female n (%)	62 (58.4)	23 (43.3)		
Smoking history n (%)	23 (21.6)	4 (7.4)	0.01*	1.3, 1.1~1.6
Contact history n (%)	23 (21.6)	5 (9.4)	0.02*	1.3, 1.0~1.6
MDR n (%)	6 (5.6)	----		

n=Number, %=Percentage, * $p < 0.05$ =Statistically significant. MDR: Multidrug-resistant, CI: Confidence intervals, TB: Tuberculosis.

Identification and comparison of CD45⁺CD14⁺ monocytes by flow cytometry

Monocytes were gated first at forward and side scatter plots followed by intersecting two antibodies gates. CD45/CD14 double positive population was gated (Figure 1).

The percentage of CD45⁺ CD14⁺ monocytes and CD14 MFI was compared among TB patients before and after anti-TB treatment and healthy controls

(Table 2). The percentage of CD45⁺CD14⁺ monocytes was compared using one-way ANOVA where its mean

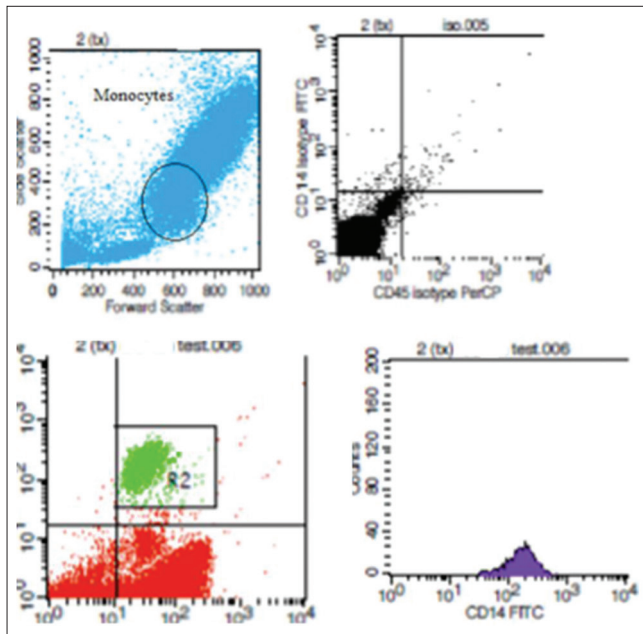


Figure 1: Representative flow cytometric analysis of peripheral blood monocytes in the study cohort. (a) Leukocytes on forward and side scatter with monocytes in encircled area. (b) Isotype control run on CD45-PerCP and CD14-FITC plot. (c) FACS plot for CD45-PerCP and CD14-FITC of representative sample showing gated double positive population in R2 and expressed in percentage of cells. (d) Histogram of CD14-FITC expression on CD45⁺CD14⁺ monocytes presented as mean fluorescence intensity

was high in TB patients on treatment than TB patients before treatment and healthy controls (6.1% as compared to 5.6% and 5.7%) and the difference among these groups was not statistically significant. The median of CD14 MFI was compared using Kruskal–Wallis test which was high in healthy controls as compared to TB patients before and after treatment and the difference was statistically significant (432 as compared to 365 and 193, $p < 0.0001$) whereas treatment naïve TB patients had high CD14 MFI than TB patients on treatment and its difference was statistically significant (365 as compared to 193, $p < 0.0001$).

Table 2: Comparison of percentage of CD45⁺CD14⁺ monocytes and CD14 mean fluorescence intensity among the studied groups

Variables	Pulmonary TB patients before treatment	Pulmonary TB patients on treatment	Healthy controls	p-value
CD45 ⁺ CD14 ⁺ (%)				
Mean±SD	5.6±2.2	6.1±2	5.7±1.8	0.2
CD14 mean fluorescence intensity				
Median	365	193	432	<0.0001*
Range	105–935	66–693	166–964	

* $p < 0.05$ =Statistically significant. TB: Tuberculosis.

CD14 (–159 C>T) SNP genotype and allele frequencies in pulmonary TB patients and healthy controls are shown in Table 3. Homozygous CC genotype was more frequent in overall study population as compared to TT genotype. While comparing

Table 3: Comparison of CD14 (–159 C>T) genotype frequencies between study groups

Variables	TB patients	Healthy controls	p-value
Genotypes			
CC n (%)	49 (46.2)	26 (49.0)	0.082
CT n (%)	29 (27.3)	15 (28.3)	
TT n (%)	28 (26.4)	12 (22.6)	
Alleles			
C n (%)	127 (59.9)	67 (63.2)	0.8
C+T n (%)	85 (40.1)	39 (36.7)	

n=Number, %=Percentage, $p < 0.05$ =Statistically significant. TB: Tuberculosis.

between groups, homozygous TT was slightly higher in pulmonary TB patients as compared to healthy controls (26.4% as compared to 22.6%), but the difference was not statistically significant. The association of exposure to T allele and pulmonary TB disease was determined using Pearson's Chi-square test. The comparison showed no statistically significant difference.

The CD14 MFI was compared with CD14 (–159 C>T) genotypes and alleles using one-way ANOVA and independent sample t-test, respectively (Table 4). There was no statistically significant difference for median CD14 MFI among CC, CT, and TT genotypes. Mean CD14 MFI was slightly higher for C allele than T allele (398 as compared to 351), but it was not statistically significant.

Table 4: Comparison of CD14 mean fluorescence intensity with CD14 (–159 C>T) genotypes and alleles

Variables	CD14 mean fluorescence intensity (Mean±SD)	p-value
Genotypes		
CC	398±201.5	0.2
CT	329±165.7	
TT	378±174.8	
Alleles		
C	398±201	0.1
T	351±170	

$p < 0.05$ =statistically significant.

Discussion

In the current study, 53 individuals in each group, i.e., TB patients before the treatment, after anti-TB therapy and healthy controls were included and compared for CD14 gene polymorphism and surface expression on monocytes. Since monocytes/macrophages have been documented as chief effector cells in defending the host against MTB infection [11], therefore, current study determined percentage of peripheral blood CD45⁺CD14⁺ monocytes in TB patients before and during treatment and in healthy controls. There was no significant difference among the groups. This finding is in contrast with the previous study which documented an increase in monocyte compartment of peripheral blood in active TB disease [21]. Rakotosamimanana *et al.* observed reduced fraction of white blood cell count and in particular, monocyte on completion of TB treatment [22].

CD14 MFI in CD45⁺CD14⁺ monocytes was compared among study groups to determine the effect of treatment on mCD14. There was highly significant difference in CD14 MFI among the three groups

($p < 0.0001$). It was noted that healthy subjects had the highest CD14 MFI followed by TB patients before the therapy whereas patients on anti-TB treatment had the lowest CD14 MFI. These findings are consistent with Guirado *et al.* who suggested that activated mononuclear phagocytes have increased PRR expression, particularly of mannose receptor, while CD14 expression was decreased during active TB infection [8].

The innate immune response is also regulated by *CD14* gene polymorphism [23]. The current study determined the prevalence of *CD14* (-159 C>T) gene polymorphism by PCR-RFLP technique and investigated its correlation with the status of TB disease. It was detected that CC genotype was more prevalent in the overall study cohort while TT genotype was more common in TB patients than in healthy controls (26% as compared to 22%). Similarly, while comparing alleles between the study groups, mutant T allele was more common in TB patients than in healthy controls, but the difference was not statistically significant. For *CD14* (-159 C>T) SNP, C allele is a reference allele in the Asian population [24]. The results of the current study are in agreement with Ayaslioglu *et al.* (2013) who also performed SNP genotyping through PCR-RFLP and could not detect significant difference in allelic as well as in genotype distribution between TB patients [19].

A study performed in Chinese population documented high frequency of T allele in C (-159) T polymorphism (OR 1.4, CI 95%) in TB patients than in controls [17]. Alavi-Naini *et al.* reported 2.3-fold increased risk of TB disease in individuals with T allele as compared to those without it [25]. In the present study, there was no significant association of T allele with TB disease susceptibility. The reason may be because the current study included genetically different population and smaller cohort.

To investigate whether *CD14* (-159 C>T) SNP genotypes have impact on expression of mCD14 on monocyte surface, the current study compared genotypes and alleles with CD14 MFI on CD45⁺CD14⁺ monocytes and no statistically significant effect of *CD14* MFI with different *CD14* SNP genotypes as well as C or T alleles was detected. However, T allele had slightly decreased CD14 MFI as compared to C allele. On the contrary to this, it was reported that mutant allele T genotype is associated with the increased expression of *CD14* [13].

Conclusion

CD14 gene (-159 C>T) polymorphism was not associated with pulmonary TB disease in a sample of Pakistani population and therefore does not support this SNP for TB susceptibility. The surface expression

of CD14 receptor on peripheral blood monocytes was decreased in active TB infection which further decreased during anti-TB treatment. It suggests the potential role of CD14 surface expression in monitoring anti-TB treatment.

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