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Association of Chemokine Genes *CXCL9* and *CXCL10* Polymorphisms with Tuberculosis in Pakistani Population

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Key words
Tuberculosis, Cytokines, Polymorphism, Genotype, Inflammation

ABSTRACT

The CXCR3 ligand family, including CXCL9, CXCL10 and CXCL11, play important role in T-cell, natural killer (NK) cell, NKT cell and dendritic cell trafficking. These chemokines were suggested for their use as alternative markers for TB diagnosis, and their possible involvement in disease pathophysiology. In the present study, we investigated the genetic association and frequency of *CXCL9* and *CXCL10* genes polymorphisms in TB patients of Pakistani population. A total of 260 study participants, including 122 with tuberculosis disease (TB) and 138 healthy control (HC) were included. Out of these 122 TB disease subjects (67%) were diagnosed with PTB (n = 82) and 33% with EPTB (n = 40). Tri and Tetra- ARMS-PCR techniques were used to genotype *rs2276886* of *CXCL9* and *rs5606198* of *CXCL10* for all the samples. The results showed significant association (OR, 2.1 (0.99 to 4.30), $p = 0.04$) of A allele of *rs2276886* with EPTB group as compared to the control. In genetic model analysis, we found strong association (OR, 0.4 (0.181 to 0.94), $p = 0.03$) of GG genotype with male with EPTB as compared to female in gender stratified dominant model. Furthermore, in context of different ethnic groups of Pakistani subpopulation, we found Punjabi speaking's to be significantly at higher risk (OR, 2.16 (95%CI, 1.0910 to 4.2901), $p = 0.02$) of developing TB as compared to other ethnic categories. Strong association of *CXCL9* polymorphism (*rs2276886* G>A) with TB disease in Pakistani population was concluded especially, Punjabi speaking participants were found to have higher risk of developing TB.

INTRODUCTION

Tuberculosis (TB) is a serious, progressive bacterial infection caused by *Mycobacterium tuberculosis* (MTB) (Loddenkemper *et al*., 2015). The disease is endemic in half of the world with a significant burden reported from the developing countries (Doherty, 2005). About two billion or nearly one-third of the world's population is infected with MTB. The global prevalence of the disease was estimated to be around 16-20 million, with 8 million new TB cases emerge every year (WHO, 2005; Corbett *et al*., 2003).Pakistan ranks sixth among the twenty-two countries with the highest burden (55%) of TB in the world (Metzger *et al*., 2010). Frequency of MTB varies among different ethnic groups, races, and genetic predisposition factor is also involved in the susceptibility of the disease (Nonghanphithak *et al*., 2015).

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Abbreviations
CXCL10, CXC ligand 10; MTB, Mycobacterium tuberculosis; TB, Tuberculosis; SNP, single nucleotide gene polymorphism; AFB, acid fast bacillus, PT (PTB), pulmonary tuberculosis disease; EPT (EPTB), extra pulmonary tuberculosis disease

Lungs have been identified as the primary site of the MTB infection. Nonetheless, the *M. bacillus* is able to disseminate into other organs following septicemia (Loddenkemper *et al*., 2015). The histopathological examination of MTB patients suggests localized, proliferative and productive inflammatory responses over the course of the infection. The exudative immunity is often followed by the cell-mediated immunity, proceeds by lymphocytes including T cells and B cells. The generation of the cytotoxic $CD4^+$ T cell response serves as the critical factor in defining the immunity (Winslow *et al*., 2008; Flynn and Chan, 2001). The chemokines are the primary cytokines responsible for immunological response, reported to play crucial role in the early inflammation of *M. tuberculosis* infection (Cooper *et al*., 2002; Flynn and Chan, 2001).

The primary cytokines responsible for the immunological response include tumor necrosis-alpha (TNF-α), interleukins, and chemokines (Cooper *et al*., 2002; Flynn and Chan, 2001). Chemokines are small chemotactic peptides, secreted by macrophages and are involved in the attraction of a wide range of immune cells, including lymphocytes, monocytes, dendritic cells, granulocytes and neutrophils to the site of infection. CXC ligand 10 (CXCL10) and CXC ligand 9 (CXCL9) are important CXC chemokines, which have been extensively studied due to their role in different pathophysiological conditions such as *M. tuberculosis* (MTB) infection, Hhimoto's thyroiditis (HT), chronic rheumatic heart disease, thyroiditis and hypothyroidism, etc. (Fae *et al*., 2013; Antonelli *et al*., 2011; Akahane *et al*., 2016). CXCL10 is a 10 kDa protein which is produced in response to interferon-gamma (IFN-γ) (Seiler *et al*., 2003). It regulates immune responses including inflammation, angiogenesis, and chemotaxis (Esche *et al*., 2005; Turner *et al*., 2014). Several studies showed the association of the potential CXCL10 promoter region SNPs including at 135 bp (135G→A) upstream of the transcription start site with MTB disease (Tang *et al*., 2009; Sheikh *et al*., 2015). Bhattacharyya *et al*. (2018) have reported significant overexpression of CXCL10 in MTB patients as compared to the control ($p = 0.0002$). Furthermore, the SNP in CXCL10 *rs4508917* (-1447 A > G) was associated with reduced expression of CXCL10, suggesting the essential role of CXCL10 in the active phase of TB infection (Bhattacharyya *et al*., 2018). A study conducted on the Polish population showed the higher levels of CXCL10, which is suggested to be used as a biomarker to screen the patients with latent MTB infection (Strzelak *et al*., 2012).

The CXCL9, also known as MIG (monokine-induced by interferon-gamma), is another IFN-γ inducible chemokine, stimulated by mononuclear cells to attract activated T cells and monocytes through the chemokine receptor CXCR3 (Ward and Westwick, 1998). CXCL9 expression regulates early granuloma formation induced by neutrophils through CXCL9 signaling pathway. On the other hand, wild type mice presenting anti-CXCL9 were found to be associated with impaired granuloma formation (Seiler, 2003). Following MTB infection, CXCL9 stimulates the expression of other chemokines as well as mediate migration of T- and natural killer cells migration (Lande *et al*., 2003). Most recently, SNP (rs2276886) within the CXCL9 (MIG) gene was associated with hypothyroidism susceptibility in Japanese populations (Akahane *et al*., 2016). Chemokines, such as CXCL9 are a class of cytokine proteins that act as signaling molecules, regulating immune and inflammatory responses and modulating cell migration properties and localization of target cells such as leucocytes. The chemokine CXCL9 produced by local cells in inflammatory lesions, plays important roles in several autoimmune diseases (Tokunaga *et al*., 2018).

In Pakistani population, there is little or no data available regarding the possible association of CXCL10 and CXCL9 polymorphisms with TB (Taheri *et al*., 2013). Based on the studies conducted on MTB patients from different ethnic groups, we hypothesize that genetic variation in chemokine genes *CXCL10* and *CXCL9* are associated with TB susceptibility. Therefore, this study was designed to investigate the risk assessment of *CXCL9* (*rs2276886*) and *CXCL10* (*rs5606198*) gene polymorphisms with TB in Pakistani population and also found their genetic association with different ethnic groups or subpopulations.

MATERAILS AND METHODS

Selection of study participants

All procedures performed in this study, involving human participants, were approved by the Independent Ethics Committee (IEC), International Center for Chemical and Biological Sciences, University of Karachi (ERC No: ICCBS/IEC-014-BS-2016/PROTOCOL/1.0). A total of 260 study participants, including 122 clinically diagnosed TB patients and 138 healthy control from Ojha Institute of Chest Diseases, Dow University of Health Sciences (DUHS) Karachi, Pakistan were collected. The blood samples from each subject was taken after written informed consent letter. TB patients were diagnosed and assessed by expert clinicians using their clinical history, radiological evidence, sputum acid fast bacillus (AFB) smear positivity and culture studies according to the WHO Tuberculosis disease and prevention guidelines (WHO Guidelines, 2019). All the collected samples were further categorized as: Group-1 PTB (pulmonary tuberculosis), Group-2 EPTB (extra pulmonary tuberculosis) and

Group-3 HC (healthy control).

PTB patients were assessed through sputum microscopy positivity for AFB or with radiological evidence of lung involvement. EPTB patients were categorized on the basis of CT scans for abdominal, skeletal and splenic involvement and MRI for meningeal involvement. The participants with any immunosuppressive conditions such as HIV, diabetes, previous history of anti tuberculosis treatment (ATT) or any other inflammatory conditions were excluded. Healthy volunteers who have any close contact with TB patients were also excluded from the study.

Blood collection and DNA isolation

About 3 mL of blood was collected from each participant (patients and healthy controls) through venepuncture procedure in anticoagulant (EDTA) tubes for experimental analyses. DNA was isolated from each collected blood sample using standard CTAB (Cetyl trimethylammonium bromide) method (Paireder *et al*., 2013). Briefly, 1 mL of CTAB buffer (100 mM Tris-HCL, 20 mM EDTA, 1.4 mM NaCl, 2% CTAB, pH was adjusted as 8.0) with 5µL of β-mercaptoethanol, 20 µL (20 mg/ mL) of proteinase K enzyme and 200 µL of whole blood were added into a vial and placed in mixing block MB102 (BIOER) at 60 ºC for 1 h. Afterwards, the tubes were centrifuged for 10 min at 12,000 rpm. Following centrifugation, supernatant was transferred in to a fresh tube and 0.8 mL of chloroform/ isoamylalcohol (24:1) solution was added. The tubes were then centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to the fresh tube and cold isopropanol was added to precipitate the DNA, and tubes were placed at -20ºC for 30 min. After that, tubes were centrifuged at -4°C (LABOGENE Scan speed 1730R) and supernatant was discarded. The pellet was washed with 70% ethanol, followed by centrifugation at 12,000 rpm for 5 min. Then, washing with 70% ethanol was repeated. Pellet was dried at room temperature and 100 µL of Tris-EDTA buffer was added in the tubes and stored at -20°C for further analysis. Spectrophotometry (UV-1700, Shimadzu Pharma Spec, Japan) was used to determine the yield and purity of DNA.

Genotyping

Tetra-ARMS PCR (*tetra*-primer amplification refractory mutation system-polymerase chain reaction) was used for *rs2276886* (G/A) polymorphism of *CXCL9* gene by using two sets of primers in a single reaction mixture tube. While for *rs5606198* (G/A) of *CXCL10* gene, Tri-ARMS PCR was applied (Taheri *et al*., 2013). The sequence of tetra- ARMS PCR primer sets were as following:

Pair-1 sense: 5′- ATATGCCATACATTGTGTAGC-3′
antisense: 3′- GTTCCAAGTCACTCCTGTAT-5′,
Pair-2 sense: 5′- CACTGAGAAGCTTTTATGACTAAC-3′
antisense:3′-TCCAATATTACTCTTCAATGCAG-5′

The resultant three genotypes of *rs2276886* (G/A) were GG (408bp), AA(232bp) and for GA both bands with control band of 594bp. For tri -ARMS PCR, three primers were used as:

Forward-1 sense: 5′-TTCCTTACCTTGAATGCCACTT-3′
Forward-2sense:5′-GGAGGCTACAATAAATAATACCTTCG-3′,
Reverse antisense:3′-GGAGGCTACAATAAATAATACCTTCA-5′

The resultant genotype of *rs5606198* (G/A) were GG, GA and AA with 295bp. The PCR amplification was performed in 96-well plates on a thermocycler (T100, Bio-Rad). The amplification conditions were set as the initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds at 54°C for *rs2276886* (G/A) while 62°C for *rs5606198* (G/A), and extension at 72°C for 1 min. The final elongation was performed at 72°C for 5 min. The PCR products were resolved in 2% agarose gel, and the genotype patterns for each subject were recorded using the visual inspection method of the gel under the ultraviolet light by using gel documentation system (Protein Simple Fluor Chem FC3).

Statistical analysis

Allele and genotype frequencies of each polymorphism were determined by direct gene counting method. Hardy-Weinberg Equilibrium (HWE) was determined by applying the equation ($p^2+2pq+q^2$) of control population for both genetic variants ($p > 0.05$). Genotype distribution between case control group and TB patients were analyzed by using Chi-square analysis and Fisher's exact tests, when the criteria for the chi-squared analysis was not fulfilled. Odd ratio (OR) was also determined by using logistic regression with their corresponding 95% CI to examine associations at the level of significance $p \leq 0.05$. All the statistical analysis of the data was performed by using SPSS version 21 (SPSS Inc. Chicago, IL, USA).

RESULTS

Genotyping of the study groups

A total of 260 study participants including 122 with TB and 138 healthy control (HC) were included in present study. Out of these 122 TB disease subjects 67% were having PTB (n = 82) and 33% were with EPTB (n = 40). The mean age of the healthy controls was 30.7 ± 7 as compare to TB patients with 33.3 ± 2.1 (Table I). Genotyping was performed by T-ARMS and tri-ARMS PCR (Fig. 1A, B). By use of gene counting method, both genotypic and allelic frequencies of *rs2276886* (G/A) and *rs5606198* (G/A) were calculated. In HC group 85% (n=117) were having GG genotype, 14% (n = 19) were with GA genotype and

1% (n= 2) were with AA genotypes. In PTB group, 78% (n = 62) were found to have GG genotype, 20% (n= 16) were with GA genotype and 2% (n= 1) were with AA genotype. The genotypic distribution in EPTB group was 71% (n = 29) with GG genotype, 27% (n =11) with GA and 2% (n= 1) with AA genotypes, respectively (Table II).

Table I. Demographic characteristics of TB patients and healthy controls.

Groups	N (%)	Gender M:F (ratio)	Mean age	SD ±
HC	138 (53)	42/96	33.7	7.5
TBD	122 (47)	54/68	33.6	2.1
PTB	82 (67)	38/44	33.6	2.5
EPTB	40 (33)	16/24	32.75	1.2

HC, healthy control; TB, tuberculosis disease; PTB, pulmonary tuberculosis disease; EPTB, extra pulmonary tuberculosis.

No departure from Hardy Weinberg equilibrium was observed for both *rs2276886* (G/A) of *CXCL9* and *rs5606198* (G/A) of *CXCL10* (χ^2 = 1.34, 1.47, $p > 0.05$). The genotypic frequency of *rs506198 (G/A)* was found to be extremely low (minor allele frequency < 0.01) in the studied group therefore, this SNP was not included in the further analysis. In *rs2276886* (G/A), no significant difference was found between genotypic frequencies of all the groups, however there was a significant difference between allelic frequency of EPTB and HC group (χ^2 = 3.3, $p = 0.04$). The minor allele A of *rs2276886* (G/A) was found significantly associated with EPTB disease group (OR, 2.1 (0.99 to 4.30) as shown in Table I. Furthermore, to assess the genotypic effect on all the groups, different genetic models including dominant, recessive and codominant were constructed. A significant association of GG genotype with EPTB group was found (OR, 0.4 (0.181 to 0.94), $p = 0.03$) as compared to the PTB and HC groups in the dominant model (Table II). No significant association was found in the remaining other genetic models. Each genetic model was further stratified for gender in each group. The male with GG genotype were found to be significantly associated (OR, 0.2 (0.060 to 0.813),

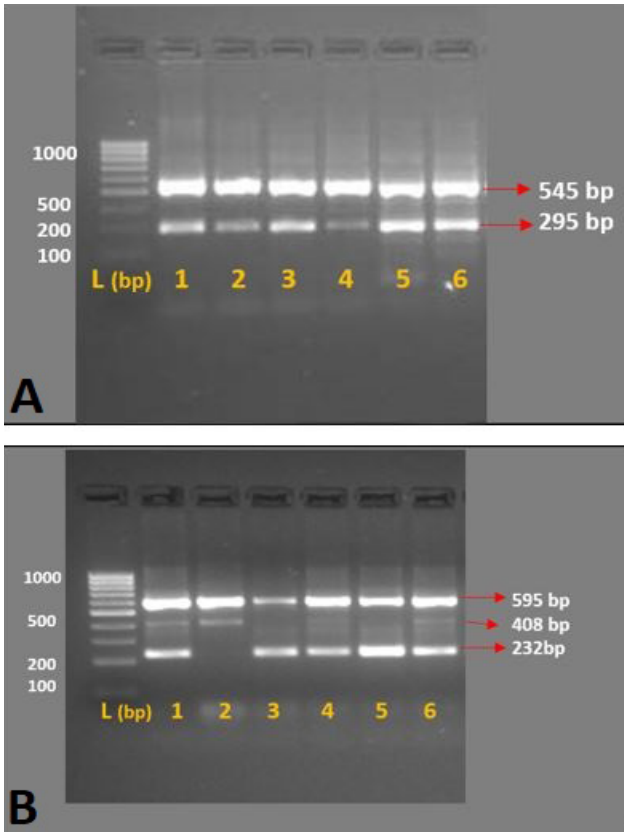

Fig. 1. (A) Tri-ARMS PCR assay based genotyping of *CXCL10 rs5606198* (*G/A*) polymorphism on 2.5% agarose gel. Lane L shows 1000 bp molecular marker, lane 1 and 2 shows positive control with GA genotype. Lanes 3, 4 and 5,6 represents GA genotype with band size of 545 and 295bp. (B) T-ARMS PCR assay based genotyping of *CXCL9 rs2276886* (*G/A*) polymorphism on 2.5% agarose gel. Lane L shows 1000 bp molecular marker, lane 1 shows positive control. Lanes 3, 4 and 5 shows GG genotype of 232 bp along with control band of 595 bp. Lanes 1, 7 represent GA genotype with both bands of 4081 bp and 232 bp along with control of 595 bp. Lane 2 represents AA genotype with both bands of 408bp and 595bp.

Table II. Distribution of genotype and allele frequencies between the PTB, EPTB and HC groups.

Gene	Groups (n)	Genotypic frequencies (%)					Allelic frequencies (%)				
		GG	GA	AA	x^2	P-value	G	A	x^2	P-value	OR (95%CI)
CXCL9	HC	117 (85)	19 (14)	2 (1)			253	23			
	PTB	62 (78)	16 (20)	1 (2)	1.5	0.45	140	18	1.09	0.29	1.41 (0.73 to 2.71)
	EPTB	29 (71)	11 (27)	1 (2)	3.4	0.12	69	13	3.3	0.04*	2.1 (0.99 to 4.30)

Note: *Significance, ($p < 0.05$).
For abbreviations, see Table I.

$p = 0.02$) with EPTB disease as compared to the PTB and HC groups in the dominant model. While no significant association was found in other gender stratified genetic models (Tables III, IV, V).

Table III. Genetic analysis of *rs2276886* (G/A) under the dominant model.

Group	GA+AA	GG	p-value	OR (95% CI)
Total				
Control	20 (15)	117 (85)		
PT-D	17(22)	62 (78)	0.19	0.62 (0.304 to 1.275)
EPT-D	12 (29)	29 (71)	0.03*	0.4 (0.181 to 0.94)
Male				
Control	7 (16)	36 (84)		
PT-D	7 (26)	20 (74)	0.32	0.6 (0.170 to 1.811)
EPT-D	7 (47)	8 (53)	0.02*	0.2 (0.060 to 0.813)
Female				
Control	14 (15)	81 (85)		
PT-D	11 (21)	42 (79)	0.35	0.7 (0.275 to 1.580)
EPT-D	5 (19)	21 (81)	0.57	0.72 (0.234 to 2.243)

For abbreviations, see Table I.

Table IV. Genetic analysis of *rs2276886* (G/A) under the recessive model.

Group	GG+GA	AA	p-value	OR (95% CI)
Total				
Control	136 (99)	2 (1)		
PT-D	78 (99)	1 (1)	0.31	3.48 (0.31 to 1.08)
EPT-D	40 (98)	1 (2)	0.13	6.4 (0.57 to 1.21)
Male				
Control	42 (98)	1 (2)		
PT-D	26 (96)	1 (4)	0.73	1.6 (0.09 to 1.95)
EPT-D	14 (93)	1 (7)	0.44	3 (0.17 to 1.19)
Female				
Control	94 (99)	1 (1)		1.8 (0.11 to 2.50)
PT-D	52 (98)	1 (2)	0.67	3.6 (0.21 to 1.79)
EPT-D	26 (100)	0	0.36	

*Significance, ($p < 0.05$).
For abbreviations, see Table I.

In the current study, the genetic association of *rs2276886* (G/A) with different ethnic groups of Pakistani population was also conducted. A regression analysis showed the significant association of minor allele (A) with Punjabi speaking ethnic group of TB disease (OR, 2.16 (1.0910 to 4.2901), $p = 0.02$) as compared to all other ethnic groups (Table VI).

Table V. Genetic analysis of *rs2276886* (G/A) under the codominance model.

Group	GG+GA	GA+AA	p-value	OR (95% CI)
Total				
Control	119(85)	22 (15)		
PT-D	78 (82)	17 (18)	0.6	0.8 (0.42 to 1.69)
EPT-D	40 (77)	12 (23)	0.22	0.6 (0.27 to 1.37)
Male				
Control	42 (86)	7 (14)		
PT-D	26 (79)	7 (21)	0.41	0.7 (0.19 to 1.96)
EPT-D	14 (67)	7 (33)	0.71	3 (0.89 to 1.05)
Female				
Control	94 (87)	14 (13)		
PT-D	52 (83)	11 (17)	0.42	0.4 (0.29 to 1.66)
EPT-D	26 (84)	5 (16)	0.65	1.29 (0.42 to 3.91)

*Significance, ($p < 0.05$).
For abbreviations, see Table I.

DISCUSSION

TB disease is generally infecting the pulmonary region, however the infection can also break into the extra-pulmonary nodules (Barr and Yates, 2017; Said *et al*., 2017). Previous data have shown that the intensity of cytokines in response to MTB antigens were affected from genetic polymorphisms (Qiu *et al*., 2001). Both the CXCL9 and CXCL10 gene SNPs are found to be associated with several diseases including TB (Tang *et al*., 2009; Slight and Khader, 2013). The current case control study was based on the rationale that these genes are located next to the interferon-gamma (IFN-γ) gene on chromosome 4 and are under the influence of this cytokine for their induction. Therefore, we investigated the influence of these SNPs at the promoter or regulatory region of CXCL9 and CXCL10 genes in TB patients. The focus was on risk of disease susceptibility due to these polymorphisms.

In the present study, we found the association of A allele of *rs2276886* (G/A) with EPTB group which suggests that a person with A allele have two times more risk to have EPTB as compared to the normal healthy control. In concordance to our study, Nonghanphithak *et al*. (2016) have also demonstrated that polymorphism in chemokine genes including *CXCL9* were associated with susceptibility to develop TB among the Asians. However, in Japanese and Chinese populations, the *rs2276886* was found to be associated with Hashimoto's thyroiditis (HT) (Akahane *et al*., 2016; Mo *et al*., 2019). In another study, Wilson *et al*. (2013) found the association of different polymorphism of *CXCL9* (*rs3733236*), located within the untranslated region, which was associated with liver fibrosis in both the humans and the animal models. Beside these facts, very limited data was available

Table VI. Regression analysis of selected polymorphism for risk of tuberculosis diseases with different ethnic groups in Pakistani population.

Ethnic groups	*CXCL9* tuberculosis group Allelic frequencies		Control group Allelic frequencies		OR (95% CI)	*p*- value
	G	A	G	A		
Punjabi	72	12	39	3	2.16 (0.5766 to 8.1419)	0.25
Sindhi	34	8	9	3	0.75 (0.1549 to 3.2170)	0.65
Pashtun	47	9	25	5	0.91 (0.2895 to 3.1663)	0.94
Urdu speaking	176	30	165	13	**2.16 (1.0910 to 4.2901)**	**0.02***
Others	15	5	5	3	0.6 (0.0962 to 3.2069)	0.51

*Significance, ($p < 0.05$).

regarding the association of *CXCL9* SNPs with TB patients. It provides the strength to our study in particular to explore the effects of genetic predisposition of CXCR3 ligands (CXCL9 and CXCL10) on TB disease in Pakistani population. We also proposed the genetic variant (*rs2276886*) of *CXCL9* as a potential genetic marker for TB patients.

In the present study, we found very low frequency of CXCL10 (*rs5606198)* SNP variant that was not included in further analysis. This low frequency might be due to the population variations with in different ethnicities. Furthermore, many cofounding factors including immunosuppressive conditions and false positive control group selection made difficult to study host genetic susceptibility to TB. In gender stratified dominant genetic models of *rs2276886* (G/A) of *CXCL9,* we found a strong association of GG genotype with male (OR = 0.2 (0.060 to 0.813), $p = 0.02$) as compared to the female of EPTB group. This represents that wild type GG genotype of *rs2276886* may provide protective effect to male from TB diseases susceptibility as compared to the female. The gender based influence on activation of several immune responses have been observed in various diseases and it might contribute to the disease susceptibility (Whitacre, 2001). Whether CXCL9 expression and function is influenced by sex has not been studied yet. In contrast to our findings, Biswas *et al*., (2020), reported another important chemokine CCL2-2518, to be significantly associated with GG genotype of female patients in South Indian population in comparison to male patients. No previous data was found with relevance to association of *rs2276886* (G/A) of *CXCL9* with TB. To validate our results, large size population based studies are recommended.

The importance of considering ethnicity can be determined by the observation of the gender-related case reports in the Eastern and Western neighbors of Pakistan, suggesting a difference in the epidemiology (Dogar *et al*., 2012). As of Karachi, being a highly populated metropolitan city of Pakistan, having people from diverse ethnic groups. So, the current study also focused on the difference in the frequency distribution of *rs2276886 (G/A)* in various ethnic groups. We found that Urdu speaking group with A allele carrier of CXCL9 variant *rs2276886* were at two times higher risk to develop TB with reference to G allele (OR, 2.16 (1.0910 to 4.2901), $p = 0.02$*). Due to the unavailability of data including plasma concentration of CXCL9 and CXCL10 proteins, we were unable to explore the genotype-phenotype relationship with respect to TB disease. To validate our results, large size population based study with different ethnic groups is recommended.

CONCLUSION

In summary, we found strong association of *CXCL9* polymorphism (*rs2276886 G>A)* with TB disease in Pakistani population. In subpopulation analysis. Punjabi speaking people have higher risk to develop TB as compared to other ethnic groups. Furthermore, in gender-based stratification, we found that male have low risk to develop TB as compared to the female. This data reinforces the critical importance of *rs2276886* (G>A) of *CXCL9* as a genetic marker for TB. In future, to validate our results, large population-based association studies are recommended to better understand the genotypic-phenotypic effects of chemokines that might pave a way to better control the TB susceptibility and to understand its deep pathobiology.

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Declarations

Compliance with ethical standards

All procedures performed in this study, involving human participants, were approved by the Independent Ethics Committee (IEC), International Center for Chemical and Biological Sciences, University of Karachi (ERC No: ICCBS/IEC-014-BS-2016/PROTOCOL/1.0). Informed consent was obtained from all participants included in the study.

Consent for publication

All authors approved the manuscript and consented to its publication.

Statement of conflict of interests

The authors have declared no conflict of interest.

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