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Interleukin-18 cytokine gene polymorphism 137G/C (rs187238) and susceptibility to tuberculosis in north India

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Abstract

Tuberculosis (TB) regained its position globally as the leading cause of mortality from a single infectious agent after being surpassed by COVID-19 for 3 years consecutively. Host genetic factors, particularly cytokine gene polymorphisms, play a significant role in influencing susceptibility to TB. Interleukin-18 (IL-18) is a proinflammatory cytokine involved in immune regulation against Mycobacterium tuberculosis. This study aimed to evaluate the association of IL-18 gene polymorphism (rs187238) with susceptibility to TB and its effect on serum IL-18 levels in a north Indian population. A case-control study was conducted with 100 newly diagnosed TB patients (pulmonary and extrapulmonary) and 100 age- and gender-matched healthy controls. Serum IL-18 levels were measured using sandwich enzyme-linked immunosorbent assay, and the IL-18 gene polymorphism at rs187238 was analyzed by polymerase chain reaction-restriction fragment length polymorphism. The association between IL-18 polymorphism, TB susceptibility, and serum IL-18 levels was statistically evaluated. Mean serum IL-18 levels were significantly elevated in TB patients (400.42±149.58 pg/mL) compared to controls (96.05±40.67 pg/mL; $p<0.01$). The distribution of IL-18 genotypes showed that individuals with GC/CC genotypes had a significantly lower risk of developing TB compared to the GG genotype [odds ratio (OR)=0.31; 95% confidence interval (CI)=0.20-0.88; p=0.0167]. Additionally, the C allele conferred a protective effect against TB (OR=0.33; 95% CI=0.22-0.51; $p<0.0001$). Serum IL-18 concentrations varied significantly with genotype, with the highest levels observed in CC genotype carriers in both cases and controls ($p<0.01$). Thus, our study suggests that IL-18 polymorphism at rs187238 significantly influences susceptibility to TB in the north Indian population. The C allele and GC/CC genotypes appear to confer a protective effect, possibly through modulation of IL-18 serum levels. IL-18 rs187238 polymorphism may serve as an independent predictive marker for TB risk, though larger studies are recommended for validation.

Key words: tuberculosis, IL-18 gene polymorphism, cytokines, genetic susceptibility, serum IL-18 levels.

Introduction

Tuberculosis (TB) is an infectious disease that is both preventable and curable, provided timely diagnosis and adequate treatment. However, in 2023, tuberculosis reclaimed its position as the world's leading infectious agent-related cause of death, accounting for nearly twice as many deaths as HIV/AIDS, after being surpassed by coronavirus disease (COVID-19) for three years [1]. Each year, almost 10 million people continue to contract tuberculosis with India accounting for 28% of the global TB burden, the highest in the world [2,3]. The progression of TB occurs due to numerous factors such as environmental, malnutrition, HIV infection, immunosuppressive therapy, Diabetes Mellitus, etc. However, only 5–10% of these cases progress to active disease, even though more than one-third of the world's population has TB. This suggests that host genetic factors may significantly influence the susceptibility to TB progression [4].

Interleukin-18 (IL18) is a pleiotropic cytokine, of the IL1 family members, produced by various hematopoietic and non-hematopoietic cells that take part in several reactions in the human body ranging from its involvement in maintaining homeostasis to a significant role in the inflammatory cascade against MTB infection. Its production in response to mycobacterial antigens (Ags) is highly associated with interferon-gamma (IFN-γ) production which forms an integral part of the defensive immunity against the development of MTB infection [5]. Further, a possible role for IL-18 in both protective immunity and human TB pathological responses has been suggested by the discovery of elevated IL-18 levels in the peripheral blood mononuclear cells of chronic refractory TB patients as well as at the site of tuberculous pleurisy [6].

The IL-18 gene is found on the long arm of chromosome 11 (11q22.2-q22.3) in humans & consists of six exons and five introns. It incorporates many genetic polymorphisms, particularly in the promotor region & cloning and transcriptional studies have demonstrated that these single nucleotide polymorphisms (SNPs) influenced IL-18 expression [7]. Furthermore, to the best of our knowledge, there are no previous studies regarding IL-18 promotor SNP effect on the susceptibility to TB among North Indian patients. Therefore, this study aimed to determine the association between IL-18 (137G/C) and the risk of newly diagnosed pulmonary or extrapulmonary TB (PTB/EPTB) among patients in North India.

Materials and Methods

This Case-Control study was conducted on adults with age >18 years of either gender recruited from the Department of Pulmonary Medicine between March 2023 and March 2024 after obtaining written informed consent from all the patients. Adults of either gender and >18 years of age without active tuberculosis were taken as controls. Patients previously treated for TB,

those who did not regularly attend the scheduled DOTS clinic for completion of the six-month treatment plan, and those with drug-resistant TB were excluded. The study included 100 TB cases and 100 age and gender-matched controls.

Sample collection, processing and storage

Venous blood of 3-4 ml was collected in a serum separator and EDTA vacutainers under all aseptic conditions. The plain vacutainers were centrifuged, and the separated serum along with EDTA vacutainers were stored at -80°C for further use. For the molecular study, DNA was isolated by solid phase extraction method from whole blood after lysis of WBCs (Figure 1a). DNA efficiency and quantity were assessed via 0.9% agarose gel electrophoresis and by UV spectrophotometer & stored at -20°C for SNP analysis.

SNP detection

The IL-18 137(G/C) gene loci was amplified using Polymerase Chain Reaction (PCR) from genomic DNA using a forward 5' ATGCTTCTAATGGACTAAGGA 3' and reverse primer 5'TTCATGAAAATAGTGATATTAC 3', under standard PCR protocol to obtain a 131 bp PCR product (Figure 1b). The PCR product was then digested by the Restriction Enzyme for 10-12 hours under 37°C followed by its inactivation at 65°C. Digested products were resolved using electrophoresis in 3% agarose gel and viewed in a gel dock (Figure 1c).

Statistical analysis

Data was analyzed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA), and a p-value of less than 0.05 was considered statistically significant and less than 0.01 was considered statistically highly significant. Differences between mean values for the demographic and biochemical parameters in cases and control groups were analyzed by unpaired t-tests. An unpaired t-test was also done to compare the genotypes between controls and cases. Allelic frequencies were determined by the gene counting method, and departure from the Hardy–Weinberg equilibrium was evaluated by the χ^2 test. We calculated odds ratios (ORs) to estimate the correlation between groups for genotypes, alleles, IL18 gene polymorphism and susceptibility to TB and serum IL18 levels. Results in the study are expressed as Mean ± S.D.

Results

The age of the case group ranged from 18 years to 45 years, with a mean age of 32.13 ± 13.87 years, and of the control group ranged from 18 years to 40 years, with a mean age of 31.49 ± 7.86 years. The difference of mean age in the cases and control was not significant with a p-value of > 0.05. The case group consisted of 56 % males and 44% females, whereas the control

group consisted of 44% males and 56% females. 39% of cases were of PTB and 61% were of EPTB. The mean value for IL18 (400.42 ± 149.58pg/mL) was higher in the case group as compared to the control group (96.05 ± 40.67pg/mL) which was statistically significant (p-value<0.01) (Figure 2). The prevalence of IL18 genotypes 137G/C was in the range of Hardy Weinberg equilibrium in both cases (Chi square=0.488, p value=0.484) and controls (Chi square=2.09243E-18, p value 0.98) (Table 1). Among cases, 60 had wild homozygous genotype GG, 28 had genotype GC and 12 were homozygous for polymorphic allele C (CC). Among controls, 43 had genotype GG, 12 had genotype GC and 45 were homozygous for allele C (Figure 3). It was found that patients with GC/CC genotypes had a significantly lower risk of developing TB than those with GG genotypes. [O.R. = 0.31, C.I. = 0.20 to 0.88, p-value=0.0167 (<0.05)] as shown in Table 2. Further on comparing the odds of susceptibility to TB to allelic distribution, it was found that the C allele rendered patients less susceptible to TB as compared to the G allele [O.R. = 0.33, C.I. = 0.22 to 0.51, p value < 0.0001], which was statistically highly significant (Table 3). Mean (±S.D.) serum IL-18 in TB patients and controls with GG genotype came to 289.58 ± 52.16pg/ml and 62.41 ± 10.41pg/ml, respectively, and in patients with GC and CC genotypes, it came to 529.43 ± 52.54pg/ml and 653.55 ± 27.06pg/ml, respectively. The controls with the same GC and CC genotypes had a mean of 82.35 ± 2.39 pg/ml and 131.84 ± 33.99 pg/ml, respectively (Figure 4). The difference between the means of the three genotypes was statistically significant (p value < 0.01) for both controls and cases.

Discussion

Tuberculosis is a multifactorial infectious disease that is both preventable and curable. Several genetic and environmental factors have been identified in its pathogenesis and progression to full-blown infection. There have been several advancements in the diagnosis and treatment modalities of tuberculosis in recent years, still in 2023 it was the world's biggest cause of mortality from a single infectious agent, killing nearly twice as many people as HIV/AIDS [3]. IL-18 is an immunoregulatory cytokine that regulates both innate and adaptive immune responses and has been shown to play an important role in host defense against MTB infection [8]. Its primary role is the induction of IFN-γ production, which in turn plays a crucial role in protection against TB by inducing macrophage activation [9]. Furthermore, IL-18 itself stimulates proliferation and activation of NK cells, T lymphocytes and CD8+ T cell cytolytic activity, thus playing an essential role in the protective immune response against intracellular pathogens [10,11].

In our study, we found that the mean serum IL-18 levels in patients with TB were 400.42 ± 149.58 pg/ml, and the levels in healthy controls were 96.05 ± 40.67 pg/ml (Figure 5). Patients

with Tuberculosis irrespective of site had statistically significant higher IL-18 levels ($p<0.01$) as well. This finding is consistent with the study by Song et al. [12], wherein pulmonary and extrapulmonary TB patients had raised IL-18 levels in their peripheral blood mononuclear cells as well as in the pleural fluid of the tuberculous pleurisy (TBP) site. Similar results were obtained by Inomata et al. [13], and Yamada et al. [14], wherein IL-18 concentrations were greater in TB cases than in healthy controls.

The presence of genotypic polymorphisms in various cytokines is known to be associated with higher susceptibility to tuberculosis infection; thus, identifying the various genetic factors influencing tuberculosis has gained popularity. Hence, we decided to study the functional polymorphism rs187238 of the IL18 137(G/C) gene and its association with TB in the North Indian population. The IL18 gene is located on the long arm of chromosome 11 and incorporates many genetic polymorphisms, particularly in the promoter region [15].

The IL-18 gene promoter region location 137 is hypothesized to represent nuclear factor binding sites for histone 4 transcription factor-1 (H4TF-1) nuclear, and a change at position -137 involving the conversion of guanine into cytosine may influence the activity of the nuclear factor H4TF-1 and result in a potential decrease in gene expression [16]. Allelic variations of cytokine genes linked to promoter region polymorphisms do not affect the protein amino acid sequence but can cause differences in cytokine production thus altering susceptibility to Mtb infection. Studies have been conducted on various populations regarding IL18 gene polymorphism and risk of TB, but results have been inconsistent.

It was seen that 40% of TB patients had the polymorphic C allele, while 57% of healthy controls showed the presence of the polymorphic allele in our study population at rs187238 SNP. A substantial protective effect against tuberculosis was seen with the CC/GC genotype in the dominant model [O.R. = 0.31, C.I. = 0.20 to 0.88 p value- 0.0167 (<0.05)] and the C allele (O.R. = 0.33, C.I. = 0.22 to 0.51, p value < 0.0001). In addition, a 6.6-fold increased risk for TB was found in the recessive model in those having GG and GC genotypes [O.R. = 6.68 C.I. = 3.26 to 13.6, p value < 0.0001]. Thus, a significant protective effect against TB was found in the CC and GC genotypes in addition to C allele. This conclusion is consistent with the data by Giedraitis et al. [16] that the IL-18 137 C allele may favor greater promoter activity of the IL-18 gene, with an increased level of IL-18, which is known to be associated with more robust TB resistance and lower TB susceptibility. Also, our findings are in agreement with the studies by Hassuna et al. in the Egyptian population and by Shen at al. in the Chinese population that found that the IL-18-137G/C SNP is associated with a lowered susceptibility to tuberculosis [17,18]. However, a meta-analysis by Zhou et al. of six case-control studies involving a total of 970 patients and 1775 controls found no association with IL18 137G/C polymorphism and susceptibility to TB [19].

Mean serum IL-18 concentrations in TB patients and controls were highest in CC genotype, followed by GC and GG genotypes, which was statistically significant (p-value < 0.01) in both cases and controls for both markers at rs187238 SNP. This is consistent with the findings of Giedraitis et al. that a nucleotide change at position -137 guanine into cytosine may influence the activity of the nuclear factor H4TF-1 resulting in a potential decrease in gene expression [16]. Further, in a study by Andrade et al. serum levels of IL-18 were found to be significantly higher in CC carriers (843.1pg/mL) compared with GG or GC carriers (303.6pg/mL and 292.0 pg/mL) respectively [20]. However, in a study in the South African population by Evans et al. found that serum IL-18 concentrations were not associated with IL-18 genotypes [21].

A relationship may exist when the polymorphism itself is functional and confers altered susceptibility to the disease or when an allele SNP is in linkage disequilibrium with an allele associated with disease susceptibility or due to a competing influence caused by population [22]. The diversity in the distribution of IL-18 gene SNPs among ethnicities and population groupings may explain why populations of different races have varying immune responses to tuberculosis and susceptibilities.

Limitations

Sample size for the current study was limited. Further studies with larger sample sizes are needed to ascertain the role of IL-18 (rs187238) gene polymorphism in tuberculosis patients. Besides, the correlation of serum IL-18 with IFN-γ levels needs to be evaluated along with their respective levels post-treatment to establish the inflammatory mechanism involved by IL-18 in tuberculosis patients, which is being taken up as the second part of the research project.

Conclusions

In the present study, it was concluded that IL18 has a role in protective immunity and human TB pathological response. A substantial protective effect against tuberculosis was seen with the CC/GC genotype and the C allele for SNP at the IL-18 137 (rs187238). In addition, a 6.6-fold increased risk for TB was found in the recessive model in those having GG and GC genotypes. The IL-18 137 (rs187238) SNP gene can be used as an independent risk predictive factor for the development of tuberculosis infection in the North Indian population. Further serum levels of cytokine IL-18 in TB patients and controls are directly influenced by genotype (GG or GC/CC) of the IL18 (rs187238) gene.

References

1. United Nations. Sustainable Development Goals. 2024. Available from: ?
2. WHO. Global tuberculosis report 2024. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024.
3. Ministry of Health and Family Welfare, Government of India. India TB report 2024. Available from: https://tbcindia.mohfw.gov.in/wp-content/uploads/2024/10/TB-Report_for-Web_08_10-2024-1.pdf.
4. Andrews JR, Noubary F, Walensky RP, et al. Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. Clin Infect Dis 2012;54:784-91.
5. WHO. Global tuberculosis report 2016. Available from: https://iris.who.int/bitstream/handle/10665/250441/9789241565394-eng.pdf?sequence=1.
6. Song CH, Lee JS, Nam HH, et al. IL-18 production in human pulmonary and pleural tuberculosis. Scand J Immunol 2002;56:611-8.
7. Zhen LB, Sun YP, Chen YY, Yin LS. IL-18 polymorphisms and tuberculosis susceptibility: a meta-analysis. Afr Health Sci 2019;19:1311-20.
8. Banda NK, Vondracek A, Kraus D, et al. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. J Immunol 2003;170:2100-5.
9. Heemskerk D, Caws M, Marais B, Farrar J. Tuberculosis in adults and children. London: Springer; 2015.
10. Dima E, Koltsida O, Katsaounou P, et al. Implication of Interleukin (IL)-18 in the pathogenesis of chronic obstructive pulmonary disease (COPD).Cytokine 2015;74:313-7.
11. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 binding protein. Front Immunol 2013;4:289.
12. Song CH, Lee JS, Nam HH, et al. IL-18 production in human pulmonary and pleural tuberculosis. Scand J Immunol 2002;56:611-8.
13. Inomata SI, Shijubo N, Kon S, et al. Circulating interleukin-18 and osteopontin are useful to evaluate disease activity in patients with tuberculosis. Cytokine 2005;30:203-11.
14. Yamada G, Shijubo N, Shigehara K, et al. Increased levels of circulating interleukin-18 in patients with advanced tuberculosis. Am J Respir Crit Care Med 2000;161:1786-9.
15. Khripko OP, Sennikova NS, Lopatnikova JA, et al. Association of single nucleotide polymorphisms in the IL-18 gene with production of IL-18 protein by mononuclear cells from healthy donors. Mediators Inflamm 2008:309721.
16. Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. J Neuroimmunol 2001;112:146-52.

17. Hassuna NA, Mohamed El Feky, Hussein M, et al. Interleukin-18 and interferon-γ single nucleotide polymorphisms in Egyptian patients with tuberculosis. PLoS One 2021;16:e0244949.
18. Shen L, Liang Z, Xu C, et al. Association between Interleukin-18-137G/C gene polymorphisms and tuberculosis risk in Chinese population: a meta-analysis. J Clin Cell Immunol 2017;08:514
19. Zhen LB, Sun YP, Chen YY, Yin LS. IL-18 polymorphisms and tuberculosis susceptibility: a meta-analysis. Afr Health Sci 2019;19:1311.
20. Cavalcante JEA, de Sousa ELH, de Oliveira Rodrigues R, et al. Interleukin-18 promoter -137 G/C polymorphism (rs187238) is associated with biochemical markers of renal function and cardiovascular disease in type 2 diabetes patients. Clin Biochem 2020;80:1-7.
21. Evans J, Collins M, Jennings C, et al. The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. Eur J Endocrinol 2007;157:633-40.
22. Wu S, Wang Y, Zhang M, et al. Genetic variants in IFNG and IFNGR1 and tuberculosis susceptibility. Cytokine 2019;123:154775.

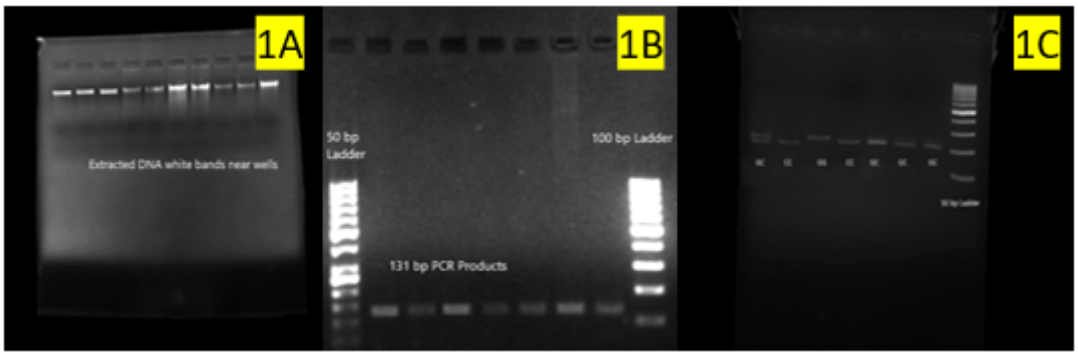

Figure 1. A) Gel picture showing extracted deoxyribonucleic acid; B) gel picture showing polymerase chain reaction products with 100 and 50 base pairs ladders; C) gel picture after digestion with 50 base pair ladders.

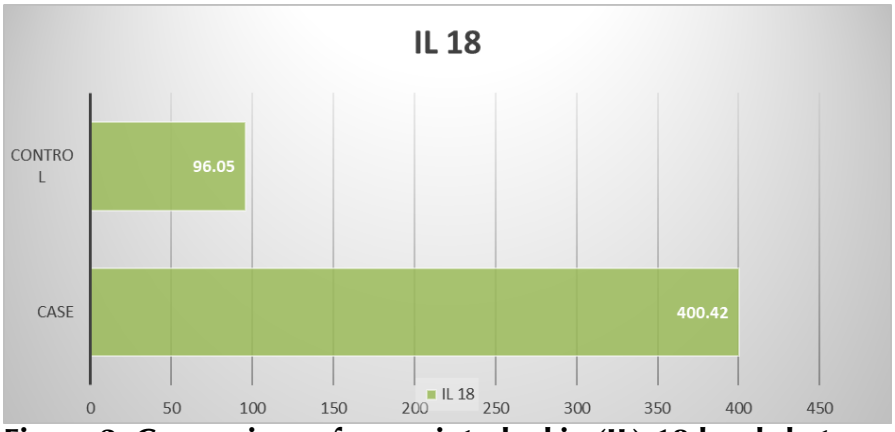

Figure 2. Comparison of mean interleukin (IL)-18 levels between cases and controls.

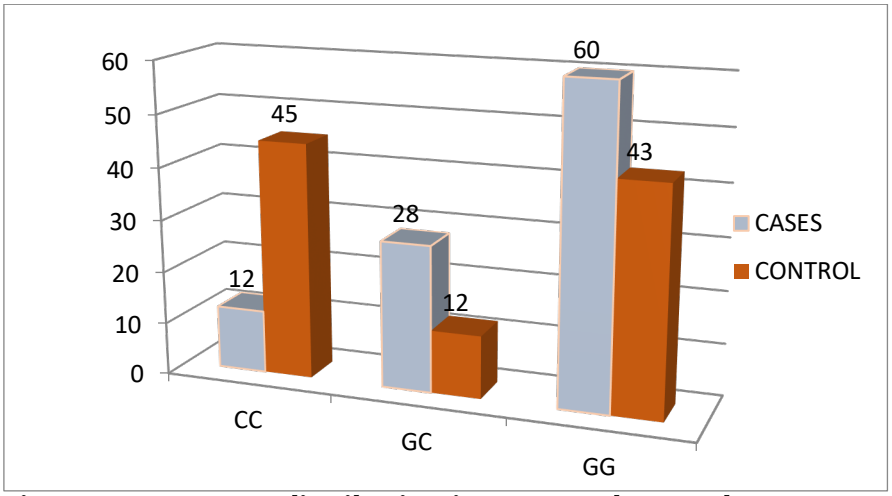

Figure 3. Genotype distribution in cases and controls.

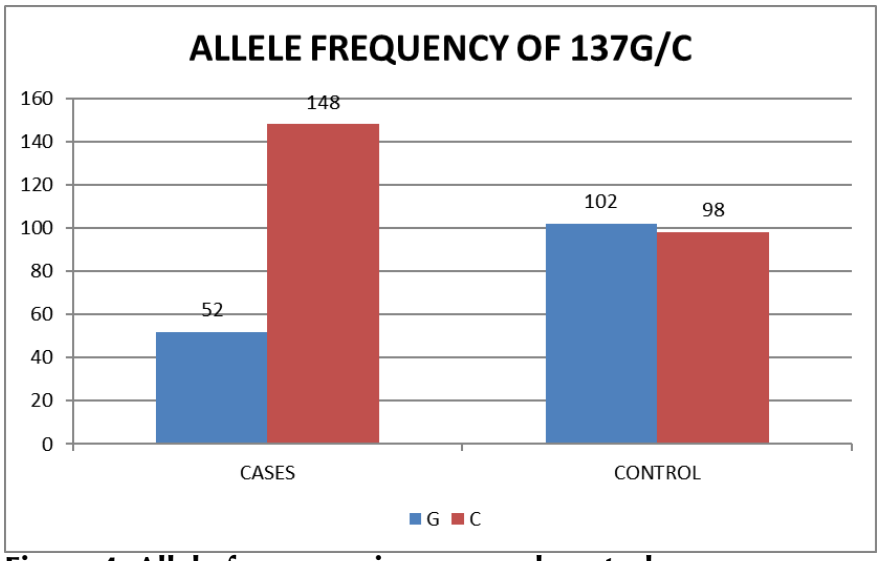

Figure 4. Allele frequency in cases and control groups.

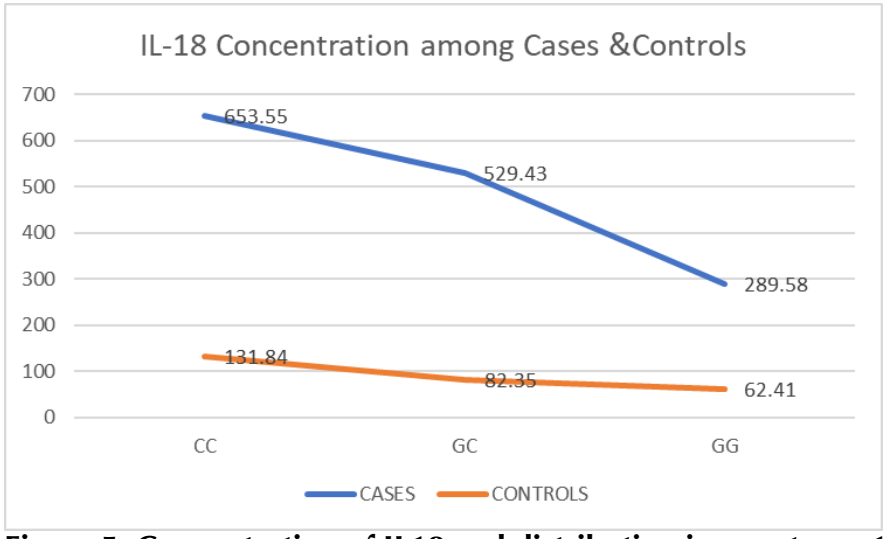

Figure 5. Concentration of IL18 and distribution in genotypes 137G/C.

Table 1. Distribution of heterozygous, homozygous wild polymorphic genotype 13G/C

Genotype	Case (n=100)	Control(n=100)	
CC	12	45	Chi-square: 65.5198 p-Value: < 0.01
GC	28	12	
GG	60	43	

Table 2. Distribution of heterozygous, homozygous wild polymorphic genotype 13G/C.

Genotype	**Cases (n=100)**	**Controls (n=100)**	**OR (with 95%CI)**	**p-value**
CC (reference)	12	45	0.31 (0.20 to 0.88)	0.016 (<0.05) (significant)
GG/GC	88	103		

Table 3. Allele frequency in cases and control groups.

Allele	Tuberculosis		
	Cases	Control	Odds ratio
C (reference)	52	102	0.33 [CI 0.22-0.51] p-value <0.0001 (highly significant)
G	148	98	
Total	200	200	