Journal of Medicine and Medical Research
Vol. 4(4): 33-40, September, 2016
ISSN: 2350-1502

www.resjournals.org/JMMR

Polymorphism in first Intron of Interferon- Gamma Gene (+874A/T) among Sudanese BCG-vaccinated Health Care Workers

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Abstract

Cytokines especially interferon-gamma (IFN-γ) are largely responsible for the regulation of the protective immune response against mycobacterial infections. Several studies have clarified the importance of common variants of IFN-γ gene regarding the susceptibility to tuberculosis. Bacille Calmette-Guérin (BCG) vaccine which is used to prevent severe forms of tuberculosis could produce local and systemic side effects. This study hypothesized that assesses the level of INF-γ among BCG-vaccinated Health Care Workers (HCWs) who were tested for (Purified Protein Derivative) PPD compared with TB- unexposed individuals. One-hundred thirty-seven HCWs (20-70 years old with ratio of TB-exposed and unexposed in females and males of 7: 3) were screened by conventional methods using microscopy, (Tuberculin Skin Test) TST, (Human Immuno Virus) HIV test and Chest X-Ray). Results of these tests showed that all subjects were apparently healthy and consequently they were allocated according to the TST results into three groups (moderate TST-positive, strong TST-positive and negative groups). DNA samples were obtained from 80 TB-exposed HCWs and 20 apparently healthy individuals. Distribution of HLA-DR, DQ alleles were determined by DNA typing and the INF-γ polymorphism (+874 T → A) was assessed by Allele Specific Polymerase Chain Reaction (AS-PCR). Frequency of A/A INF-γ genotype was totally absent among the strongly positive group, while it was 8% among the TST negative group. Polymorphism at *+874* was identified using AS-PCR. The variations in allelic frequencies of IFN-γ gene polymorphisms were also shown in the different population groups and genetic polymorphisms associated with tuberculosis had yielded conflicting results in different ethnic groups.

Keywords: Human leukpcyte allele (HLA), tuberculosis (TB) Bacille Calmette-Guérin (BCG), Interferon-Gamma Gene**,** Purified Protein Derivative (PPD).

INTRODUCTION

IFN-γ may be a key cytokine in the activation of macrophages for mycobacterial stasis and killing (Ambreen *et al*, 2009). The type 1 cytokine interferon (IFN)-γ plays a pivotal role in the defense against viruses and intracellular pathogens and in the induction of immune-mediated inflammatory responses (Billiau *et al*., 1998). IFN- γ production is critical in the control of *Mycobacterium tuberculosis* infection, whether IFN- γ is produced as a by-product of the activation of immune

defense mechanisms early in infection, or by antigen-specific T cells following the induction of specific immunity (Collins and Kaufmann, 2001).

Bacille Calmette-Guérin (BCG) vaccine has been used to prevent tuberculosis (TB) since 1921, and it also seems to be effective against the disseminated disease and meningitis in childhood TB. BCG vaccine has a low incidence of serious adverse reactions and is considered to be a safe vaccine (Parvaneh and Pourakbari, 2009). The possible genetic factors that affect the outcome of BCG vaccination in individuals' using correlate the degree of immune responses as measured by tuberculin skin test (TST) with INF-γ levels in TB-exposed HCWs and control subjects (Lio *et al*, 2002). T- helper (Th) lymphocytes could be divided into three subsets: Th1 clones characterized by the production of IFN-γ, Th2 clones, characterized by the production of interleukin 4 (IL-4) and Th17 lineage, the arm of the CD4 (Harrington *et al*, 2006). In mycobacterial infection, Th1-type cytokines seem to be essential for protective immunity (Cooper *et al*, 1993), and individuals lacking receptors for IFN-γ suffer from recurrent, sometimes lethal mycobacterial infections (Flynn *et al*, 1993, Holland *et al*, 1998). Th2-type cytokines inhibit the *in* vitro production of IFN-γ (Lucey *et al*, 1996), as well as the activation of macrophages (Appelberg *et al*, 1992**)**, and may therefore weaken host defense (de Waal Malefyt *et al*, 1993). IFN-γ is one of the most important cytokines involved in macrophage activation, stimulating antitumor and antimicrobial activities, as well as it stimulates the synthesis and expression of MHC-II.

There is an association between the genetic background of the human host and the susceptibility or resistance to *M. tuberculosis* infection (Stead, 2001). Low synthesis of INF-γ has been associated with active tuberculosis (Antonio et *al*, 2010). The protective role of IFN-γ in tuberculosis is well established (Flynn *et al*, 1993), primarily in the context of antigen-specific cell-mediated immunity (Andersen *et al*, 1997). Mycobacterial antigen-specific IFN-γ production *in vitro* can be used as a surrogate marker of infection with *M. tuberculosis* (Van Crevel *et al*, 1999). In principal, naive individuals do not show purified protein derivative (PPD)-stimulated IFN-γ production *in vitro* (Van Crevel*et al*, 1999). However, in both PPD-positive and PPD-negative individuals, *M. tuberculosis*-infected monocytes stimulate lymphocytes for the *in vitro* production of IFN-γ (Johnson and McMurray, 1994). This *M. tuberculosis* sonication stimulates production of monocyte-derived cytokines like TNF-α and IL-1β. These, as well as IL-12 and IL-18, may act as co-stimuli for age-independent IFN-γ production (Van Crevel *et al*, 2000). Acquired cell-mediated immunity after vaccination with *Mycobacterium bovis* vaccine (BCG) is more effective in disseminated infection compared with the pulmonary disease (Colditz *et al*, 1994). Similarly, naturally acquired cell-mediated immunity does not prevent exogenous reinfection of the lung (Vankayalapati *et al*, 2000). Although TB is known to be endemic in Sudan, little information is available about the magnitude of the disease and its epidemiology. Health care workers who are in direct or indirect contact with the TB patients and co-patients have no reliable medical record for their general health conditions and specifically for TB vaccination. More importantly there is no regular check by TST. In the light of the key role played by IFN- γ in the control of tuberculosis, The aim of this study was to study the degree of immune responses as measured by tuberculin skin test (TST) with INF-γ levels between TB-exposed HCWs and unexposed controls , in the present paper we have evaluated the distribution of the functional in IFN-γ gene polymorphism (at position +874) SNP was investigated among TB-exposed HCWs. This study examines the hypothesis that if there is any association between polymorphism of INF-γ gene and its product, INF-γ level between exposed and unexposed control individuals. Although TB is known to be endemic in Sudan, little information is available about the magnitude of the disease, its epidemiology. Health care workers who are in direct or indirect contact with the TB patients and co-patients have no reliable medical record for their general health conditions and specifically for TB vaccination. More important there is no regular check by TST.

MATERIALS AND METHODS

Subjects

Gene polymorphisms were analyzed in 137 HCWs (90 females and 47 males) who were vaccinated with BCG at birth.Tthey were selected after confirmation of acid-fast bacilli (AFB). All examined specimens (137) which did not show the characteristic AFB appearance of serpentine cords using oil immersion lenses which considered as negative. HIV, Chest X- Ray, TST negative subjects were revaccinated with BCG. They were considred as healthy, tuberculosis – free according to the results of the above tests.

Control group

All the TB-exposed subjects and unexposed (controls) were selected from Sudanese healthcare workers.The ages of TB-exposed and non exposed HCW groups were between 20 and 70 year. TB-exposed HCW who participated in the

study were from different categories, 60 (43.8%) were nurses, 57 (41.6%) were laboratory technicians, 9 (6.6%) were security staff, 6 (4.4%) were doctors, and 5 (3.6%) were Janitors. Tuberculin skin test was performed on all TB-exposed subjects, 107 out of 137 (78.1%) were positive and 30 (21.9%) were negative. All subjects were further classified into three groups according to the diameter size obtained (strongly positive (15mm- 25mm), moderately positive (6mm and < 15mm), negative(≥ 6mm). HCWs were screened by conventional methods (microscopy, TST, HIV test and Chest X-Ray). All subjects were apparently healthy and allocated according to the TST results into three groups (moderate TST-positive, strong TST-positive and negative groups). Eighty seven exposed healthcare workers out of one hundred thirty seven were vaccinated at birth and 50 HCWs were not vaccinated at birth from which 9 were revaccinated during the study.

Typing of IFN-γ gene polymorphism at position +874.

Ethical aspects

All individuals were briefed about the study and singed an informed consent form before sample collection. The present study was approved by the Human Ethics Committee of the Institute of Tropical Medicine Diseases, International Center for Research, Ministry of Science and Technology, Sudan.

Blood Collection

Five ml of venous blood were taken by a syringe from each individual (study and control groups) and kept at 4Cº in EDTA coating plastic disposable vacutainer tubes until used.

Determination of the INF-γ +874

Genomic DNA was obtained from whole blood by the QIAamp Blood Kit (QIAamp® DNA Mini Kit, QIAGEN INC., Chtworth. Cat # 51106. Lot No. 42471530, USA). according to the manufacturer's instructions using conventional salting out method. Polymorphism at position *+874* of *IFN*-γ gene was identified using allele specific polymerase chain reaction (AS-PCR). AS - PCR was preformed according to Parvaneh, *et al* (2009) as a rapid tool for correlation determination between the 12- CA-repeat allele in the first intron of IFN-γgene and the presence of the T allele at a single nucleotide polymorphism (SNP) at the +874 position (*+874T/A*) from the translation start site, that is important in the induction of constitutively high IFN-γ production. The assay targeted the *A/T* alleles at two different regions, 260 bp bands corresponding to IFN-γ A or T allele and the 100 bp bands corresponding to β-globin (Internal control). The PCR reaction was performed in total volume of 15.1 µl included 0.3 µl of 8 mM dNTPs,1.5 µl of 3.5 mM Mgcl2, 0.6µl 5U/ µl Taq DNA polymerase and 0.3 µl of 10µM from each specific antisense primers (antisense: TCA ACA AAG CTG ATA CTC CA; sense+874T: TTC TTA CAA CAC AAA ATC AAA TCT; or sense +874A: TTC TTA CAA CAC AAA ATC AAA TCA), sense +874A and sense +874T). Then 0.3 µl of 2 µM of each internal control primers amplify a human β-globin sequence (BGF: ACA CAA CTG TGT TCA CTA GC; BGR: CAA CTT CAT CCA CGT TCA CC), followed by the addition of 5 µl of the template DNA. The mixture was gently mixed, and then transferred to the PCR machine. Amplification was performed using a touchdown method that included initial denaturation at 95 ºC for 1 min followed by two loops; loop 1 which consisted of 10 cycles with the following program: 95 ºC for 15 sec, 62 ºC for 50 sec, and 72 ºC for 40 sec and the loop 2 included 20 cycles with the following program: 95 ºC for 20 sec, 56 ºC for 50 sec and 72 ºC for 50 sec and a final extension step at 72 ºC for 10 min. The PCR products (15 µl) was loaded into the gel wells) was visualized by electrophoresis at 100 V for 25 min. in 1.5 % agarose with 0.5 mg/ml of ethidium bromide and photographed (Figure 1).

Statistical analysis

Collected data were analyzed using the Statistical Package for Social Science (SPSS, Chicago, IL, USA; Version 16).The genotype distribution of the INF-γ gene in TB-exposed HCWs among different work durations in TB wards was

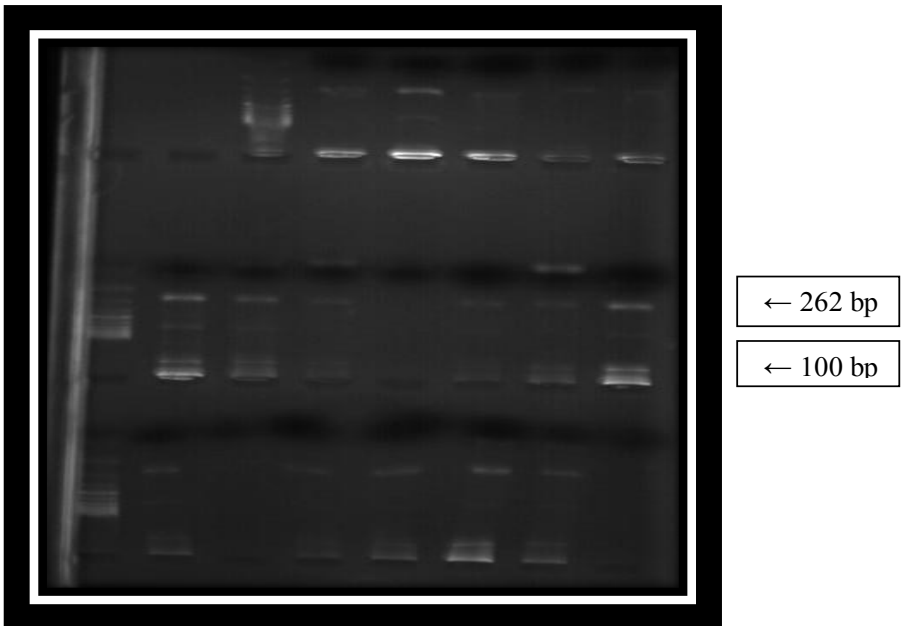

Figure 1: Agarose gel electrophoresis of AS- PCR products for INF-γ (+874A/T) polymorphism; 262bp corresponding to INF-γ A or T allele and the100bp bands corresponding to the β- Goblin (internal controls).

Table I: INF-γ (+874T/A) genotypes distribution in exposed HCWs (n= 57) and control group (n= 17)

INF-γ genotype	Exposed Group (%)	Unexposed Control Group (%)
A/A	7 (12.3%)	0.0
A/T	43 (75.4%)	17 (100%)
T/T	7 (12.3%)	0.0
Total	57	17

analyzed using one-way ANOVA. Charts were done using Excel software. The results considered significant at p-value equal to or less than 0.05.

RESULTS

INF-γ genotype was determined in 80 TB-exposed HCWs and 20 apparently healthy individuals using allele specific-polymerase chain reaction (AS- PCR) amplification of the genomic DNA (Figure 1). The results revealed a 100-bp product for human β-globin as a control gene, and a 260-bp product for +874 (homozygous for allele T; TT). Also they were A874 (homozygous for allele A; AA), or both alleles T and A (heterozygous; AT). The TB-exposed and unexposed groups were categorized for+874 polymorphism into three genotypes by discriminating between the presence and absence of the A/T alleles. The results of genotyping for the exposed and control groups were presented in table I.

Genotypes distribution among different groups of TST

The results revealed that there was no association between genotype and the allele frequencies of the +874T/A gene polymorphism in exposed healthcare workers among the three groups (strongly positive, moderately positive, negative to TST). Odds ratio for the A874 allele was : $p > .05$, $\chi^2 = 1.5$ (Table II).

Table II: Frequency of the Genotyping of the T874A polymorphism of the INF-γ Gene in all the exposed healthcare workers.

TST Results in Groups	Genotypes			Total
	AA (n= 7)	TT (n= 7)	AT (n= 43)	
Strong Positive group (n=10)	0.0 (n = 0)	20% (n =2)	80% (n = 8)	100%
Moderate positive group (n=22)	22.72% (n= 5)	13.63% (n = 3)	63.64% (n= 14)	100%
Negative group (n= 25)	8% (n = 2)	8% (n = 2)	84% (n = 21)	100%

Table III: INF-γ (+874T/A) allele and genotype frequencies (n, %) in exposed healthcare workers and controls

Target group and control	Genotypes		
	AA (n= 7)	TT (n= 7)	AT (n= 60)
Exposed healthcare workers group (n= 57)	12.3% (n = 7)	12.3% (n = 7)	75.4% (n=43)
Control group (n=17)	0%	0%	100% (n=17)

The genotyping frequency of INF-γ gene in exposed healthcare workers and unexposed (controls) showed that A/T heterozygote (75.4%, n=43) in exposed healthcare workers and all controls (100%, n= 17). The T/T genotype was presented in the 12.3% (n=7) among exposed healthcare workers while A/A genotype frequency was 12.3% (n=7), the exposed healthcare workers group showed a similar frequency of A/A and T/T genotype. No significant difference was observed ($p= 0.08$, $\chi^2=4.9$) between exposed individuals and unexposed controls (Table III).

Work duration and INF-γ genes at position +874

The genotype distribution of the INF-γ gene in (57) TB-exposed HCWs with different work durations in TB wards was analyzed using one way ANOVA. The results showed that there was no significant differences in frequency of A/A, T/A and T/T genotypes ($p= 0.06$) between exposed individuals and unexposed controls. There was only A/T alleles in TB-exposed HCWs who worked for ≤ 5 years but subjects working for ≥ 10 years had A/A, T/T and A/T genotypes.

DISCUSSION

The knowledge of the genetic basis of susceptibility to disease is an expanding field (Casanova *et al,* 2007 and Hill,1998).A functional cytokine network is the central element in the homeostasis of the immune response, and its change may lead to abnormal or ineffective immune response, as seen in human infection by *M. tuberculosis* (Antonio *et al*, 2010). The aim of this study was to examine the degree of immune responses as measured by tuberculin skin test (TST) with INF-γ levels in TB-exposed HCWs and unexposed controls. The TB-exposed HCWs included in the present study were smear negative or in other words all collected sputa were negative on microscopy, and all workers were HIV-negative. Chest X- Ray results were normal, most of them were vaccinated by BCG at birth and the others were immunized later. In the present study, it was found that about 78.1% of the subjects were positive to TST. It has been shown that a single nucleotide polymorphisms (SNPs) located in the promoter or coding regions of cytokine genes result in differential cytokine secretion due to altered transcriptional activation (Ambreen *et al*, 2007). IFN-γ may be a key cytokine in the activation of macrophages for mycobacterial stasis and killing. However, other studies in humans have shown that there is single nucleotide polymorphisms (SNPs) located in the first intron of the IFN-γ gene (at position

+874) whicvh have shown variable associations with susceptibility and severity of TB and its severity (Ambreen*et al*, 2009). Recent study showed that single-nucleotide functional polymorphisms in cytokine genes display variable associations with LTBI and active TB disease. Combinations of cytokine SNPs with the *IFN-γ* +874 T/A SNP markedly influence the severity and outcome of TB. These results may reflect the polygenic aspects of a predisposition to severe and active TB (Yi Hu *et al*., 2015).

The exposed HCWs were classified into three sub-groups namely, strongly positive, moderately positive and negative subjects based on the TST results. The A/A INF-γ genotype was totally absent among the strongly positive group while its frequency was 8% among the negative group while T/T INF-γ genotype showed the highest frequency among the strongly positive. On the other hand the A/T was the only genotype present among the control subjects (100%) and showed the highest frequency in the strongly positive group among the exposed HCWs. These variations in allelic frequencies of IFN-γ gene polymorphisms also were shown in different populations (Bagheri*et al*, 2006). It may be concluded that there is no surprising that genetic polymorphisms associated with TB have yielded conflicting results in different ethnic groups (Takiff, 2007). INF-γ polymorphism (+874 T → A) is the most studied polymorphism in terms of association with TB sites and severity. However, the reports are conflicting in that A allele was more common in patients with TB and T allele more common in controls in Italian (Lio*et al*, 2002), South African (Rossouw*et al*, 2003), In recent study showed that the genotype TT showed a protective effect while genotype AA may be associated with increased susceptibility to developing tuberculosis . Hong Kong Chinese (Tso*et al*, 2005) and Spanish populations (Lopez-Maderuelo*et al*, 2003). In Turkey a study showed an association with the A allele (Oral *et al*, 2006). In Croatia an association with INF γ A allele was found only in microscopy and culture positive versus negative TB patients. On the other hand, no association has been reported in Caucasians in Houston, Texas (Moran *et al*, 2007) and in South India (Vidyarani*et al*, 2006). There is only one study in Colombia (Henao*et al*, 2006) which showed that there was an association of INF-γ +874 T allele with the more localized pleural disease. An Iranian study showed that the effect of INF-γ T allele in TB-affected patients is restricted to pulmonary patients with minimal/ moderate disease and it is directly related to people who were exposed to risk factors more than others. Some of the differences could be due to the influence of other genes linked to susceptibility to TB (Ambreen*et al*, 2009). It was reported that T- helper (Th) lymphocytes could be divided into two subsets: Th1 clones characterized by the production of IFN-γ, and Th2 clones, characterized by the production of interleukin 4 (IL-4) (Mosmann*et al*, 1986). In mycobacterial infection, Th1-type cytokines seem to be essential for protective immunity (Cooper *et al*, 1993), and individuals lacking receptors for IFN-γ suffer from recurrent, sometimes lethal mycobacterial infections (Flynn *et al*, 1993, Holland *et al*, 1998 and Newport *et al*, 1996). A Th2-type cytokine inhibits the *in* vitro production of IFN-γ (Lucey*et al*, 1996), as well as the activation of macrophages, and it may therefore weaken host defense (Appelberg*et al*, 1992**)**.

CONCLUSION

Based on the importance of IFN-g in the protective immunity against tuberculosis, our results demonstrated that the associations of tuberculosis exposure among Sudanese healthcare workers who were vaccinated at birth and increased PPD skin test reactivity explained by the immaturity of the immune systemTST results into three groups moderate TST-positive, strong TST-positive and negative groups with low production of INF-γ g among the negative group while highly produced among the strongly positive group. The A/T was the only genotype present among the unexposed controls.

ACKNOWLEDGEMENT

We thank all subjects who involved in the study. This work was supported by the Biotechnology Laboratory, Ahfad University for Women, we would like to thank all of them for their valuable helps and constructive advice during the bench work. Thanks for excellent technical support by staff members of the laboratory of Ashaab hospital and medical staff of Ummdawban Hospital - Khartoum State, for there valuable help during the the blood collection.

REFERENCES

Ambreen A., Najeeha T., Bushra J., Zahra H., Tashmeem R., Ghaffar D and Rabia H (2009). Cytokine gene polymorphisms across tuberculosis clinical spectrum inpakistani patients. *PloS ONE* 4: 4778.

Ambreen A., Najeeha T., Bushra J., Zahra H., Tashmeem R., Ghaffar D and Rabia H. (2007). Cytokine Gene Polymorphisms across Tuberculosis Clinical Spectrum in Pakistani Patients. *PLoS ONE 4(3): e4778.*

Andersen P (1997). Host responses and antigens involved in protective immunity to Mycobacterium tuberculosis. *Scand. J. Immunol. 45:115-131.*

Antonio CR, Vallinoto AC., Graça ES., Araújo MS., Azevedo VN., Cayres-Vallinoto I., Machado LF., Ishak MO and Ishak R (2010). IFNG _874T/A polymorphism and cytokine plasma levels are associated with susceptibility to *Mycobacterium tuberculosis* infection and clinical manifestation of tuberculosis. *Human Immunology*.71: 692–696.

Appelberg R., Orme IM., Pinto de Sousa, M. I. and Silva, M. T. (1992). In vitro effect of interleukin-4 on interferon-gamma- induced macrophage activation. *Immunology* 76: 553-559.

Bagheri M., Abdi-Rad I., Omrani D and Khalkhali HR (2006). Heterogeneity of cytokine single-nucleotide polymorphisms among the Iranian and in the other East-South Asian populations. *Transfusion Medicine 16: 192–199.*

Billiau A., Heremans H., Vermeire K and Matthys P (1998). Immunomodulatory properties of interferon-gamma. An update.*Annals of the New York Academy of Sciences*, 856, 22.

Casanova JL and Abel L (2007). Human genetics of infectious diseases: a unified theory. *EMBO J,* 26(4): 915-22.

Colditz GA., Brewer TF., Berkey CS., Wilson ME., Burdick E., Fineberg HV and Mosteller F. (1994). Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA.2;271(9):698-702.*

Collins HL and Kaufmann SH (2001). The many faces of host responses to tuberculosis. *Immunology*, 103, 1.

Cooper AM., Dalton DK., Stewart TA., Griffin JP., Russell DG and Orme IM (1993). Disseminated tuberculosis in interferon gamma gene-disrupted mice.*J. Exp. Med.* 178:2243-2247.

de Albuquerque AC., Rocha LQ, de Morais Batista AH., Teixeira AB.., Dos Santos DB and Nogueira NA (2012). Association of polymorphism +874 A/T of interferon-γ and susceptibility to the development of tuberculosis: meta-analysis. *Eur J Clin Microbiol Infect Dis. 2012 Nov;31(11):2887-95.*

de Waal MR., Figdor CG and de Vries JE (1993). Effects of interleukin 4 on monocyte functions: comparison to interleukin 13. *Res. Immunol. 144:629-633.*

Flynn, JoAnne L., John Chan, Karla J. Triebold, Dyana K. Dalton, Timothy A. Stewart, and Barry R. Bloom (1993). An Essential role for Interferon gamma in resistance to Mycobacterium tuberculosis infection. J Exp Med 178: 2249–2254.

Harrington LE., Mangan PR and Weaver CT (2006). Expanding the effector CD4 T-cell repertoire: the Th17 lineage. *Curr Opin Immunol 18:349-356.*

Henao MI., Montes C., Paris SC and Garcia LF (2006). Cytokine genepolymorphisms in Colombian patients with different clinical presentations of tuberculosis*. Tuberculosis* 86: 11–19.

Hill AV (1998). The immunogenetics of human infectious diseases. *Annu Rev Immunol*, 16: 593-617.

Holland SM., Dorman SE., Kwon A., PithaRowe IF., Frucht DM., Gerstberger SM., Noel GJ., Vesterhus P., Brown MR and Fleisher TA (1998). Abnormal regulation of interferon-gamma, interleukin-12, and tumor necrosis factor-alpha in human interferon-gamma receptor 1 deficiency.*J Infect Dis*178: 1095-104.

Johnson BJ and McMurray DN (1994). Cytokine gene expression by cultures of human lymphocytes with autologous Mycobacterium tuberculosis-infected monocytes. *Infect. Immun. 62:1444-1450.*

Lio D., Marino V., Serauto A., Gioia V., Scola L., Crivello A., Forte G., Colonna-Romano G., Candore G and Caruso C (2002). Genotype frequencies of the +874T2.A single nucleotide polymorphism in the first intron of the interferon-gamma gene in a sample of Sicilian patients affected by tuberculosis.*Eur J Immunogenet* 29: 371–374.

López-MD, Arnalich F., Serantes R., González A., Codoceo R., Madero R., Vázquez JJ and Montiel C (2003). Interferon-gamma and interleukin-10 gene polymorphisms in pulmonary tuberculosis 5691.*Am. J. Respir. Crit Care Med* 167: 970–975.

Lucey DR., Clerici M and Shearer GM. (1996). Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases.*Clin.Microbiol.Rev.* 9: 532-562.

Melanie JN., Clare MH., Sara H., Catherine MH., Ben AO., Robert W and Michael L (1996). A Mutation in the Interferon-γ –Receptor Gene and Susceptibility to Mycobacterial Infection. *N Engl J Med 335:1941-1949*.

Moran A., Ma X., Reich RA and Graviss EA (2007). No association between the +874T/A single nucleotide polymorphism in the IFN-g gene and susceptibility to TB. *Int J Tuberc Lung Dis* 11: 113–115.

Mosmann TR., Cherwinski H., Bond MW., Giedlin MA and Coffman RL (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins*. J. Immunol.* 136:2348-2357.

Oral HB., Ferah B., Esra KU., Bilkay B., Ahmet B., Halis A., Ercüment E., Beyza E and Güher G (2006). Interleukin-10 (IL-10) gene polymorphism as a potential host susceptibility factor in tuberculosis.*Cytokine* 35: 143–147.

Parvaneh1 N., Pourakbari1 B., Daneshjoo K., Ashraf1 H., Salavati1 A and Mamishi1 S (2009). Polymorphism in the First Intron of Interferon-Gamma Gene *(+874T/A*) in Patients with BCG Adenitis.Iranian J Publ Health, 38(3):12-16.

Rossouw M., Nel HJ., Cooke GS., van Helden PD and Hoal EG (2003). Association between tuberculosis and a polymorphic NfkappaB binding site in the interferon gamma gene. *Lancet* 361: 1871–1872.

Stead WW (2001). Variation in vulnerability to tuberculosis in America today: Random, or legacies of different ancestral epidemics? *Int J Tuberc Lung Dis. 5:807–14*

.

Takiff HE (2007). Host Genetics and Susceptibility. In: Palomino JC, Leao SC, Ritacco V, eds (2007) Tuberculosis 2007 – From Basic Science to Patient Care. *Brazil.* Pp 207–262.

Tso HW., Chong WP., Tam CM and Chiang AK (2005). Association of *Tuberculosis* 4: 411–27.

van Crevel R., Karyadi E., Preyers F., Leenders M., Kullberg BJ., Nelwan RH., van der Meer JW (2000). Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. *J Infect Dis. 181(3):1194-7.*

van Crevel R., Van der Ven Jongekrijg J., Netea MG., de Lange W., Kullberg BJ and van der Meer JW (1999). Disease-specific ex vivo stimulation of whole blood for cytokine production: applications in the study of tuberculosis. *J. Immunol. Methods 222:145-153.*

Vankayalapati R., Wizel B., Weis SE., Samten B., Girard WM and Barnes PF (2000). Production of interleukin-18 in human tuberculosis. *J. Infect. Dis. 182:234-239.*

Vidyarani M., Selvaraj P., Prabhu AS., Jawahar MS., Adhilakshmi AR and Narayanan PR (2006). Interferon gamma (IFNc) & interleukin-4 (IL-4) gene variants & cytokine levels of pulmonary tuberculosis.*Indian J Med Res 124: 403–410.*

Yi H., Linlin W., Dange L., Qi Z.,Weili J and Biao X (2015). Association between cytokine gene polymorphisms and tuberculosis in a Chinese population in Shanghai: a case–control study. *BMC Immunology201516:8.*