RESEARCH

Cytokine production and polymorphisms in the IFN γ, IFN γR1 and IL12 genes

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ABSTRACT

One third of the world's current population has been infected with *Mycobacterium tuberculosis*. However, most of these cases will not develop the full-blown active disease and remain asymptomatic, known as latent tuberculosis infection (LTBI). Although the LTBI individuals are asymptomatic, there is a chance of reactivation and 5-10% of them develop active tuberculosis (TB). The risk of reactivation is high among the household contacts of active TB. Thus, household contacts constitute an important target for early prevention and control of tuberculosis. Cytokines play an important role in protective immunity and may aid in identification of diagnostics and new vaccines. The role of IL-12 and IFN-γ has been established in protection against mycobacterial infections. The balance between the secretions of IL-12/IFN-γ appears to be essential for the regulation of inflammation in response to *Mycobacterium tuberculosis*. The aim of the present study was to evaluate the cytokine production in serum and culture supernatants and the single nucleotide polymorphisms (SNPs) in the IFN-γ and IL-12 genes.

The cytokine levels of IFN-γ and IL-12 were estimated in serum (n=150) and culture supernatants (n=25) stimulated with r32kda *Mycobacterium bovis* BCG in active pulmonary tuberculosis patients (APTB), household contacts (HHC) and healthy controls (HC) by enzyme-linked immunosorbent assay (ELISA) and their genetic polymorphisms (n=150) were studied by amplification refractory mutation systems polymerase chain reaction (ARMs PCR).

The mean serum levels of IFN-γ and IL12 were high in APTB and HHC compared to HCs. The mean stimulated levels of IFN-γ were significantly low in APTB and HHC compared to HC. IL-12 cytokine levels were significantly high in APTB and HHC as compared to HCs. The AT genotype in IFN-γ +874 A/T, CC and TC genotypes in IFNgR1-56C/T were found to be associated with TB. In conclusion, cytokine levels and cytokine gene polymorphisms affect the susceptibility to tuberculosis in household contacts.

KEYWORDS: Household contacts; Single nucleotide polymorphisms; IL-12/IFN-γ; IFNgR1, cytokine secretion.

Citation: Joshi et al. Cytokine production and gene polymorphisms of IFN γ, IFN γR1 and IL12. Polymorphism 2020; 7: 1-12.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by bacterium *Mycobacterium tuberculosis (Mtb)*. According to the WHO 2017 report, there were an estimated 1.3 million TB deaths and 10.4 million people fell ill with TB in 2016 [WHO, 2017]. A relatively small proportion of people infected with *Mtb* develops active TB disease, while majority of the infected individuals develop clinically latent TB infection which is characterized by the absence of disease symptoms [Erick Wekesa Bunyasi et al, 2017]. The transition between the initial control of infection and the establishment of latent TB infection, which may lead to active TB, is still unknown. Hence early diagnosis of TB infection is essential for the control and spread of the disease.
The progression of active TB is associated with the immune status and various cytokines have been implicated in the protective immunity, pathophysiology and development of the disease [Egidio Torrdao et al, 2013]. The cytokine environment present at the time determines the differentiation of Th cells. Thl cells develop when IL-12 is present along with antigen stimuli and Th2 cells develop if IL-4 is present along with antigen stimuli [Rukhsana Jabeen et al, 2013]. Thl cytokines, such as IFN-γ, IL-2 and IL-12 through enhancement of T-cell function and macrophage activation may prove to be potent immuno-therapeutic agents [Therwa Hamza et al, 2010]. IL-12 induces T cells and NK cells to produce proinflammatory cytokines such as IFN-γ and TNF-α while also regulating the production of IL-17 [Trinchieri G, 1995]. IFN-γ is a key cytokine in activation of macrophages for mycobacterial stasis and killing [Ambreen Ansari et al, 2009]. IL-12 is important in mediating protective immunity against TB [Cooper AM et al, 1997]. IL-12 is crucial for optimal differentiation and maintenance of IFN-γ-secreting antigen-specific Th1 cells and in controlling mycobacterial infections in mice and men.

The IL-12/ IFN-γ axis is determinant in which IL-12, produced early in infection by APCs, promotes the generation of Th1 lymphocytes, activation of macrophages by T cells, and IFN-γ production and further elimination of bacteria. The balance between the secretions of IL-12/IFN-γ appears to be essential for the regulation of inflammation in response to *Mtb* [Yone Vila Nova Cavalcanti et al, 2012]. A series of mutations associated to these axis components were identified in humans: these include mutation in the IL-12Rβ1, IL-12p40, IFN-γR2 genes [Noe Ramirez-Alejo et al, 2014]. Also, human genetic deficiencies in IL-12, IFN-γ, IFN-γ receptor axis result in increased susceptibility to mycobacterial diseases.

The allelic variants at 3'UTR and promoter have different effects on IL12B mRNA [Morahan G et al, 2001]. Deleterious mutations in genes of IL-12B encoding IL-12p40, a subunit of IL-12 and IL-12R have been shown to be associated with susceptibility to infections. An A/C single nucleotide polymorphism at position-1188 has been found to have biological significance in *M.tb*. IFN-γ and IFN-γ R form a vital complex in determining the outcome of the biological effects of IFN-γ [Dorman SE et al, 2004]. A previous study showed that defects in either of these two genes influence the availability of IFN-γ and therefore individuals were more prone to mycobacterial infections [Ottenhoff et al, 1998]. It has been reported that the production of IFN-γ is genetically controlled and +874 T/A was found to be one of the well-known polymorphisms of this gene. Also, several studies have reported the associations between gene polymorphisms and ex-vivo cytokine production investigating their role in modulating the overall cytokine response. The present study was aimed to assess whether the genotype variants of IFN-γ, IFN-γ R1 and IL-12 influences the cytokine secretion and together contribute to disease progression or resistance towards the disease.

MATERIALS & METHODS

Study groups

The study was carried out at free chest clinic and PPM-DOTS center, Mahavir Hospital and Research Center, Hyderabad, India. Based on the data analysis over 10 years (1998-2008) at the center, it has been observed that most of the tuberculosis patients treated were in the age group of 15 to 25 yrs. Hence the present study was designed to include the subjects in the age range of 15 to 25 yrs. A total of 490 cases were enrolled during 2009-2012, of which 150 were active pulmonary tuberculosis patients (APTB), 190 Household contacts (HHC), and 150 Healthy controls (HC). All patients had positive acid-fast bacilli (AFB) smear microscopy. The bacterial sputum gradation was based on the number of Acid-Fast Bacilli (AFB) observed on the slide under the microscope as per the Revised National Tuberculosis Control Program (RNTCP) guidelines. The household members of the patient of the same age group have been taken as contact who were asymptomatic to TB. Healthy controls with 15-25 years age and with no history of TB were considered for the study.

Ethical statement

All the study protocols were reviewed and approved by the independent institutional ethical committee of Mahavir Hospital and Research Center, Hyderabad, India. Informed written consents were taken from all the participants enrolled in the study. Human immunodeficiency virus (HIV), Renal Transplant, Diabetic, Hypertension, malignancy and cardiac patients were excluded from the study.

Sampling

A total of 6ml blood was collected and separated in serum, heparin and EDTA tubes. The serum tubes were centrifuged at 4000 rpm for 10 min and serum was separated and stored at -80°C in cryovials until analysis. For assessing T cell proliferation 3ml of blood was drawn in Heparin tubes and 1ml of blood was collected in EDTA tubes for DNA isolation.

PBMC proliferation assay

Peripheral blood mononuclear cells (PBMC's) were isolated from heparinized blood by density gradient centrifugation using a Histopaque-1077 (Sigma, St. Louis, MO). Cells were then cultured in RPMI 1640 complete medium (Invitrogen Corporation, Grand Island, NY) at a concentration of 1 X 10^6 cells/ml (Falcon Products, Becton Dickinson, Oxnard, CA) and stimulated with 4 ul (3 mg/ml) *M. bovis* BCG r32-kDa Ag and 30 ul (1mg/ml) Concanavalin A (Sigma Aldrich, St. Louis, MO), the latter as a positive control for cell reactivity. The cells were incubated for 5 days and 3 days respectively, at 37°C and in an atmosphere of 5% CO2. Supernatants and cells were collected and stored at -80°C for further studies. After the addition of MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], the optical density (OD) was recorded by using an ELISA reader (Bio rad laboratories) of a dual-wavelength at 570nm and 620nm reference filter. Data were expressed as the stimulation index (SI), i.e., the ratio of the mean OD of experimental cultures to the mean OD of control cultures, which was considered positive if the value was ≥2.

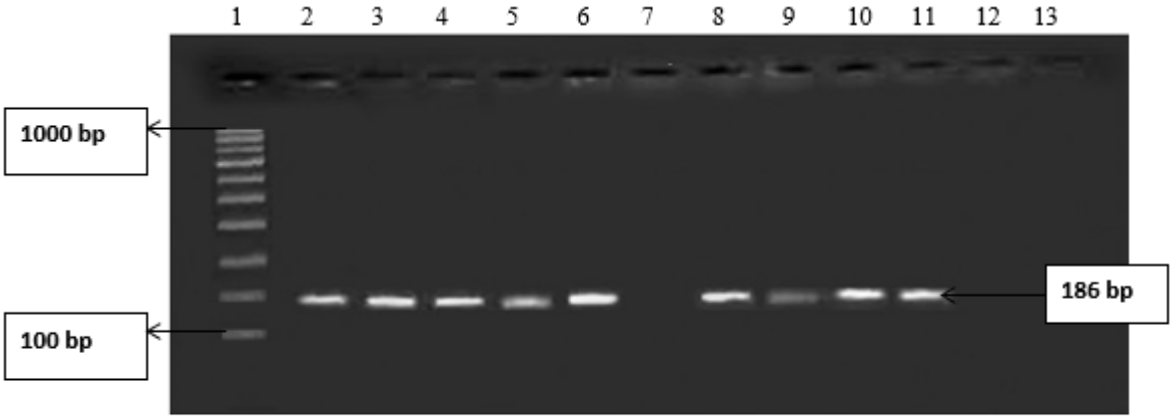
Cytokine levels of IFN-γ and IL-12

The concentrations of *IFN-γ* and *IL-12* genes were measured in serum and culture supernatants by enzyme-linked immunosorbent assay (ELISA) using commercially available kits for cytokine detection (BD Opt EIA for human *IFN-γ* and *IL-12*). The preparation of all reagents, and the working standards and protocol were followed according to the manufacturer's instructions. The absorbance was read using an ELISA reader (BIO-RAD) at 450nm and 570nm dual filter. All the samples were assayed in duplicate.

Single nucleotide polymorphism genotyping

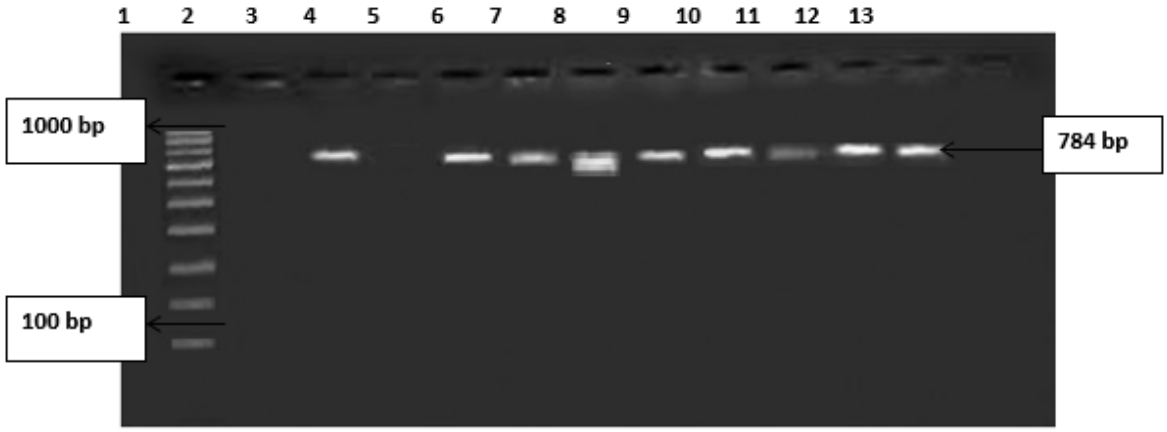
Genomic DNA was extracted from whole blood collected in EDTA tubes using Flexi gene kit

according to the manufacturer's protocol. Quantity of DNA was confirmed by Nano Drop and DNA was stored at -20°C. *IFN-γ* (-874A/T) and *IL-12*(-1188C/A) genotyping was carried out by ARMS-PCR (Amplification refractory mutation system-polymerase chain reaction). IFN-γ(+874A/T) amplification was performed by using common primer 5'- tcaacaaagctgatactcca- 3' A allele primer 5'- ttcttacaacacaaaatcaaatca -3' and T allele primer 5'ttcttacaacacaaaatcaaatct -3' with cycling conditions of 95°C for 1 min followed by 30 cycles at 95°C for 20sec, 62°C for 1 min, 72°C for 50sec and finally for 5 min extension at 72°C. A product size of 186bps was detected (Fig 1a). IL-12 (+1188A/C) genotyping was done using common primer 5'-atcttggagcgaatgggc at -3', C allele primer 5'- ttgtttcaatgagcatttagcatc t -3' and A allele primer 5' - ttgtttcaatgagcatttagcatc g -3' with cycling conditions of 94°C for 3 min followed by 35 cycles at 94°C for 30sec, 65°C for 45sec, 72°C for 45sec and finally for 5 min extension at 72°C. A product size of 784bps was detected. Electrophoresis of PCR products was carried out on 2% agarose gel stained with ethidium bromide, and presence or absence of fragments was visualized by UV transilluminator (Fig. 1b).



Lane 1-100 base pairs ladder;
Lane 2-3;4-5;8-9;10-11 - AT genotype.
6-7 - AA genotype.

Fig 1a - Agarose gel picture showing amplified ARMS PCR products of IFN-γ +874 T/A



Lane 1-100 bp ladder
Lane 2-3;4-5 - CC genotype.
Lane 6-7;8-9;10-11 - AC genotype.
Lane 12-13 AA genotype.

Fig 1b- Agarose gel picture showing amplified ARMS PCR products of IL-12 +1188 A/C.

Statistical analysis

The serum and culture supernatant levels of cytokines were measured by ELISA. Shapiro Wilk test was run to check the normality distribution for each group of the independent variable, and the difference between the groups was analysed by non-parametric Mann-Whitney U, t-test and unpaired t test. Mean and SD were calculated for each group. Graphpad prism was used to perform the test of significance. Multivariate regression analysis was used to find the correlation between gene polymorphisms and serum levels using IBM SPSS Statistics (version 20). The conventional 5% level of significance was used for all statistical tests. SNP stats was used to test the associations between SNPs and the risk of TB. All statistical tests were two-sided, a value of ≤0.05 was considered significant, and analyses were performed using Epi Info 6 SNPStats.

RESULTS

Clinical characteristics were studied in a total of 150 patients, 190 household contacts and 150 healthy controls. For all these subjects, age, sex, BCG scar, body mass index (BMI) and tuberculin skin test were taken into consideration for the analysis of the data. Males were found to be predominant compared to females in all the three categories and a significant difference was seen in APTB & HHC compared to HC. The mean age of APTB, HHC and HC were (22.74±7.71; 27.67±10.34; 23.49±1.86), and no significant difference was observed between the ages of APTB and HHC when compared to HC. The number of BCG scar negative individuals was more in APTB while in HHC and HC scar positive individuals were high and a significant difference was observed in scar status between the groups. The number of TST-positive individuals was more both in APTB and HHC. The BMI showed a significant difference between the groups (Table 1).

Single nucleotide polymorphisms of IFN-γ, IFN-γ R1, IL-12

The single nucleotide polymorphisms of IFN-γ (-874A/T), IFN-γ R1 (-56C/T), IL-12(-1188A/C) were studied in 150 APTB, 190 HHC & 150 HCs. The genotype distribution in APTB and HHC deviated from Hardy Weinberg equilibrium ($p<0.05$) but in HC were in HWE ($P>0.05$).

The AT genotype of IFN-γ (+874 A/T) (Fig:1) was significantly associated with disease in APTB and HHC ($p<0.0097$ OR-1.90; CI-1.16-3.10) ($p<0.04$ OR-1.62; CI-1.01-2.60) Table 2a. APTB were found to be 1.9 times at higher risk and HHC at 1.6 times for developing the disease compared to controls. The alleles of IFN-γ (+874 A/T) have not shown any significant difference (Table 2b).

The genotypes and alleles frequencies of IL-12 -1188C/A (Fig:2) did not show any significant difference between the subjects (Table 3a, 3b). For IFN-γ r1 (Fig:3), CT genotype was more frequent in APTB and HHC, whereas in HCs the CC genotype was more frequent. The Co-dominant, dominant and over dominant models were significantly associated with the disease and were showing a protective response towards the disease both in APTB and HHC. The co-dominant model was significant at ($p<0.05$ OR-0.55 CI-0.34-0.91); ($P<0.000$ OR- 0.42 CI- 0.26-0.66), dominant at ($p<0.03$ OR-0.61 CI-0.39-0.97); ($P<0.000$ OR-0.42 CI-0.26-0.66) and over dominant at ($p<0.01$(OR-0.57 (0.36-0.91); ($P<0.000$ OR-.0.44 CI- 0.28-0.68) Table 4a. There was no significance for the alleles in APTB while in HHCs both the alleles were significant; T allele was positively associated at $p<0.04$ (OR-2.198 CI-1.016-2.101) whereas C allele was showing a negative association at $p<0.04$ (OR-0.715 CI-0.5197-0.9845) Table 4b.

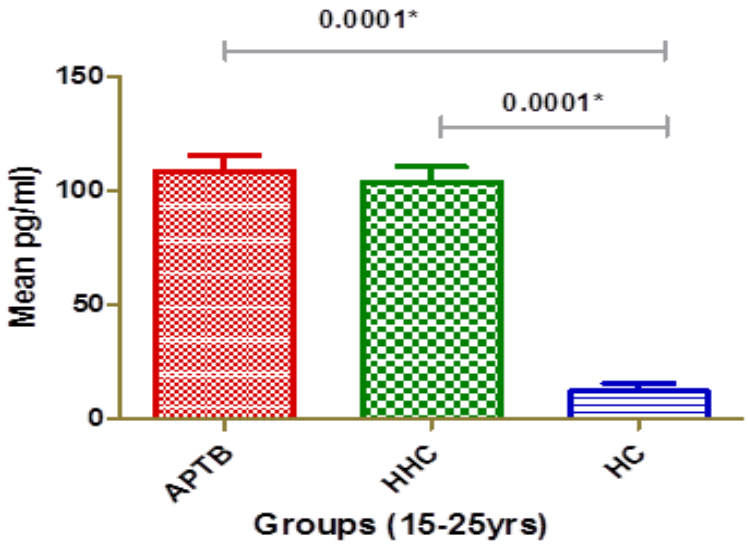


Fig:1 IFN γ serum levels in pulmonary Tuberculosis. APTB-active pulmonary tuberculosis patients, HHC-household contacts; HC-healthy controls, Bars indicate the mean &SD for each group. Horizontal lines indicate a statistically significant difference between groups. Difference between the groups were analyzed by Mann-Whitney U test. *P<0.05 was considered statistically significant.

ELISA of IFN-γ and IL-12 in serum samples

The IFN-γ and IL-12 genes serum levels were estimated by ELISA in 130 APTB, 130 HHC and 100HCs. The serum levels of IFN-γ were highly significant in APTB and HHC compared to HCs and their mean concentrations were 186±6.822 pg/ml, 103.7±6.67 pg/ml and 12.34±3.44 pg/ml at p=0.0001 and p=0.0001. The mean concentration of IL-12 in APTB and HHC were 118.8±14.21 pg/ml & 114.6±14.99 p*g/ml* and were significant at p=0.0001 and p=0.0002 compared to HCs 36.11±9.23 p*g*/ml Fig.1 &2.

The result of ANOVA revealed a statistically significant difference at p<0.05 in the IFN-*γ* and IL-12 levels between the subjects. Post hoc comparisons using the Tukey HSD and Bonferroni test also indicated that there was a significant difference in their mean levels between APTB, HHC and HC.

Pearson correlative analysis was performed to assess the associations between single nucleotide polymorphisms and circulating serum levels of IFN-*γ* and IL-12 and examined whether these variant genotypes were correlated with serum concentrations. The results showed weak correlation between serum and SNP's for both IFN- *γ* and IL12.

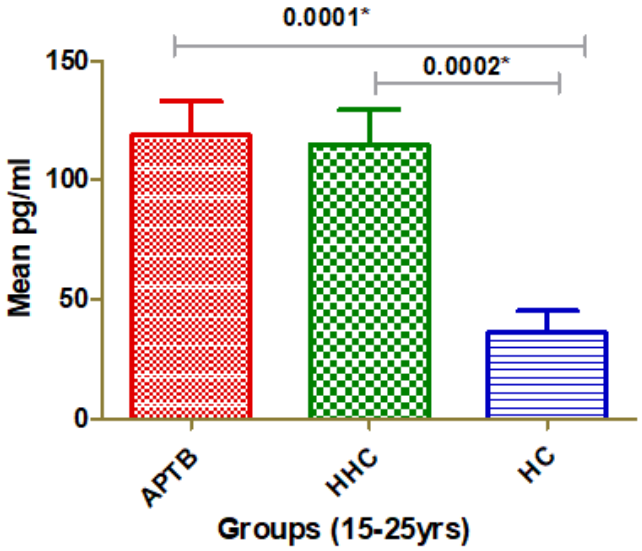


Fig:2 IL-12 serum levels in pulmonary Tuberculosis. APTB-active pulmonary tuberculosis patients, HHC-household contacts; HC-healthy controls. Each bar in the cross-sectional study represents one individual. A horizontal bar with whiskers indicates the mean and SD of each group. Bars above the plots represent the statistical differences between the groups. Difference between the groups were analyzed by Mann-Whitney U test. *P<0.05 was considered statistically significant.

The IL-12 levels showed significant negative correlation with genotype variants in APTB (r= -0.449; p<0.008) and HC (r=-0.374; p<0.04) and insignificant in HHC. IFN-γ serum levels showed insignificant weak positive correlation with the genotype variants.

The result of ANOVA revealed a statistically significant difference at p<0.05 in the IFN-γ and IL-12 levels between the subjects. Post hoc comparisons using the Tukey HSD and Bonferroni test also indicated that there was a significant difference in their mean levels between APTB, HHC and HC.

Pearson correlative analysis was performed to assess the associations between single nucleotide polymorphisms and circulating serum levels of IFN-γ and IL-12 and examined whether these variant genotypes were correlated with serum concentrations. The results showed weak correlation between serum and SNP's for both IFN-γ and IL12. The IL-12 levels showed significant negative correlation with genotype variants in APTB (r= -0.449; p<0.008) and HC (r=-0.374; p<0.04) and insignificant in HHC. IFN- γ serum levels showed insignificant weak positive correlation with the genotype variants.

ELISA of IFN-γ and IL-12 in culture supernatants stimulated with r32kd antigen

The IFN-γ and IL-12 cytokine levels in culture supernatants were measured in a total of 75 cases, APTB (n=25), HHC (n=25) and HC (n=25). The mean stimulated levels of *IFN-γ* were significantly low in APTB (29.93±4.94 pg/ml) and HHC (30.04±5.35 pg/ml) compared to HC (67.00±8.76 pg/ml) at p=0.0003 & 0.0006. While the *IL-12* cytokine levels were high in APTB (77.14±27.12 pg/ml) and HHC (75.19±8.87 pg/ml) compared to HCs (66.70±19.35 pg/ml) and were significant at p=0.0001 & 0.0002 Fig.3 &4.

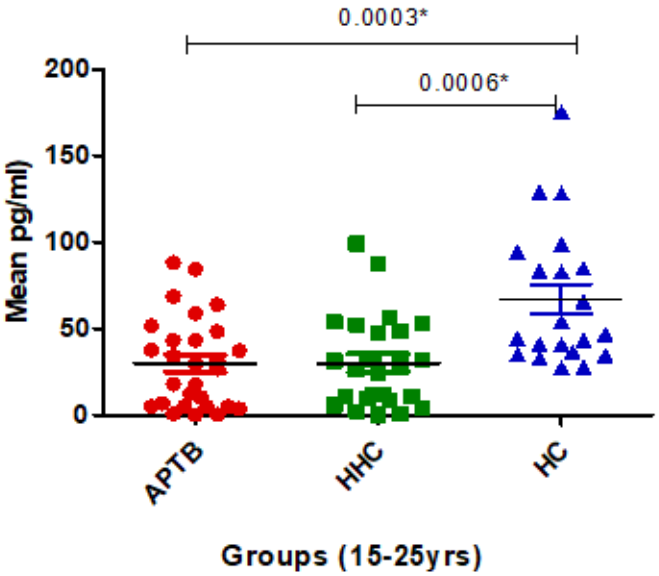


Fig: 3 IFN-γ Level in culture supernatants stimulated with r32KdaM.bovis BCG. APTB-active pulmonary tuberculosis patients, HHC-household contacts; HC-healthy controls, Bars indicate the mean &SD for each group. Horizontal lines indicate a statistically significant difference between groups. Differences between the groups were analyzed by Mann-Whitney U test. *P<0.05 was considered statistically significant.

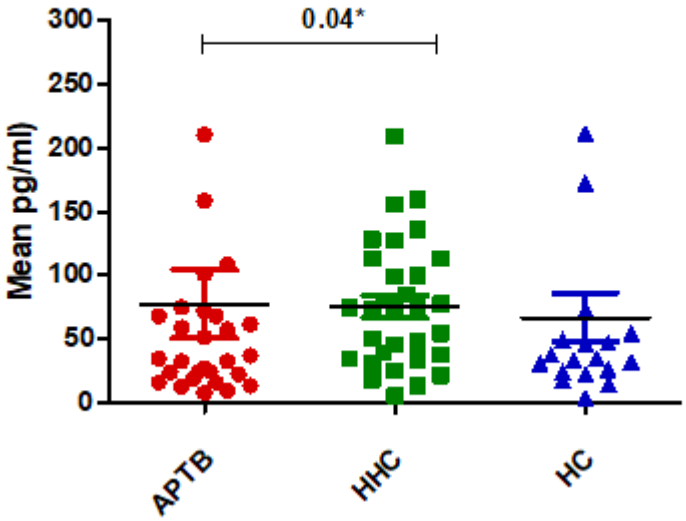


Fig:4 IL-12 level in culture supernatants stimulated with r32Kda *M. bovis* BCG
APTB-active pulmonary tuberculosis patients, HHC-household contacts; HC-healthy controls. Each dot in the cross-sectional study represents one individual. A horizontal bar with whiskers indicates the mean and SD of each group. Bars above the plots represent the statistical differences between the groups. Differences between the groups were analyzed by Mann-Whitney U test. *P<0.05 was considered statistically significant.

The result of ANOVA revealed a statistically significant difference at $p<0.05$ in the IFN-γ levels between the subjects. Post hoc comparisons using the Tukey HSD and Bonferroni test also indicated that there was a significant difference in their mean levels between APTB, HHC and HC. While IL-12 levels did not show any significant difference using ANOVA and Post hoc comparison.

DISCUSSION

IFN-γ producing Th1 cells are essential to control mycobacterial replication [Salgame P et al,2005; Flesch I et al, 1987; Serbina NV et al,2008]. A series of clinical and experimental studies have demonstrated the importance of IFN-γ production in the control of tuberculosis. The loss of function alleles, or their receptors and signaling molecules of cytokines, can cause Mendelian susceptibility to mycobacterial disease [Casanova JL et al, 2002]. We have observed significantly raised serum levels of IFN-γ in APTB, HHC compared to HC. Recently Chin Chung shu have also reported increased serum levels in TB patients at diagnosis than IGRA positive and negative contacts [Chin-Chung Shu et al, 2013]. Also, a study by Muralidhar A Katti observed significantly high IFN-γ levels in patients and contacts compared to controls [Muralidhar Katti et al, 2011]. Contradictory to our study Amal Elhaj et al. have reported no significant difference in the serum levels between patients and controls [Amal Elhaj et al, 2013]. The high levels of IFN-γ in our results indicate Th1 response at the site of the disease. The levels may also vary accordingly based on clinical characteristics. Also, abnormalities in the IFN—γ R may reduce IFN—γ response in the Mtb infected individuals leading to high serum levels. In contacts, the high IFN— γ levels may be due to occurrence of necrosis instead of apoptosis due to infected macrophages, which may further lead to disease progression if not detected, or due to low immune status.

When the production of IFN- *γ* in stimulated cells with different mycobacterial antigens is considered the levels of this cytokine have been found to decrease in the PBMCs in TB patients in several studies. Similarly, in our study we observed decreased levels of *IFN- γ* in culture supernatants stimulated with *r32kd Ag* in APTB and HHC compared to HC. Other reports [Sahiratmadja E et al, 2007; Vankayalapati R et al, 2003; Ji-Sook Lee et al, 2007] also showed decreased levels of *IFN- γ* in patients compared to controls with *M.tb* sonicate antigen, heat-killed *M.tb* Erdman strain and *M.tb* antigens, respectively. While Giuseppe Nunnar [Giuseppe Nunnari et al, 2003] reported no difference in *IFN- γ* levels when stimulated with *PPD* between patients and healthy controls. IrajNikokar [Iraj Nikokar et al, 2006] showed less IFN- *γ* response in patients with cavitary tuberculosis compared to healthy tuberculin positive individuals. IL-12 is an important inducer of IFN-γ and the decreased production of IFN-γ in patients and HHC may be due to immunosuppression caused by sequestration of cells producing this cytokine.

In the present study AT genotype of IFN-γ was significantly associated with resistance to pulmonary TB both in APTB and HHC. Similar studies were reported in Tunisian and Turkish population, respectively [Walid Ben Selma et al, 2011; Sallakci N et al, 2007]. In contrast Vidya rani et al, Haluk O B et al reported no significant difference in genotypes or alleles between patients and controls [Vidyarani et al, 2006; Haluk B Oral et al, 2006]. The correlation between the molecular pathology, mycobacterial virulence, and clinical phenotype in inherited IFNgR1 deficiency suggests that more subtle variation in IFNgR1 could contribute to *M tb* disease susceptibility in an outbred population [Awomoyi A et al, 2004]. In our study we have found that -56C/T is significantly associated with APTB and HHC. The CC and TC were shown to be protective genotypes & T allele was found to be the risk allele. Several studies have shown the association of this genotype with the disease. Similarly, in Sudan population, it was reported that C allele and

CC genotype were protective for the disease. [Attalla M et al, 2011]

IL-12 plays an essential role in *Th1* protective immunity. It forms a key link between activated mononuclear phagocytes and T-cells. *IL-12* induces the production of *IFN-γ*. The present study showed high serum levels of IL-12p40 in APTB patients and HHCs compared to HCs. These results were in concordance with the North Indian population [Abhimanyu et al, 2011]. In contrary to our study Verbonn et al reported no significant difference in the serum levels of active TB patients compared to contacts and controls [Verbon A et al, 1999]. The high IL-12 levels of HHC indicate that they were likely to be at an early stage of infection or exposed to secretory Mtb antigens and may manifest the disease during their lifetime.

Despite being a Th1 cytokine IL-12 have shown high stimulated levels in both APTB and HHC; similar studies were reported with *10.4Ag* in active TB patients compared to tuberculin positive household contacts [Sutherland JS et al, 2010]. Conflicting results were observed by Chang-hwa [Chang-Hwa S et al, 2000] showing depressed production of *IL-12* in patients compared to controls with *30&32kd Ag*. The high IL-12 levels indicate susceptibility to tuberculosis in both APTB and HHC.

The IL-12B gene mutations have been found to cause inherited impairment of immunity to mycobacterial infection. Several studies have reported the association of IL-12B (3'UTR -1188C/A) polymorphism with the risk of TB. In our study, we have found lack of association of IL-12 at -1188 positions between APTB, HHC and HCs. These results were similar with a study reported in South Indian population [Prabhu Anand et al, 2007] and in Whites and African American population with no significant difference between patients and controls [Xin Ma et al, 2003]. On the contrary Tso et al. have found association between TB and specific haplotypes of IL12B gene [Hoi W Tso et al, 2004]. This genotypic difference may be due to environmental variables in different proportions, penetrance, and logic of association studies.

CONCLUSION

In summary, the stimulated and serum levels of IFN-γ in household contacts similar to patients indicate the presence of infection in a latent form. The high serum levels of IL-12 indicate the susceptibility of household contacts towards the disease while high stimulated levels indicate the balance in the cytokines for protective immune response. The genotypes of IFN-γ and IFN-γ receptor identifies the risk genotype and alleles in household contacts associated with the disease.

Acknowledgements

We thank the Late Dr. K.J.R. Murthy and Dr. P.S. Raju and also Staff of the free chest clinic Mahavir PPMDOTS, Tuberculosis Unit (T.U), Research Director, Chairman and Managing Trustees of Bhagwan Mahavir Medical Research Centre.

Authors' contributions

(1) the conception of the study by Late Dr. K.J.R. Murthy and design of the study by GSL & LKC analysis and interpretation of data by LJ & GSL (2) revising it critically for important intellectual content by GSL, LJ (3) final approval of the version to be submitted by GSL.

Conflict of interest

Authors have no conflict of interest.

Source of funding

Financial support was provided by DBT- RGYI (Sanction no: 102/IFD/PR/2029/2007-2008 dated 18/01/2008) and COE (Sanction No: BT/01/COE/07/02, dated 30/12/08).

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