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MUTATIONAL ANALYSIS OF INTERFERON-GAMMA GENE IN INDIAN WOMEN WITH FEMALE GENITAL TUBERCULOSIS

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ABSTRACT

FGTB is usually a silent disease evidencing itself only when really looked for. It usually affects females of reproductive age group. Disruption of the IFN-gamma gene in mice infected with *M. tuberculosis* has resulted in exacerbation of disease, progressive and widespread tissue destruction and necrosis with numerous bacteria. We therefore proposed, to study the possible association of IFN- γ gene polymorphism in Indian women with female genital tuberculosis. It is a prospective case-control study. Screening of genomic DNA samples were carried out from clinically definite 106 FGTB patients and 100 unaffected patients aged between 18 to 40 years. +874 (T→A) IFN- γ genotyping was carried out by using sequence specific primer polymerase chain reaction (SSP-PCR) method. Statistical tests were performed using pantaray software systems.

According to our investigation, FGTB patients showed more or less similar TT (30.18% vs. 30.0%), higher AA (19.81% vs. 9.0%) genotypes compared to controls and the frequency of AT genotypes decreased significantly. Distribution of IFN- γ genotypes between patients and controls were have statistical disparity. This study suggests that IFN- γ +874 T to A polymorphism have an etiological association with susceptibility of female genital tuberculosis.

Keywords: *Mycobacterium tuberculosis*; Interferon- γ gene polymorphism; Female Genital Tuberculosis (FGTB)

INTRODUCTION

Female genital tuberculosis (FGTB) is usually a symptom-less disease diagnosed during investigations for infertility (Namavar Jahromi et al., 2001). It represents 15-20% of extra pulmonary tuberculosis (Rajamaheshwari, 2009). In 80-90% cases, FGTB affects women between 18-38 years of age with menstrual irregularities accounting for nearly 27% of manifestations of FGTB (Chakrabarti et al., 1998). Primary infection may occur when the male partner has active genitor-urinary TB and transmission takes place by sexual intercourse (Richards and Angus, 1998). It is usually a

result of reactivation of a silent bacillema, primarily from lungs and also thought to be from cervical TB infections (Richards and Angus, 1998; Sutherland et al., 1982). The seeding of bacilli usually occurs immediately after puberty as blood supply to the pelvic organs increases and as a result, more bacilli can reach genital organs and infect them (Crofton et al., 1992). Infection of vulva, vagina and cervix may result from direct inoculation and ascending spread to other genital organs may occur (Haas et al., 2002). The incidence of infertility in genital TB worldwide varies from 44-74%; in India it is reported to be 58% (Dam et al., 2006) and majority are in the same age group (Crofton et al., 1992). In western countries the incidence of FGTB is estimated to be <1%, whereas in some

African and Asian countries it reaches 15–19% (Punnonen et al., 1983; Giannacopoulos et al., 1998). Recently, Jindal et al., 2010 have been suggested the use of Endo TB-PCR for high specificity and early diagnosis of female genital tuberculosis as performed by laparoscopy and so laparoscopy can be avoided in TB-PCR-positive patients for diagnosis keeping the PCR-negative cases in mind (Majumdar and Satwik, 2011). Due to its rarity and mild clinical picture, the index of suspicion for the diagnosis of FGTB among gynecologists is usually low. Some times, immune and genetic susceptibility of host may not help in early detection by Endo TB-PCR. Therefore, remains to be an increasing public health concern worldwide. *Mycobacterium tuberculosis* (*M. tuberculosis*) is a facultative intracellular pathogen capable of producing both a progressive disease and an asymptomatic latent infection (Parrish et al., 1998). The role of IFN gamma (IFN- γ) as the main macrophage-activator Th1 cytokine has been clearly established in animal models infected with *M. tuberculosis* by Flynn et al. and Dalton et al., in 1993. In human, single nucleotide polymorphisms (SNPs) located in the first intron of the IFN- γ gene (at position +874) has shown variable associations with disease susceptibility and severity (Pacheco et al., 2008). On other hand, several studies have demonstrated that ethnicity and cytokine polymorphism plays a significant role in the susceptibility to a wide range of diseases (Hoffmann et al., 2002; Newport et al., 1996) including FGTB. IFN- γ modulates a number of functions in addition to MHC expression, including the activation of macrophages, NK cells and the inhibition of the Th₂ phenotype in T cells (Maher et al., 2007). Exactly which function is undermined in +874A individuals have not been determined yet. Pravica *et al.*, 2000 noted a novel single nucleotide polymorphism (SNP), T to A, located at the +874 position from translation start site in the first intron of IFN- γ gene, which coincides with a putative NF- κ B binding site that could play a

fundamental role in the induction of constitutively high IFN- γ production. The differences in the magnitudes of the responses that were seen may reflect the environment in which the cohorts live, or they may reflect the nature of the patients' infections (Cahn et al., 2003). Disruption of the IFN-gamma gene in mice infected with *M. tuberculosis* has resulted in exacerbation of disease, progressive and widespread tissue destruction and necrosis with numerous bacteria (Dalton et al., 1993). Therefore, alteration in IFN- γ production may influence the susceptibility to FGTB and this alteration could be due to gene polymorphism. The homozygous T/T, A/A and heterozygous A/T alleles are associated with any increase or decrease in production of IFN- γ , this cytokine can affect the outcome of the disease severity. We therefore, hypothesized that the IFN- γ +874T/A gene polymorphism might be associated with female genital tuberculosis. This gene was chosen due to its essential central regulator role in response to infection that may be involved in FGTB pathogenesis and their potential regulation on gene expression.

MATERIALS AND METHODS

This is a prospective case-control study, which recruited women visiting the gynecology clinics at two collaborating centers, which register cases from all over the region of Andhra Pradesh, India, complaining for infertility and suspected of having genital tuberculosis (TB) on clinical grounds. During the period of our study (2006–2011), the samples from the consecutive women in these two centers were analyzed. The ethical committee of Hospital and Research Centre approved the research protocol. Informed written consents were obtained from all the participants. Proforma to obtain information on the general, obstetric and gynaecological details including family history, marital status, age at menarche, length of menstrual cycle, associated symptoms, duration and amount of blood loss, duration of infertility, and socio demographic

details like social status, occupation, lifestyle, age, body mass index (BMI), limited information on diet was used and a thorough clinical examination done. General characteristics for all patients were recorded in the medical chart. Apart from routine hematological investigations, specialized investigations consisting of transvaginal sonography of uterus and adnexa, hormone profiles, immunological assays, and endoscopy were performed as and when needed. Surgically removed tissue was taken from both groups for laboratory examination, including AFB using light microscopy following concentration, staining by Ziehl–Neelsen stain as well as culture and *M. tuberculosis* specific PCR (Abebe et al., 2004), the diagnostic criteria by which tuberculosis was confirmed. All patients met the **inclusion criteria**: 18-40 years of age having irregular periods, with past history of having Genital TB and Tubal blockage, experiencing infertility (in >60% of cases), pelvic pain and scanty menstruation and amenorrhoea, and histopathological evidence in biopsy of premenstrual endometrial tissue or demonstration of tubercle bacilli in culture of menstrual blood or endometrial curetting. **Exclusion criteria were as all the following**: Women above 40 years of age, symptoms suggestive of pulmonary TB/Extra pulmonary TB except infertility, with normal abdominal and vaginal examinations, other chronic disease, pregnancy or nursing, severe psychiatric dysfunction, multiple sclerosis or other autoimmune disorders, pulmonary infections, HIV co-infection, women with diabetes, malnutrition and other medical disorders like hypertension were excluded. Details of laparoscopy findings like unilateral or bilateral tubal block with hydrosalpinx, omental adhesions, frozen pelvis, tubo-ovarian masses, tubercular salpingitis and tubercles were noted in **Table 1**. Symptoms included pelvic pain, irregular menstrual bleeding, scanty menstruation, dysmenorrhoea oligomenorrhoea, amenorrhoea and infertility. A pelvic mass in variable combination aroused a

suspicion. Constitutional symptoms such as sweating, increase in temperature and weight loss were not major complaints while local organ dysfunction manifested in amenorrhoea, omental adhesions and bilateral tubal blockage seen on hysterosalpingographic study. The median age of the subjects was 29 (range 18-40) years. All subjects were HIV negative and normal for pulmonary TB on the basis of complete history, physical examinations; chest X-ray, lung plain X-ray and by appropriate tests such as tuberculin test, sputum smears and sputum cultures (Raut et al., 2001; Saracoglu et al 1992). The study population is from the state of Andhra Pradesh, which is known for ethnic variations.

Study group: Tube ovarian biopsy was taken from 106 women during laparoscopy; from 45 women endometrium was obtained by curettage and 61 women with biopsy for smear microscopy, histopathology, culture and PCR for mycobacterium. All these women were infertile: primary infertility in 81 (76.4%) women and secondary infertility in 25 (23.58%) women with mean age of 29.16 ± 3.73 years, mean age at menarche of 12.53 ± 1.01 years, mean duration of infertility of 3.97 ± 2.91 years. Other gynaecological pathology like dysmenorrhoea in 49 (46.2%) women, tubal block with hydrosalpinx in 57 (53.77%) women, omental adhesions in 41 (38.68%) and tubercular salpingitis in 51 (48.1%) were reported. Blood samples were collected in heparinised tubes. The specimens were received and preserved in 10% formalin, processed in routine manner and embedded in paraffin wax. Three-micron thick sections were cut and stained by haematoxylin and eosin (Namavar Jahromi et al., 2001). The diagnosis was undertaken on morphological grounds (Raut et al., 2001). Erythrocyte sedimentation rate (ESR) was performed on all the patients, which showed readings of between 57 and 123. Tissue specimens were examined by a pathologist for granulomatous reactions, fibrosis suggestive of mycobacterium disease.

Control group: Out of one hundred women who attend the same clinic for other gynaecological disorders and tubal sterilization, 92 (92%) women were proven fertile. Eight (8%) women were infertile: primary infertility in 7 (7%) women, secondary infertility in 1 (1%) women with other gynaecological pathology (3 polycystic ovaries, 1 idiopathic infertility, 4 pelvic inflammatory disease) and were laparoscopically confirmed to be without female genital tuberculosis. All the women in

this group were asymptomatic with mean age of 26.11 ± 4.57 years, mean age at menarche of 12.03 ± 0.84 years and mean duration of infertility of 0.174 ± 0.184 years. The following symptoms were also present in the control group: abdominal pelvic pain was observed in 2 (2%) women, dysmenorrhoea in 25 (25 %), oligomenorrhoea in 3 (3%), there were 2 (2%) mild menorrhagia, and 1 (1%) general malaise cases. There were no severe cases as shown in the **Table 1**.

Table I. Comparison of Socio-Demographic and Clinical details among Female Genital TB cases and Controls

Characteristics	Female Genital TB Cases (n=106)	Control group (n=100)
Age (years)	29.16 ± 3.73	26.11 ± 4.57
Body mass index (kg/m ²)	24.34 ± 1.46	23.86 ± 1.85
Age at menarche (years)	12.53 ± 1.01	12.03 ± 0.84
Infertility Symptoms		
Duration of infertility (years)	3.97 ± 2.91	0.174 ± 0.184
Primary infertility [n (%)]	81 (76.4)	7 (7)
Secondary infertility [n (%)]	25 (23.58)	1 (1)
Proven fertility [n (%)]	NA	92 (92)
Menstrual irregularity		
Dysmenorrhoea [n (%)]	49 (46.2)	25 (25)
Mild [n (%)]	28 (26.4)	17 (17)
Moderate [n (%)]	11 (10.37)	6 (6)
Severe [n (%)]	9 (8.5)	2 (2)
No dyspareunia & dysmenorrhoea [n (%)]	27 (25.47)	48 (48)
Amenorrhea [n (%)]	7 (6.6)	1 (1)
Menorrhagia [n (%)]	4 (3.77)	2 (2)
Oligomenorrhea [n (%)]	13 (12.26)	3 (3)
Abdominal pain [n (%)]	15 (14.15)	2 (2)
General malaise [n (%)]	5 (4.7)	1 (1)
Laparoscopy findings		
Tubal block with Hydrosalpinx [n (%)]	57 (53.77)	NA
Omental Adhesions [n (%)]	41 (38.68)	NA
Frozen pelvis [n (%)]	27 (25.47)	NA
Tubo-ovarian mass [n (%)]	23 (21.7)	NA
Tubercular salpingitis [n (%)]	51 (48.1)	NA

Note: Some patients had more than one abnormal finding

Data are presented as mean \pm SD, NA: not applicable

Culture

Homogenized samples were cultured on Lowenstein Jensen egg medium for acid-fast bacilli and incubated for 3 to 8 weeks. Ziehl-Neelsen staining was used to identify the bacilli (Abebe et al., 2004).

DNA Preparation

Five ml of whole blood from patients and controls were used for DNA extraction by a modified proteinase-K/salting-out method (Miller et al., 1988). Polymorphism at position +874 of IFN- γ gene was identified using sequence specific polymerase chain reaction (PCR-SSP) as described by Pravica with some modifications (Pravica *et al.*, 2000). Briefly, red blood cells were lysed using cold Lysis buffer-I (0.3 M sucrose, 10mM Tris-HCl (pH: 7.4), 5 mM MgCl₂, 1% Triton x-100). The pellet was washed with phosphate buffer saline (PBS) once. To the pellet 3 ml Lysis buffer-II (10mM Tris-HCl, 400mM NaCl, 2mM Na₂-EDTA), 200 μ l of 10% SDS and 40 μ l proteinase-K were added and incubated in 37°C overnight. To remove proteins, 1 ml of 6M NaCl was added and centrifuged for 5 min at 1500g. For extraction of DNA, 2 volumes of absolute ethanol were added to the supernatant. The extracted DNA was washed twice in 70% ethanol, dried at 37°C, and recovered in sterile water. Extracted DNA was stored at -20°C until utilization.

PCR-SSP Amplification

IFN- γ polymorphism at position +874 in the first intron (T versus A) was determined by

sequence-specific primer-PCR (PCR-SSP) according to manufacturer's recommendations (QPS Bioserve India (P) Ltd, Hyderabad, India). Briefly, the PCR was performed in a final volume of 50 μ l with 100-200ng of isolated genomic DNA as template in reaction mixture containing 200 μ M (each) dNTPs and 0.5 U Taq DNA polymerase, 1X reaction buffer (Bangalore Genie, Bangalore, India), 3.5mM MgCl₂ (GENETIX, New Delhi, India), 0.5 μ M each specific primers (antisense: TCA ACA AAG CTG ATA CTC CA; sense +874 T: TTC TTA CAA CAC AAA ATC AAA TCT; or sense +874A: TTC TTA CAA CAC AAA ATC AAA TCA), and 0.2 μ M of each internal control primers (QPS Bioserve India (P) Ltd, Hyderabad, India). Internal control primers amplify a human β -globin sequence (forward primer: ACA CAA CTG TGT TCA CTA GC; reverse primer: CAA CTT CAT CCA CGT TCA CC). PCR amplification was performed using a touch down method that included initial denaturation at 95°C for 5 minutes followed by two loops; loop 1 which consisted of 10 cycles with the following program: 95°C for 30 seconds, 62°C for 50 seconds, and 72°C for 40 seconds and loop 2 included 20 cycles with the following program: 95°C for 30 seconds, 56°C for 50 seconds and 72°C for 40 seconds and a final extension step at 72°C for 5 minute. The amplified products were run on 1% agarose gel that was in a buffer containing 0.5 μ g/ml ethidium bromide (**Figure-1**). Later it was visualized under UV light and photographs were documented.

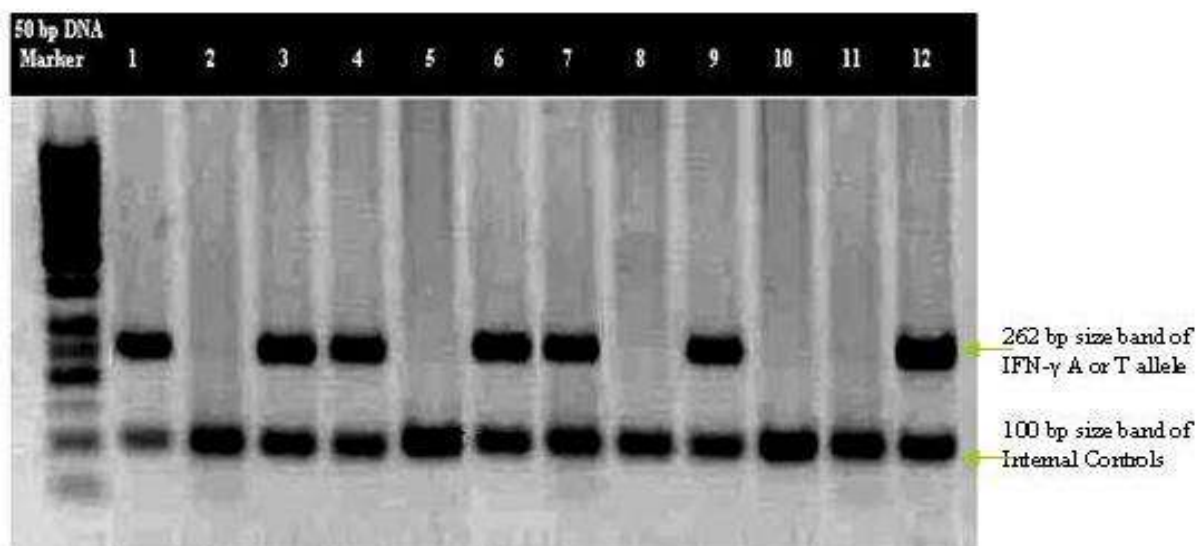


Figure-I: PCR-SSP Amplified product of IFN- γ (+874 T \rightarrow A) gene SNP from FG TB patients were electrophoreses on 1% agarose gel; 262 bp size bands correspond to IFN- γ A or T allele and the 100 bp size bands correspond to internal controls.

50bp DNA marker is loaded in first well; lanes 1 and 2 show homozygosity for T allele; lanes 3 and 4 show heterozygosity for A and T alleles; lanes 5 and 6 show homozygosity for A allele; lanes 7, 8, 9 and 10 show homozygosity for T allele; lanes 11 and 12 show homozygosity for A allele.

STATISTICAL ANALYSIS

Statistical analysis was performed using pantaray software systems (Uitenbroek and Daan, 1997). Comparison of age, menarche age, body mass index, duration of infertility in the study groups and control group was performed using the independent two-sample Student's t test and data are presented as mean \pm SD. The odds ratio (OR) and p-values were used to measure the strength of the association between genotypes and female genital tuberculosis. Hardy– Weinberg equilibrium (HWE) analysis was performed to compare genotypes frequencies between patients and controls by using χ^2 analysis (df=1). All odds ratios (OR) were calculated as estimates of the confidence intervals (CI) were calculated at the 95% level (95% CI). p -value <0.05 is considered significant.

RESULTS

A total of 206 women were enrolled in the study. Symptoms are found mild and local,

such as abdominal pain or menstrual irregularities, tubal blockage, tubercular salphingitis and infertility are the most common consequences (Namavar Jahromi et al., 2001), clinical signs of the FG TB patients (case group) versus control groups were given in the **Table 1**. Once fibrosis is established, fertility is generally difficult to restore even with appropriate treatment (Lamba et al., 2002). Therefore, this prospective large case-control cohort study was commenced for the SNPs of IFN- γ +874T/A in Indian women with FG TB (n=106) for the first time along with 100 controls. The 106 (51.45%) of FG TB patients were confirmed to have the evidence of *M. tuberculosis* infection by either AFB smear microscopy, or positive culture, or histopathology, or PCR or a combination of these (Abebe et al., 2004). 93.44% (57/61) from biopsy specimens and 68.89% (31/45) of positive cases from the curettage specimens were identified by PCR for mycobacterium as shown in **Table-II**.

Table-II: Comparison of the diagnosis yield of female Genital TB by different methods				
Type of Specimens	Smear Microscopy	Culture	Histopathology	PCR for mycobacterium
Case (n=106)				
Curettage (n=45)	0	3	11	31
Biopsy (n=61)	19	32	42	57
Total	19 (17.92%)	35 (33.01%)	52 (49.05%)	88 (83.01%)
Control (n=100)				
Curettage (n=37)	Not detected			
Biopsy (n=63)	Not detected			

The IFN- gamma (+874) genotypes and allele frequencies of all FGTB patients and controls are shown in **Table-III**. Distribution of the genotypes in all groups was consistent with the Hardy-Weinberg equilibrium. The *IFN-γ* +874AA genotype was overrepresented in FGTB patients 19.81% when compared with the controls (9.0%) (Raut et al., 2001). Most of the FGTB patients and controls showed TA genotype (50.0% and 61.0% respectively) which is associated with intermediate IFN-γ

production. However, FGTB patients also showed slight increase in frequency of TT (30.18% versus 30.0%) in comparison with controls. An increasing number of studies have shown that single nucleotide polymorphisms (SNPs) located in the promoter or coding regions of cytokine genes result in differential cytokine secretion due to altered transcriptional activation. It may be possible that different stimuli result in differential transcription of the same gene (Henao et al., 2006).

Table III: 2x2 Contingency analysis of IFN gamma (+874) genotypes and allele frequencies distribution in Female Genital TB and Healthy Controls ^a							
IFN-γ genotype/Alleles	AA	AT	TT	TT+AT	AA+AT	874A	874T
Case (%) n=106	21 (19.81)	53 (50)	32 (30.18)	85 (80.18)	74 (69.81)	37 (34.91)	42 (37.73)
Control (%) n=100	9 (9.0)	61 (61.0)	30 (30.0)	91 (91.0)	70 (70.0)	35 (35.00)	46 (46.00)
P^b value	NA	0.0219	0.0943	0.0279	0.0625	NA	0.645
X² value		5.254	2.798	4.834	3.469		0.212
OR (95%CI)		2.685(1.132-6.367)	2.187(0.866-5.523)	2.498(1.0839-5.7574)	2.207(0.9467-5.146)		0.8636 (0.463-1.611)

Note:

^a Values are given as number (percentage) unless otherwise indicated.

The P^b value was evaluated by χ^2 test with a 2 x 2 contingency table (genotypes) and a 2 x 2 table (allele frequencies) versus control women. ^b- Significance set at $P \leq 0.05$.

Analysis of our results showed that there was significant association in IFN-γ genotypes (AA vs. AT) between FGTB patients and control women (p value=0.0219, χ^2

value=5.254, OR=2.685, 95% of CI=1.132-6.367). i.e., individuals with *IFN-γ* +874 AA and (TT+AT) genotype had chi- squared value equals to 4.834, p value equals to 0.0279 and

OR (95%CI) =2.498 (1.084 -5.757). No significant differences was established in IFN- γ +874AA vs. TT and AA vs. (AA+ AT) genotype frequencies between the case-control groups (p value=0.0943, X^2 =2.798, OR (95%CI) =2.187 (0.866-5.523) and p value=0.0625, X^2 =3.469, OR (95%CI) = 2.207 (0.946-5.146) respectively). Statistical analysis using risk of A and T alleles frequencies of FGTB patients with controls demonstrates no association with susceptibility to FGTB per se (p value=0.645). Our results are in the line with recent studies which have been reported an association between the +874 A/T SNP in the first intron of the IFN- γ gene and pulmonary TB, (Rossouw et al., 2003) suggesting that the TT genotype which is associated with lower IFN- γ production confers susceptibility to FGTB. Therefore, higher levels of IFN- γ can cause more effective cell-mediated immunity against mycobacterium. A single nucleotide polymorphism (SNP), T to A, located at position +874 in the first intron could influence IFN- γ production levels. The association of different genotypes at this position, with a low (AA), medium (AT) and high (TT) cytokine production has been shown in vitro (Lopez-Maderuelo et al., 2003). This is the first study investigating the genetic association of polymorphisms in the +874 IFN- γ gene with FGTB patients using SSP-PCR.

DISCUSSION

Female genital tuberculosis is an important cause of infertility, rarely diagnosed in developed countries. It often has low-grade symptoms with very few specific complaints have been explained in the **Methods and Materials**. IFN- γ is required for host defense against a broad range of pathogens and is especially critical for mycobacterial immunity. Lack of production and mutations in the cytokine gene (Cooper et al., 1993) is associated with the most lethal forms of infections and increase the susceptibility to

develop the disease. IFN- γ +874A allele has been previously reported to be associated with infectious diseases such as tuberculosis, hepatitis B virus infection, and parvovirus infection, (Tso et al., 2005; Ben-Ari et al., 2003) revealing its potential role in host defense against microbial infections. The mechanism by which the IFN- γ +874T/A allele influences the susceptibility to FGTB may depend on its role in the regulation of IFN- γ production. The T allele of IFN- γ +874A/T provides a binding site for the transcription nuclear factor- κ B (NF- κ B), which is able to regulate IFN- γ expression (Pravica et al., 2000). In particular, the +874A polymorphism in the gene for IFN- γ results in decreased IFN- γ expression (relative to the +874T variant) and has been associated with susceptibility to TB in some but not all studies (Moran et al., 2007; Vidyarani et al., 2006). Heterozygous carriers have an intermediate phenotype, suggesting that more subtle variation in the IFN- γ response pathway may underlie susceptibility to TB in outbreed human populations (Levin et al., 1995). Our observation shows that the individuals with IFN- γ +874 AA genotype is more prevalent in patients with FGTB associated clinical findings like tubal block with hydrosalpinx, tubercular salpingitis and Omental adhesions. These were not distinguished in pulmonary TB and controls as well. This nature of disease may be due to genetic variations in the bacterial strain and extraordinary virulent nature of mycobacterium. Depending upon the geographic locality and ethnicity of a population, variations have been reported in various studies regarding the occurrence and frequency of extrapulmonary tuberculosis (EPTB) in the two sexes, in different age groups and the organs involved (Kadivar et al., 2007; Sreeramareddy et al., 2008). The differences in transmissibility and virulence among *M. tuberculosis* strains are related to the genetic background and different lineages with specific geographical regions of the organisms (Caminero et al., 2001; Gagneux

and Small, 2007). Animal infection models suggests haematogenous dissemination of infection occurs before the onset of T-cell mediated immunity (Chackerian et al., 2002) and supports the hypothesis that the ability of different strains of *M. tuberculosis* to produce different clinical phenotypes varies dependent upon their interaction with the host innate immune response, but the relevance of these findings to human disease remains uncertain (Manca et al., 2005). Nonetheless, it is possible that more common genetic variants such as promoter region polymorphisms that influence gene expression are associated with the disease. Therefore we suggests that, the *IFN-γ* +874 (A/T) alleles polymorphism were significantly associated with tuberculous bacillus infectivity and likely plays role as a genetic risk factor for the pathogenesis of FG TB in Indian women. It is also possible that low *IFN-γ* production may impair antimycobacterial response against FG TB infection, rendering these individuals more susceptible to Tuberculous bacillus infection other than pulmonary TB (Vidharani et al., 2006). Although *IFN-γ* can overcome these phenomena in vitro, *M. tuberculosis* can interfere with *IFN-γ* signaling and down regulate the transcription of *IFN-γ* inducible genes (Ting et al., 1999). Further, we also suggested that asymptomatic nature of the disease, accessibility of reproductive clinics, and elucidation of genes associated with virulence, pressure of susceptible factors, detection of intraspecies differences in genome sequences and gene expression studies should not be neglected during the description of FG TB.

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