CHAPTER 1

INTRODUCTION AND REVIEW LITERATURE
1.1 Tuberculosis and worldwide distribution

Tuberculosis (TB) is an infectious disease, caused by the *Mycobacterium tuberculosis* (*M. tb*) which is transmitted through inhalation. TB remains a chief challenge to global public health in the 21st century (WHO, 2015). TB is emerging as a significant health problem with 10.4 million incident TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children (WHO, 2016). India alone estimated one quarter (26%) of the worldwide TB incidence (Rao V G et al., 2012). Annually, around 1.7 million people die due to TB (WHO, 2017). Figure 1.1 and 1.2 shows the worldwide and Indian prevalence in year 2015. TB can affect any organs, tissue and therefore called as a multisystem disease excluding only the hairs and nails (Kulchavenya E, 2014).

![Figure 1.1 Worldwide TB prevalence](https://www.tbfacts.org/mdr-tb/)

**(Adopted from https://www.tbfacts.org/mdr-tb/)**
India has highest burden countries of pulmonary TB

(Adopted from: http://facts.net/tuberculosis/)

1.2 Extra pulmonary Tuberculosis (EPTB) & Female Genital TB (FGTB)

Apart from most infectious pulmonary TB, EPTB is also increasing day by day throughout the world (Sharma J B et al., 2015; Kumar Set al., 2015). TB exists in two forms; PTB and EPTB (Lee J Y et al., 2015). PTB predominately presents with pulmonary disease, although EPTB is also not uncommon (Aka N et al., 1997; Gatongi et al., 2005). FGTB is one of the forms of EPTB. FGTB shows different prevalence rate in different regions of India (Ara K C et al., 2016).
1.3 Prevalence of EPTB & FGTB

The prevalence of genital tuberculosis worldwide has been increased from 22 million to 1.86 billion (Khanna Aet al., 2011). Various epidemiological surveys reported the prevalence of FGTB from less than 1% to over than 20% in different countries (Melanat M et al., 2004; Avan B I et al., 2001). FGTB is now a major global health problem mostly in developing countries including India and it has also been declared as “public health emergency” by World Health Organization (WHO) in 1993 (Vithalani N et al., 1982; WHO, 2017). Due to lack of report available in literatures the prevalence of FGTB is as much higher as one might imagine and majorly it account as significant case with female infertility (Namavan B B et al., 2001; Shahzad S et al., 2012). The worldwide incidence of infertility in FGTB varies from 10 to 85 % with 58% endemic in India Figure 1.3 (mani R et al., 2003; Tripathi S M et al., 2002).

![Image](https://www.sciencedirect.com/science/article)

Figure 1.3 Prevalence of EPTB in India

(Adopted from: Indiahttps://www.sciencedirect.com/science/article/)
1.4 Pathogenesis of FGTB

The pathogenesis of the disease is based on the interaction between the bacilli and the host. In humans \textit{M.\textit{t}b} is a causating agent of infection. Infection in the body starts either from direct bacterial invasion to any organ called as primary infection or secondary products may be generated by mycobacterium (Kennedy D H et al., 1989). Most of the cases (80\%) presenting pulmonary disease as active TB with concurrence EPTB as secondary infection although in others EPTB represents as primary infection (Padberg J et al., 2015). Sometimes tubercles remain dormant in latent phase and reactivated further when body immunity is compressed. The numbers of factors are responsible for the difference in infectivity like, the type of mycobacterium species, size and infectivity of the strain (Alan J W et al., 2006). Pulmonary infection mainly occurs via respiratory tract through inhalation of aerosol, which contains a number of viable \textit{M.\textit{t}b}. These viable \textit{M.\textit{t}b} are disseminates its path through blood stream to different organ and remain dormant in latent foci (Kurman R J et al., 2002). These latent foci break down and further infection or reactivation of TB occurs as consequences of decreased cellular immunity.

1.5 Presentation and symptoms of disease (Organs affected in FGTB)

Individual affected by disease mainly below 40 yrs in reproductive age group within 21-30 year. It is estimated that at least 11\% of the patients lack symptoms and its often detected diagnostic workup (Vijaya N et al., 2005). High index of suspicion is required for early diagnosis due to asymptomatic presentation of FGTB disease (Neonakis I K et
The typical presentation of FGTB includes pelvic pain, menstrual irregularity, general malaise and most common is infertility with the pelvic adhesion and perihepatic adhesion i.e., Fitz Hugh Curtis Syndrome (FHCS) (Sharma J B et al., 2007 and 2008). Genital organs which most damaged during *M.tb* infection are fallopian tubes in almost 90% followed by the endometrium in 50-80%, ovaries in 20-30%, cervix in 5-10% and vagina and vulva in <1% (Sharma J B et al., 2008). Short and long term sequale of *M.tb* causes atrophy of endometrium causes Asherman syndrome and shrunken uterine leads to infertility.

1.5. i) Mode of spread

- **Hematogenous:** From a primary site, 90% of the spread occurs in this route. If this coincides with the growth spurt of pelvic vasculature the reproductive organs (mostly the fallopian tubes) get affected.

- **Lymphatic/Direct:** Direct involvement of pelvic organs or through the lymphatic's from the infected organs (peritoneum, bowel and mesenteric nodes)

- **Ascending:** Sexual transmission from a male with urogenital tuberculosis causes vulval, vaginal or cervical lesion.
1.6 Diagnosis of FGTB

The diagnosis of FGTB needs number of test with an appropriate sampling premenstrual Endometrial Aspiration (EA) of tissue is the best sample used for detection of FGTB by using conventional and molecular test (Bhanothu V et al., 2014). AFB, LJ, MGIT culture are the most commonly used as gold standards. It’s universally agreed that rate of positivity in AFB is very rare or sometimes it 1-2% in 100 due to minimal bacterial load. Culture method (LJ and MGIT) gives a definitive diagnosis of tuberculosis, but it’s very time consuming due to very slow growth of M.tb (Gautam I et al., 2016). Molecular methods used for early detection of M.tb like Polymerase Chain Reaction (PCR) is highly specific and sensitive as compared to conventional methods. False positivity is
the major problem with this molecular method. As Laparoscopy is an invasive procedure although it is the most reliable tool to diagnosed FGTB as it showed high sensitivity, specificity and negative predictive value, when compared to PCR (Sharma J B et al., 2008). The WHO banned the usage of any serological test in individual suspected of any form of TB. Due to non-specific clinical and laboratory findings the diagnosis of genital TB is often difficult and frequently delayed (Goldin A G et al., 1985; Schaefer G et al., 1970). In genital TB transmission of disease could be possible through its male partner to female and vice versa. Early diagnosis may be associated with a more favorable result before extensive genital damage occurs (Ramachandran R et al., 2003).

**Table 1.1 Frequency of symptoms associated with FGTB**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Asymptomatic</td>
<td>11%</td>
</tr>
<tr>
<td>Infertility</td>
<td>45%-55%</td>
</tr>
<tr>
<td>Pelvic pain</td>
<td>50%</td>
</tr>
<tr>
<td>Poor general conditions</td>
<td>26%</td>
</tr>
<tr>
<td>Menstrual irregularities</td>
<td>20%</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>04%</td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td>3.8%</td>
</tr>
<tr>
<td>Ulcer on vulva</td>
<td>0.2%</td>
</tr>
</tbody>
</table>
1.7 Resources and importance of Vitamin D in human body

Malnutrition has always been playing a very important role for all infectious diseases including TB (Kumar V et al., 2007; Lawn S D et al., 2011; Hood M L H et al., 2013). Along with malnutrition vitamin deficiencies are the biggest challenges in the developing countries where burden of TB is very high (Chakraborty S et al., 2014). Vitamins are bio-molecules which maintain body’s physiology and responsible for a spectrum of vital functions they boost up the protective immune system in the body due to their anti-oxidant, pro-oxidant, anti-inflammatory role. A number of vitamins are well known like vitamin A, C, B, E, D etc. (Brambilla D et al. 2008; Bowayed J et al., 2010; Ciccone M M et al., 2013; Wahlqwist M I et al., 2013)

Figure 1.5 Resources of vitamin D
(Adopted from: https://www.youtube.com/watch?v=cV0FhKANIdg)
A well known role of vitamin D is studied in bone mineral density (BMD) and calcium homeostasis in the body (Aranow C et al., 2011). The significance of vitamin D has been escalating in recent year because of its role in the response of immune system against \textit{M.tb} which can work as important predictor of tuberculosis (Martineau A R et al., 2007). Vitamin D modulated macrophages functions which further activate human anti-mycobacterial activity (Chan T Y K et al., 2000).

**Figure 1.6 Signs & causes of vitamin D deficiency**

*(Adopted from:https://www.inlifehealthcare.com/2015/12/17/vitamin-d-deficiency-causes-symptoms-diseases/)*

Figure 1.6 shows vitamin D deficiency presents with number of symptoms. Deficiency of vitamin D (25-hydroxycholecalciferol) has long been implicated in activation of
tuberculosis (Talat N et al., 2010). Vitamin D serum level is also lower in TB patients than in healthy controls (Davies P D et al., 1985). Inconsistently, prolonged treatment of TB also causes a turn down in serum vitamin D levels (Nnoaham K E et al., 2008). Activation of monocyte /macrophage occurs through vitamin D receptor and it is one of the few mediators that have shown to impaired the growth of \textit{M.tb} in the macrophages (Selvaraj P et al., 2000).

1.8 How vitamin D exerts its effect

1. 25-dihydroxyvitamin D3, the activated form of vitamin D, is a potent immune modulator (Toubi E et al., 2010). VDR is a nuclear hormone and a member of the super family of steroid receptors (Ohyama Y et al., 2016). Vitamin D receptor (VDR) which is present on human monocytes and activated T and B lymphocytes, interacted with vitamin D which acts like a hormone, by binding a nuclear receptor VDR. The binding of vitamin D with VDR further modulates monocyte-macrophage activity by binding to VDRs, which are responsible for both intracellular replication of \textit{M.tb} and the destruction of \textit{M.tb} by acting as Antigen Presenting Cells (APCs) (Zeng J et al., 2015). Vitamin D & VDR exerts their antimicrobial action through the release of major component i.e., cathelicidin peptides which have antimicrobial as well as anti-endotoxin activity (Dima et al., 2011). Vitamin D can also stimulate the expression of potent antimicrobial peptides, such as cathelicidin and β defensin. Cathelicidin is effective against gram-positive and gram-negative bacteria, fungi and mycobacteria (Bartley J, 2010). Subjects having 25(OH) D optimal level levels less than 20 ng/ ml may be unable to fully express cathelicidin (Jeng L et al., 2009). Due to this monocyte-macrophage
activity associated with human innate immunity to infectious agents like *M.tb*. The vitamin D is now a days in great particular of interest (Singla N et al., 2015).

![Diagram of Vitamin-D exerts its effect through VDR](https://www.sciencedirect.com/science/article/pii/)

**Figure 1.7 Vitamin-D exerts its effect through VDR**

*(Adopted from: https://www.sciencedirect.com/science/article/pii/)*

1.9 Transformation of inactive to active form

Vitamin D is one of the micronutrient derived from some dietary source as well as from dermal ultraviolet B (UVB) radiation (Ralph A P et al., 2013). Mainly, it is monitored by calcidiol, 25-hydroxycholecalciferol 25(OH)D serum level i.e., inactive form of vitamin
D, which reflects also the skin synthesis due to the solar radiation. Liver metabolizes Vitamin D into 25-hydroxyvitamin D3 calcidiol /25(OH) 2D3 i.e., inactive form of vitamin D, which is further hydroxilated by the kidney to the active form 1,25-dihydroxycholecalciferol (1,25[OH]D) or calcitriol (Figure 1.8) (Moroti R et al., 2012). Risk of TB infection & its progression from latent to active TB has been hypothesized to decrease with use of vitamin D sufficiency (Gibney K B et al., 2008; Davies P, 2010). As well as the effectiveness of treatment also improves with supplementation of vitamin D (Ralph A et al., 2008; Martineau A R et al., 2011; Wejse C et al., 2009).

![Figure 1.8 Conversion of inactive to active form of Vitamin D](https://www.sciencedirect.com/science/article/pii/)

(Adopted from: https://www.sciencedirect.com/science/article/pii/)
1.10 Vitamin D level in human

Serum level of 25-hydroxyvitamin D up to or more than 30ng/ml is sufficient for the conversion of its inactive form to bioactive form, which enhances the expression of cathelicidin (antimicrobial peptide). Simultaneously, if levels of 25(OH)D found to be less than 20 ng/ml, patient is immunocompressed. Addition of vitamin D to standard Anti tubercular therapy (ATT) drug regimen results in faster clearance of the infection. Previous evidence suggests that hypovitaminosis D is associated with the susceptibility for cancer, autoimmune disease, diabetes and cardiovascular disease, which indicates the importance of sufficient vitamin D level (Gautam S et al., 2017).

Table 1.2 Vitamin D doses for different age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Recommended Dietary Allowance (RDA) per day</th>
<th>Tolerable Upper Intake Level (UL) per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants 0-6 months</td>
<td>400 IU (10 mcg) *</td>
<td>1000 IU (25 mcg)</td>
</tr>
<tr>
<td>Infants 7-12 months</td>
<td>400 IU (10 mcg) *</td>
<td>1500 IU (38 mcg)</td>
</tr>
<tr>
<td>Children 1-3 years</td>
<td>600 IU (15 mcg)</td>
<td>2500 IU (63 mcg)</td>
</tr>
<tr>
<td>Children 4-8 years</td>
<td>600 IU (15 mcg)</td>
<td>3000 IU (75 mcg)</td>
</tr>
<tr>
<td>Children and Adults 9-70 years</td>
<td>600 IU (15 mcg)</td>
<td>4000 IU (100 mcg)</td>
</tr>
<tr>
<td>Adults &gt; 70 years</td>
<td>800 IU (20 mcg)</td>
<td>4000 IU (100 mcg)</td>
</tr>
<tr>
<td>Pregnancy &amp; Lactation</td>
<td>600 IU (15 mcg)</td>
<td>4000 IU (100 mcg)</td>
</tr>
</tbody>
</table>

*Adequate Intake rather than Recommended Dietary Allowance.

(Adopted from- https://blog.paleohacks.com/the-ultimate-guide-to-vitamin-d/)
1.11 Vitamin D Genetic variants and its association with FGTB

The VDR gene is located on the long arm of the human chromosome number 12 (Alexandra S G et al., 2012). Labuda et al., in 1992 first elucidated the location of VDR gene on physical map of chromosome number 12 and later on its more centromeric (12cen-q12) by Fluorescent In Situ Hybridization (FISH) and radiation hybrid mapping by Taymans et.al in 1999 (Szpirer J et al., 1991; Taymans S E at a., 1999). Sequence variations that frequently occur in the population are referred to as “polymorphism” can have self-effacing and slight but true biological effects. Human population has high frequency of allelic variants in human genome. Due to this most of the research made them a burning target to explain the possible association in any disease with genetic variation (Andre G et al., 2004). Vitamin D receptors are associated with differential susceptibility to TB and other infectious diseases (Chan T Y, 2000).

Various case control studies have been studied with reference to tuberculosis, among which are NRAMP1 (Natural Resistance Associated Macrophage Protein One), HLA-DQB1 (MajorHistocompatibility Complex, Class II, DQ beta-1) and VDR (Attapon C et al., 2009). More than 62 polymorphisms have been reported in VDR gene dispersed throughout the gene, in the 5′ regulatory region of VDR in and around exons 2–9 and in the 3′ Untranslated Region (UTR) (Fang Y et al., 2005). Polymorphisms in the 5′ regulatory region (Cdx2 and A-1012G) are known to influence the transcriptional activity of VDR gene while polymorphisms in the 3′ UTR (BsmI, ApaI, and TaqI) have been shown to be associated with stability of VDR mRNA (Alagarasu K. et al., 2008). The majority of four common variants of VDR gene are FokI, BsmI, ApaI and TaqI.
being most intensively investigated (Selvaraj P et al., 2000; Selvaraj P 2003; Selvaraj P 2004). The polymorphisms identified in the gene cluster of VDR region are (BsmI site, alleles B and b; ApaI site, alleles A and a; TaqI site, alleles T and t; B, A and T are wild type alleles and b, a and t, mutant alleles; FokI site, allele F and f) (Selvaraj P et al., 2000). FokI polymorphism located in the translation initiation codon and affects the protein function of VDR gene by increasing in length into three more amino acids. The transcription of this allele is 1-7 times less efficient than the F allele (Singla N et al., 2015). The ApaI SNP, found in intron 8, results in a T→G change (the T allele is designated ‘A’ while the G allele is designated ‘a’). Because ApaI is intronic, away from the intron-exon boundaries, it is not known to produce splicing errors and, therefore, it is unlikely to have functional consequences (Nejentsev S et al., 2004). The TaqI polymorphism has been shown to be functionally more important is located within exon-9 and a single base change C to T in codon 352 at the 3’ end of the VDR gene leads a silent mutation (Verbeek W et al., 1997). Literature showed less common allele of TaqI site designated as t mutant allele has been associated with higher levels of mRNA expression (Morrison N A et al., 1994). TaqI polymorphism similarly to ApaI is unlikely to alter VDR function. BsmI polymorphism is located in intron 8 of VDR gene. Add protein function of BsmI. Apart from all four polymorphism, FokI SNP is most important in term of disease susceptibility of TB, which can regulate the transcriptional activity of the gene (Cao Y et al., 2015). VDR variant gene and the immune response against TB show vitamin D–related gene-environment interactions in the host response to TB. In this way, vitamin D can modulate immune expression in response to an M.tb
immune challenge a classical intracrine mechanism (Wang T T et al., 2004; Gombart A F et al., 2005). The conclusion from these studies was that individuals with low serum 25(OH)D with polymorphism in VDR gene will be less able induction of antibacterial activity and may therefore be at greater risk of infection (Hewison M, 2012).

Figure 1.9 VDR gene structure with different types of variants

(Adopted from: http://www.indianjurol.com/article.asp?issn=0970-1591;year=2012;volume=28;issue=4;spage=377;epage=381;aulast=Manchanda)

1.12 Combinatorial role of Vitamin D & Toll Like Receptor (TLR) in immune response

Toll-like receptor 2/1 (TLR2/1) are up regulates the vitamin D receptor expression as well as 25-hydroxyvitamin D-1α-hydroxylase by M.tb or other infectious agents (Azam F et al., 2016). TLRs play are directly associated with VDR which is a nuclear receptor
and exerts both first line or innate immune response and adaptive immune response to keep the pathogen inactive during its latent period. The TLR antimicrobial pathway was dependent on the presence of 25-hydroxyvitamin3 (25D3), which was converted in monocytes and macrophages by the CYP27b1-hydroxylase to 1,25D3 (Liu P T et al., 2007).

Figure 1.10 Role of TLR coupled with VDR in immune response
(Adopted from: https://www.researchgate.net/figure/49782143_fig1_Figure-1-Vitamin-D-induced-cathelicidin-expression-and-monocyte-bacterial)
1.13 TLR and *M. tb* with immune response

Innate immunity plays an important role in recognition of *M. tb* by cells and the host defense against *M. tb* (Biyikli O O et al., 2016). TLRs belongs to family of type-I membrane proteins and contain an extracellular domain with leucine-rich repeats whereas its cytoplasmic portion shows very much homology to interleukin (IL)-1 receptor family (Faridgohar M et al., 2017). The recognition of mycobacteria as invading pathogens, followed by activation of innate host defense responses, and the subsequent initiation of adaptive immune responses (Kleinnijenhuis et al., 2010). To protect themselves from the immune response each bacilli develop antagonizing and immune response avoiding mechanisms. TLRs were first described in Drosophila species; later in 1997 human analogues were found to be an important component of natural immunity (Means T K et al., 1999).

1.14 TLR and its members

TLRs are a family of PRRs consisting of 12 members in mammals. Pattern Recognition Receptors (PRRs) consist several classes in the recognition of *M. tb*, including TLRs, C-type Lectin Receptors (CLRs), and Nod-Like Receptors (NLRs) (Kleinnijenhuis et al., 2010. There are some more receptors are also present in human bodyapart from TLRs and PRRs such as NOD2, Dectin-1, Mannose receptor, and DC-SIGN. Eleven TLRs have been described and every TLR has specific ligand binding activity (Biyikli O O et al., 2016). TLR family, TLR1, TLR2, TLR4, TLR6 and TLR9 and possibly TLR8 their adaptor molecules MyD88 play roles in the recognition of *M. tb* and initiation of the
immune response against tuberculosis (Davila S et al., 2008; Means T K et al., 2001; Bafica A et al., 2005). Mostly TLRs are expressed on the surface of the cell membrane or on the membrane of endocytic vesicles of mainly immune cells including dendritic cells (DCs) and macrophages cells.

**Table 1.3 TLRs and their cognate ligands**

<table>
<thead>
<tr>
<th>Toll Like Receptors (TLRs)</th>
<th>Location in Human cell</th>
<th>Type of ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-1</td>
<td>Plasma membrane</td>
<td>Triacylated lipopeptides</td>
</tr>
<tr>
<td>TLR-2</td>
<td>Plasma membrane</td>
<td>Peptidoglycan, Lipoteichoic acid</td>
</tr>
<tr>
<td>TLR-3</td>
<td>Endosome</td>
<td>ssRNA and dsDNA</td>
</tr>
<tr>
<td>TLR-4</td>
<td>Plasma membrane</td>
<td>LPS, LTA, fibronectin, mannan(Candida)</td>
</tr>
<tr>
<td>TLR-5</td>
<td>Plasma membrane</td>
<td>Flagellin</td>
</tr>
<tr>
<td>TLR-6</td>
<td>Plasma membrane</td>
<td>Diacylated lipopeptides, fungal</td>
</tr>
<tr>
<td>TLR-7</td>
<td>Endosome</td>
<td>ssRNA, Imidazoquinoline</td>
</tr>
<tr>
<td>TLR-8</td>
<td>Endosome</td>
<td>ssRNA</td>
</tr>
<tr>
<td>TLR-9</td>
<td>Endosome</td>
<td>Bacterial and viral unmethylated CpG motif</td>
</tr>
</tbody>
</table>


The interaction itself does not lead to immediate ingestion of the mycobacterium (Underhill D M et al., 1999). After the infection of two-to-three week’s interaction of *M. tb* with TLR which leads to T-cell immunity and antigen-specific T lymphocytes reached at infection site, and release proinflammatory cytokines like interferon-γ (IFN-γ) that will further activate macrophages (Crevel R V et al., 2002). In the signaling pathways of TLR and *M. tb* interaction MyD88 plays important as primary response
protein specific for mycobacterium. After the activation of macrophages phagocytosis of \textit{M.tb} takes place, which involved different types of receptors such as the scavenger receptors, the Mannose Receptor (MR), and complement receptors (Weikert L F et al., 1997; Hirsch C S et al., 1994; Schlesinger L S et al., 1990). In our study, we studied on TLR-2 and TLR 8. As in VDR genetic variants found same as TLRs also have some genetic variants.

![Figure 1.11 Different Members of TLRs](https://www.sciencedirect.com/science/article/pii/)

**1.15 TLR-2 and genetic variants**

In human genome TLR-2 gene is located on chromosome 4q32 with two noncoding exons and one coding exon (Aderem A et al., 2000). In macrophages TLR-2 is one which mediated host’s immune response to \textit{M.tb}. (Yoshida A et al., 2009). Most of the bacterial proteins or lipids such as 19 kDa lipoprotein of \textit{M.tb}, lipoarabinomannann and LprG lipoprotein recognized by TLR-2 (Gaustein A et al., 2015; Bukhari M et al., 2015;
Infection caused by certain mycobacterial species such as *M.tb* and *M.leprae* mostly eradicated by TLR2. (Khan A U H et al., 2014). Glycolipids of mycobacterial species, Lipoproteins [LprA, LprG, LpqH (19-kDa lipoprotein) and PhoS1 (38-kDa lipoprotein)] act as agonists of TLR (Drage et al., 2009). TLR-2 forms heterodimers with either TLR-1 or TLR-6. These heterodimers further recognized mycobacterial cell wall specifically glycolipids like LAM, LM, 38-kDa, and 19kD mycobacterial glycoprotein, and Phosphatidylinositol Mannoside (PIM), triacylated (TLR-2/TLR-1), or diacylated (TLR-2/TLR-6) lipoproteins (Jones B W et al., 2001; Uszynski S et al., 2001). TLR2 is believed to be important through stimulatory effects on TNFα production in macrophages and in IL-12 release in macrophages for initiation of innate host defense (Underhill D M et al., 1999; Bafica A et al., 2005). TLR2 was found to play a significant role for the stimulation and release of IL-1β production (Kleinnijenhauis J et al., 2009; Pompei L et al., 2007). More than 175 SNPs for the human TLR-2 have been reported. The expression of TLR-2 on macrophages is important to determine the fate of innate immune responses to *M.tb* (Drage et al., 2009). TLR-2 have two work, first its high expression on macrophages worsen the outer of infection and the another side, it maintains *M.tb* to its dormant stage avoids activation of *M.tb* from latent phase. Many studies have been conducted on various TLR-2 SNPs, often with varying and even contradictory results in different ethnic groups (Schurz H et al., 2015).

The A allele of the TLR-2 rs11938228 polymorphism has been associated with TB disease (allelic and recessive model) in European and Asian populations, but not African
or Hispanic populations while another study in an Asian cohort found no association (Zhang Y et al., 2013; Sanchez D et al., 2012; Xue Y et al., 2010). Molecular biology and immunological studies have resulted in identification of several functional Single Nucleotide Polymorphisms (SNPs) modulating infectivity and differential clinical presentations exist for genes encoding several types of proteins (Azad A K et al., 2012). Out of which, TLR-2 and interferon-γ (IFN-γ) genes are concern challenges due to their role in immune system, reproductive physiology and basic pathology at multiple levels (Bhanothu V et al., 2015). Recent data suggests that SNPs of TLR-2 genes play an important role in susceptibility to TB among different populations and subsequently, in the development of infertility (More S A et al., 2003; Witkin S S et al., 2010; Bansal C et al., 2012).

Figure 1.12 Activation of TLR and phagocytosis of *M.tb*

(Adopted from: https://www.researchgate.net/figure/262055729)
1.16 TLR8 and genetic variants

Immunological role of TLRs with innate host defense started in 1981 with the seminal discovery in insects (Casanova J L et al., 2011). TLR8 (TLR-3, TLR-7, TLR-8, and TLR-9) is one of the intracellular TLR, which directly involved in nucleic acids recognition and were shown to evolve under strong purifying selection. On the basis of genomic structure and sequence similarities TLR-9 forms a subfamily with TLR-7 and TLR-8, but the natural ligands for TLR-7 and TLR-8 are still not known. After a long time, it was now reported that TLR8 activated under specific conditions, which gives up new field of investigation. Gene variants of TLR-8 have overt relevance to viral infection diseases, but few polymorphisms have been reported in the Chinese population (Mihai G N et al., 2012). Till date, not a single publication have been reported which could be stated that on what form of TLR-8 protein is expressed in people and whether this changes the TLR8 gene expression or protein functional level.

A recent genetic study has correlated the presence of a TLR-8 SNP (rs3764880: A>G; Met-Val) with the development of active TB, suggesting a role for TLR-8 in the detection of phagosomal bacteria (Gantier M P et al., 2010). Only with the exceptions are 19 amino acids in the N-terminus, TLR-8 variant 1 and variant 2 (TLR8v1 and TLR8v2) proteins similar. Mostly TLR-8 is expressed in monocytes/macrophages and myeloid dendritic cells. A recent genetic study indicates that polymorphic variations in the TLR-8 gene locus are the only ones to be significantly correlated with active TB development, among 149 polymorphisms across other TLRs and important signaling
adaptor molecules. Up-regulation of TLR-8 mRNA levels in the blood of patients with active TB was also observed (Davila S et al., 2008).

Figure 1.13 TLR 7/8 activation with other member of TLR family

(Adopted from:https://www.frontiersin.org/articles/10.3389/fimmu.2014.00079/)
1.17 **Hypothesis**

Host genetic polymorphism in vitamin D receptor (VDR) & Toll like receptor (TLR) gene may modulate the female genital tuberculosis disease.

1.18 **Aim of the study**

To identify host genetic risk variant, that predisposed to female genital tuberculosis in females presenting as infertility in North India.

1.19 **Objectives of the proposed study**

1. To assess the association of sequence variants in vitamin D receptor (VDR) & Toll like receptor (TLR) genes with female genital tuberculosis, in North Indian infertile women.

2. To assess the impact of host serum vitamin D level on manifestation of probable FGTB susceptibility.

3. To evaluate the possible association of VDR & TLR gene variants, with the serum vitamin D level as genetic risk factor for FGTB patients.