TO STUDY THE PROPORTION AND RISK OF LATENT TUBERCULOSIS IN PATIENTS DIAGNOSED WITH RHEUMATOID BEFORE STARTING DISEASE-CROSS SECTIONAL STUDY

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"TO STUDY THE PROPORTION AND RISK OF LATENT TUBERCULOSIS IN PATIENTS DIAGNOSED WITH RHEUMATOID ARTHRITIS BEFORE STARTING DISEASE-MODIFYING ANTIRHEUMATIC DRUGS (DMARDS):
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<u>ABSTRACT</u>

<u>BACKGROUND AND AIM:</u>

Tuberculosis (TB) is one of the most common infectious killers. According to the 2019 Global TB Report, despite the severe medical measures taken in an effort to control it, the incidence of TB in India is as high as 27 lakhs. Patients with latent tuberculosis have mycobacterium tuberculi infection but no visible signs of active TB. These patients do not exhibit any clinical signs or symptoms of active TB. When compared to the general population, people with rheumatologic diseases who are harbouring LTBI are up to four times more likely to develop active tuberculosis. In addition, it was discovered that individuals receiving biological therapy for underlying rheumatologic disorders had a risk of latent tuberculosis infection reactivation of 2% to 30% per life year (1). Our study will aid in determining the burden of latent TB in rheumatoid arthritis patients. More rigorous screening can be ensured, which will reflect in regulating the seedbed of TB, if it is discovered that the proportion in the study group is higher than the proportion of LTBI in the general population.

METHODS:

Our study involved a cross-sectional analysis of Rheumatoid Arthritis patients who have been treated in the past but have never used biologic medications. The participants were assessed for active TB using an AFB smear (if symptomatic) and a chest X-ray; if active TB was found, the participants were excluded from the study and the appropriate therapy was started. QuantiFERON GOLD (QFT) and TST LTBI tests were performed on study participants. After administering the PPD for 48–72 hours, the TST findings were read.

RESULTS:

From our study we inferred that the proportion of latent tuberculosis infection in patients with rheumatoid arthritis and was 51.5%,This was estimated by positive test results by QFT. Proportion of LTBI by TST only was 39.4% . This was from 17 LTBI positive cases from 33 patients with RA, and 7.7% converted to Fulminant TB.

CONCLUSION:

From our study we conclude that the proportion of latent tuberculosis among the risk population with rheumatologic diseases like rheumatoid arthritis is 51.5% and 7.7% converted to Fulminant TB. In the absence of a gold standard test to diagnose latent tuberculosis infection we would recommend testing by tuberculin skin test and IGRA to avoid not diagnosing LTBI which have potential to reactivate to activeTB disease in people with altered immune status like our study population.

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ABBREVATIONS

TB – Tuberculosis

MTB – Mycobacterium tuberculosis

LTBI – Latent tuberculosis infectionRA

– Rheumatoid Arthritis

AS – Ankylosing Spondylitis

DMRDS – Disease modifying anti rheumatic drugs

bDMARDS – Biological disease modifying anti rheumatic drugs

csDMARDS – Conventional synthetic disease modifying anti rheumatic drugsTST –

Tuberculin skin test

QFT – QuantiFERON gold test.

IGRA – Interferon gamma release assay

RIF – Rifampicin

WHO – World health organisation

HIV – Human immune deficiency virusCD

– Cluster of differentiation

MHC – Major histocompatibility complexNK

cells – Natural killer cells

Man LAM - Mannose capped lipoarabinomannanINF

– G – Interferon Gamma

IL – Interleukin

MIP – Macrophage inflammatory protein MCP –

Monocyte chemo attractant protein

TNF – Tumor necrosis factor

TLR – Toll like receptor

TNF R – Tumor necrosis factor receptor

BCG – Bacillus Calmette Guerin

NTM – Non tuberculosis mycobacterium

NOS – Nitrogen oxide synthase

EPTB – Extra pulmonary tuberculosis

PPD – Purified protein derivative

TU – Tuberculin units

ELISA – Enzyme linked immune sorbent assay

ESAT 6 – Early secretory antigenic target

CFP 10 – Culture filtrate antigen PCR –

Polymerase chain reaction

NSAIDS – Non steroidal anti-inflammatory drugs

HLA – Human leukocyte antigen

BMI – Body mass index

SD – Standard deviationCI

– Confidence interval

Introduction

Despite decades of efforts to control tuberculosis (TB), India has the greatest global burden of tuberculosis (TB), and it continues to spread [1,2]. The results of an infection with Mtb can have one of three distinct outcomes depending on how the environment, host, and pathogen-specific variables interact. cure, latency or active disease. Although much remains unknown about the pathophysiology of latent tuberculosis, it is defined by immunologic evidence of mycobacterium tuberculosis infection in a continuum between self-cure and asymptomatic, to active tuberculosis (TB) diseases. The virulence of the strain, the extent of exposure to the index case, the amount of bacterial inoculum, and various host features including age and co-morbidities all have a role in where one stands on the continuum [3].TB remains among the topmost cause of infectious disease related deaths. Latent tuberculosis infection serves as a reservoir for the spread of mycobacterium tuberculosis, which in turn allows the disease cycle to continue on a population-wide scale [3–5]. When compared to the general population, people with rheumatologic illnesses who are harboring LTBI are up to four times more likely to develop active tuberculosis. It was also discovered that individuals receiving biological therapy for underlying rheumatologic disorders had a risk of latent tuberculosis infection reactivation of up to 25%[6,7]. Patients with latent TB disease are infected with Mtb but do not show symptoms clinically.

LTBI patients possess no signs of active TB. Therefore, screening with TST or QFTs is recommended prior to beginning treatment that includes steroidsand other biological agents that will change patient's immunological status. The patient may be more susceptible to developing an active tuberculosis infection due to the patient's Weakened immune system[5,7,8].

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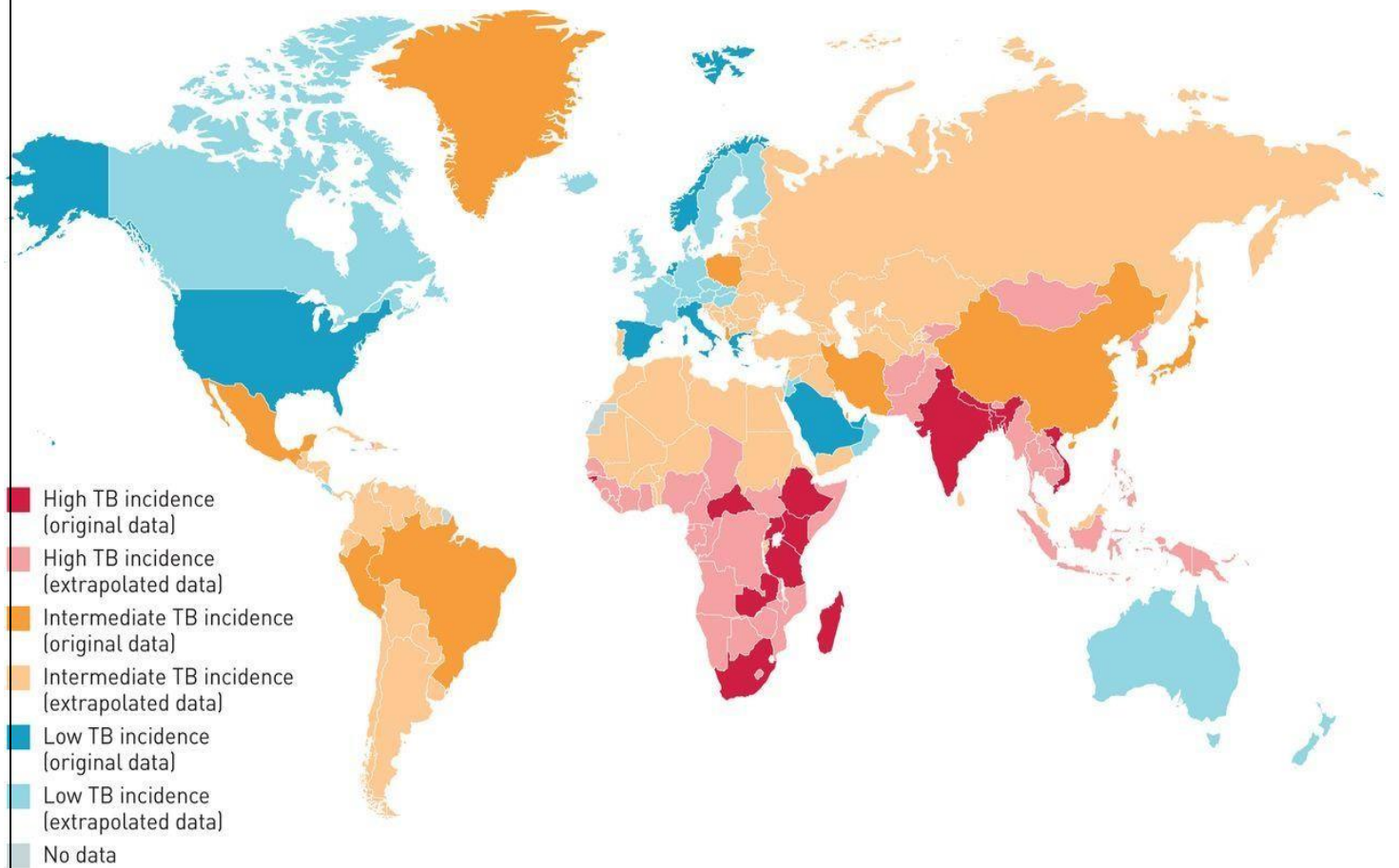
Figure 1 : Global prevalence of latent TB infection[9].

Our research shall aid in determining proportion of latent TB in rheumatoid arthritis subjects. If the proportion in the study population is found to be more than the proportion of LTBI in general population, increasing the screening process may help. More importantly, to avoid reactivation, patients needing steroid and immunosuppressive medication should be given LTBI prophylaxis. In order to determine the proportion of LTBI in people with rheumatoid arthritis, this study is designed.

<u>AIMS AND OBJECTIVES</u>

<u>Aim:</u>

- To find the proportion of Latent tuberculosis infection in patients diagnosed with Rheumatoid Arthritis, by using Interferon Gamma Release Assay and Tuberculin Sensitivity Test.
- To find out risk of latent tuberculosis converting into fulminant tuberculosis by the use of steroids for the treatment of RA.

Review of literature

TUBERCULOSIS

Mycobacterium tuberculosis is the infectious agent that causes tuberculosis (TB). It is a facultative intracellular, non-spore-forming, aerobic, non-motile, alcohol, and acid-fast bacteria. The high lipid content of this bacillus is responsible for its distinctive characteristics. TB, also known as extra-pulmonary TB, typically infects the lungs but can also affect other human organs (10). Aerosol routes are used to spread the illness when ill persons cough or otherwise release bacteria into the air, which can cause the disease. Overall, only 5–15% of the subjects, 1.7 billion Mtb infections globally will result in TB disease throughout the course of a person's lifetime (11). Sputum smear microscopy, molecular testing, and culture-based techniques are all used as TB diagnostics.

Tests available for TB that is immune to first- and second-line anti-TB medications. One of these is XpertMTB/RIF, which concurrently assesses TB and rifampicin resistance, the most potent first-line anti-TB medication.

The World Health Organization (WHO) estimated that 10.0 million (range 9.0 - 11.1 million) new cases of TB occurred and 1.3 million (1.2 - 1.4 million) individuals died from the disease in 2017. This was despite several preventive attempts to minimise the burden and impact. More than 70% of these new cases occurred in developing countries, with Africa having the greatest mortality per population rates (11).

The current TB epidemic is being sustained and fueled by two important factors: the human

HIV infection and its relationship to active tuberculosis disease, as well as Mycobacterium tuberculosis strains' increasing resiliency to the most effective (first-line) anti-TB drugs (11,12). Population growth, low case identification, cure rates in developing nations, active transmission in crowded hospitals, jails, public spaces, emigration from nations with high incidence rates owing to war or famine, drug misuse, social disintegration, and homelessness are other contributing causes. Patients with active illness and positive sputum smears the primary infection in a population is pulmonary TB. Only around 10% of people with M. tuberculosis primary infection develop clinical illness. The immunological response that follows stops M. tuberculosis from growing in the remaining instances.

90% of the time, the immune response only succeeds in containing the infection, while just 10% of people achieve total eradication of the disease. This is due to the fact that some bacteria are able to avoid immune system defences (like phagosome-lysosome fusion, CD1 molecules, production of nitric oxide, antigen presentation by MHC class I, class II, and other reactive nitrogen intermediates) and persist in non-replicating (dormant or latent) states in old lesions.

When a human has latent tuberculosis, they are infected yet do not exhibit any symptoms. But between 5 and 10%, of those with latent tb go on to acquire active illness (13). This pool of latently infected people is where the majority of active disease cases in countries with low TB incidence originate.
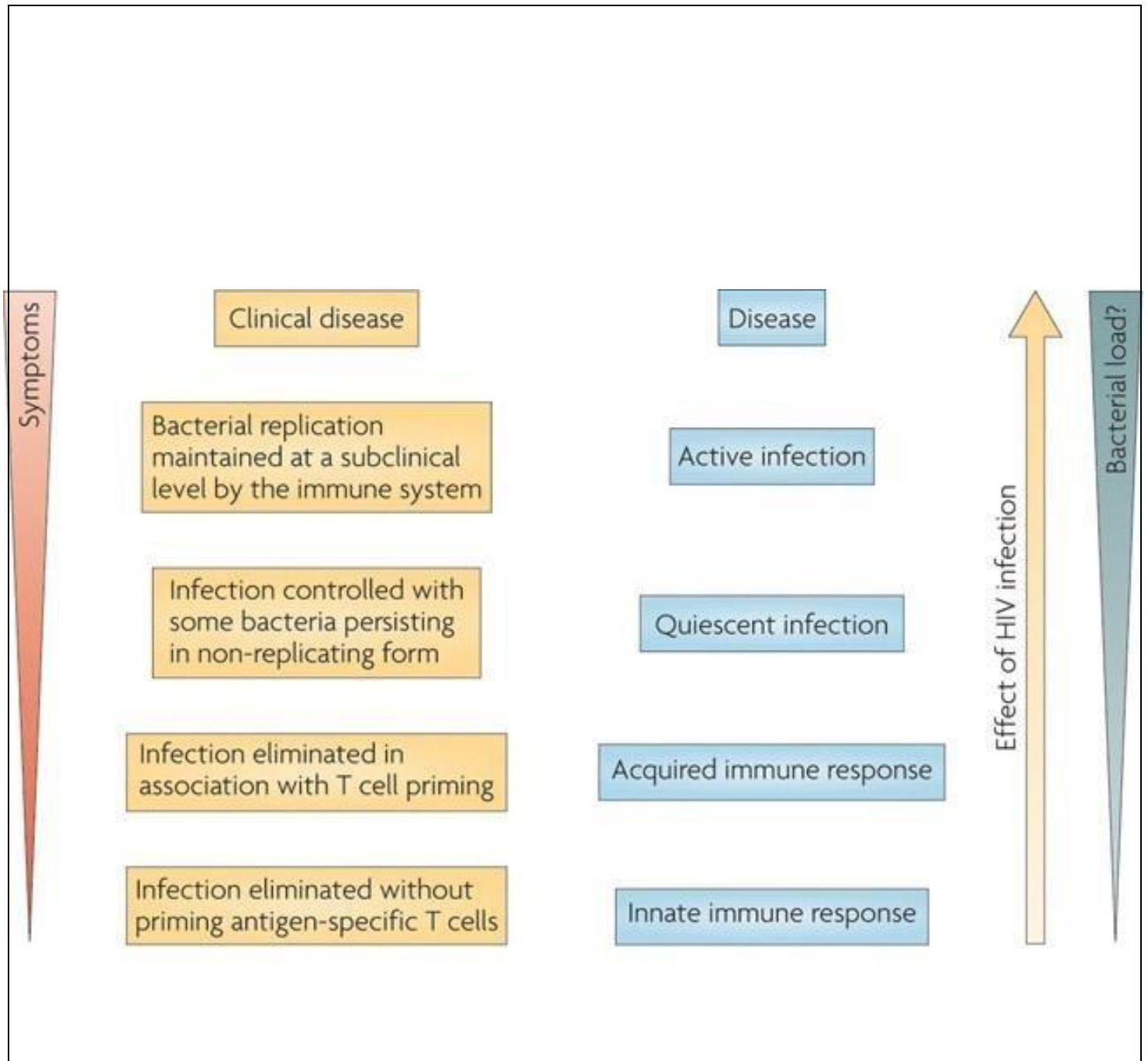
Figure 2 : Spectrum of TB infection(14)

LATENT TUBERCULOSIS

Clemens von Pirquet coined the phrase "latent TB infection" (Wagner, 1964). (15). Koch's tuberculin was the name of the skin test he developed using a rudimentary combination of mycobacterial antigens. He proposed this classification to describe a child who tested positive for tuberculin on the skin but did not exhibit any extrapulmonary or pulmonary TB symptoms. Latently infected people are not contagious, unlike patients with active TB disease, and their chest radiographs don't show any abnormalities or indications of cured TB disease.

The WHO defines LTBI as a state of persistent immune response to stimulation by mycobacterium tuberculi antigens without evidence of clinically manifested active TB[16]. Recent estimates indicate that latent TB infections affect about one-quarter of the world's population [17]. Even healthy people can live a lifetime with a latent TB infection due to the highly varied latency period. Reactivation happens in a small percentage of cases (5%–15%), frequently in the first two–five years after an infection that the immune system was able to contain [18]. Reactivation is when dormant, nonclinical infection becomes an active case of tb. Consequently, those who have latent tuberculosis infection serve as a significant source of new instances of active TB [19]. Although the underlying factors that lead to LTBI reactivation and their related aetiologies are not fully known, they undoubtedly entail bacterial, host, and environmental factors [20]. While the person's lifetime risk for turning a latent TB infection into active TB is estimated to be between 5% and 15% in otherwise healthy people with proven LTBI, a number of comorbidities and risk factors are linked to heightened likelihood of developing active TB. The most significant known and documented risk factor is, Infection with the human immunodeficiency virus (HIV).

HIV and latent TB co-infection raise a person's risk of getting active TB disease by more than a hundred times [21]. The danger is still quite high even when antiretroviral medication is effective [22,23]. According to their related risk factors, other comorbidities and conditions linked to LTBI reactivation have been classified as having a high, moderate, slightly elevated, low, or extremely low risk [20,24,25]. High-risk patients include those who have silicosis[28], chronic renal failure individuals who require haemodialysis[26], transplant recipients who are on immune suppressants[27], and others.

The subjects on glucocorticoids or tumour necrosis factor-alpha (TNF-alpha) inhibitors[29,30] (used to treat a variety of autoimmune and inflammatory conditions), people with any type of DM , and under the age of four children who recently infected are at moderate risk [31]. Latent TB reactivation is slightly more in people who misuse alcohol, cigarettes, are underweight, or are malnourished [32]. These groups have greater rates of TB than the overall population [4,11,24]. Suppressed immunity is a trait shared by the majority of these disorders that raise the likelihood of reactivatio

PATHOGENESIS OF LATENT TUBERCULOSIS:

There exist a great variability in the course of mycobacterium tuberculi infections among Homosapien sapiens[33]. Despite continuous exposure to infectious TB patients, some people do not become infected, as shown by a negative TST and IGRA for LTBI [34]. Infection sets in when the bacilli enter the alveoli and are phagocytized by the alveolar macrophages and resident dendritic cells. As he bacteria move from the distal part of airways to the draining lymph nodes, which are the mediastinal lymph nodes, the dendritic cells will now transport the bacteria and antigens, where they initiate the T cell mediated response of the immune system.

In order to establish a granuloma, more immune cells such as lymphocytes and macrophages move to the main infection site. In the majority of TB animal models, bacterial growth rises logarithmically before plateauing just as the T cell response begins and granulomas start to appear histologically[35].

The granuloma is the hallmark of TB, It is a focal collection of inflammatory cells that have a specific architectural structure in humans. Granulomas are believed to act as a physical and immunologic barrier to confine infection and stop its spread.

A granuloma is a structural organisation of distinct immune cells, including macrophages, T cells, B cells, dendritic cells, neutrophils, natural killer (NK) cells, fibroblasts, in response to pulmonary inflammation caused by the stimulation of host cell mediated immunity with mycobacterial antigens [36,37]. The immune system maintains the granulomas by a dynamic process of continual immunologic regulation of bacterial reproduction. The granuloma forms when host macrophages phagocytose the mycobacterium and release pro-inflammatory cytokines like TNF- alfa to recruit additional cells [38].

Recruited macrophages either consolidate to create multinucleated giant cells or develop into epithelioid cells inside the granuloma that has formed[39]. The outer circle of lymphocytes, including CD4 T cells of the adaptive immune response, surrounds the aforementioned cells and may increase the ability of macrophages to kill bacteria by releasing IFN-gamma [38]. It is seen that the granuloma is enclosed in a tight layer of fibroblasts at a later stage [39]. The result of mycobacterial infection in the host is determined by the adaptive cell-mediated immune response and the appropriate establishment of the granuloma. The host response is sufficient in 90% of those with Mycobacterium tuberculi infection to prevent the TB illness [33, 37, 40]. Mycobacterium tuberculi effector proteins and glycolipids have a role in a number of metabolic alterations that are associated with the persistence of TB bacilli in granulomas [41, 42]. The persistent bacilli in granuloma are subject to a variety of stressful situations, including hypoxia, nutritional shortage, an acidic pH, and nitric oxide-inhibited respiration. All of these elements trigger the production of genes that cause the mycobacteria to enter a dormant stage[43]. The metabolic and replicative activities of these latent bacilli can be reduced, and they can also impede growth and development. Additionally, they develop immunity to immune defence and effectively escape immune cell eradication [44]. Tuberculosis bacilli can resurface after several years or even decades of latency, alter their metabolism, and change the pressure on granulomas, which results in necrotic cell death [41]. According to certain theories, the correct granuloma development in tuberculosis infections is crucial for regulating mycobacterial growth and influencing tissue damage and transmission, the two most important features of active TB disease [45].

The localisation of lymphocytes that are specific to an antigen within the lungs may be affected by an inadequate upregulation of the adhesion molecules on circulating lymphocytes. This has an impact on the ability of the proper granuloma to inhibit Mycobacterium tuberculosis from growing [46]. The intricate mechanisms directed at the antigen presentation determine the strength of the T cell response in the granuloma. Mannosecapped lipoarabinomannan (Man-LAM), trehalose dimycolate (cordfactor), 19- kDa lipoprotein, other mycobacterial components can influence how mycobacterial protein and glycolipid antigens are processed and presented by cells with MHC class I, MHC class II, and CD1 molecules [47]. In this manner, bacilli may prevent macrophages from presenting antigens to T cells [48, 49]. CD4+, CD8+, gamma and delta T-lymphocytes, as well as CD1 restricted as well as cytotoxic T cells are insufficiently activated, which impairs macrophages' ability to kill bacteria and alters the capacity of other immune cells involved in the inflammatory response, causing damage to the tissue and the spread of infection [45, 47, 49].

Most people develop a specific acquired cell-mediated immunity after their first contact to mycobacteria, which inhibits mycobacterial growth without completely eliminating it [50]. These people still have inactivated TB bacilli in their system. For an immune-competent patient, the lifetime chance of latent tuberculosis infection reactivating into active tuberculosis is in the range of 10%. [37, 40].

The main element of the host's defense against tuberculosis is cell-mediated immunity. Controlling tuberculosis infection in those people who are resistant, depends upon the formation of a Th-1 immune response. This type immune response includes the participation of host alveolar macrophages, dendritic cells, T lymphocytes which include TCD4+, TCD8+, and Tγδ.

Interlukin-8, monocyte chemoattractant protein 1 (MCP--1), and macrophage inflammatory protein--1 alpha (MIP--1α), as well as pro inflammatory cytokines interferon--gamma (IFN-gamma), interleukin-2 (IL--2), interleukin-12 (IL--12), interleukin-18 (IL--18), and tumour necrosis factor-alpha (TNF--alpha), are released.

All of the abovementioned cells are required for the recruitment of fresh cells to the infection site in order to develop a granuloma that harbours and destroys tuberculosis bacilli. Additionally, it develops the long-term niche required for the growth of LTBI [36,51].

Latent infection can develop into active disease when a host experiences a number of favourable conditions. Among the known risk factors for latent TB reactivation to active tuberculosis are HIV infection, immunosuppressive therapy with glucocorticoids, anti-TNF therapy, anti-cancer medications, malnutrition, smoking, alcoholism, malignancy, insulin-dependent diabetes, and renal failure [37,43,52].

THE ROLE OF INNATE IMMUNE RESPONSE:

The activation of the innate immune system and the ensuing response, which includes the bacillus coming into touch with the macrophages and dendritic cells of the innate immune system, always signal the start of the host's resistance against mycobacterial infection.

It has been established that toll-like receptors play a crucial role in mycobacterium TB infection as pattern recognition receptors. Increased susceptibility to MTB in MyD88-deficient mice first indicated that Toll-like receptors were important in the initial host response[53,54], Although there is debate regarding the precise Toll-like receptors involved. However, expression of the innate immunological signal transduction adaptor MyD88-dependent IL-1R is also essential for mycobacterium tuberculosis resistance [55]. Toll-like receptors--2, Toll-like receptors--4, and Toll-like receptors--9 have been indicated being crucial in the response to MTB in vitro and in vivo studies in murine models[54,56–62], while other research have unable to support these findings [63,64]. TLR gene variants have been linked to a higher risk of mycobacterium TB infection or illness [65,67]. TLR activation causes the vitamin D dependent formation of antimicrobial-peptides with bactericidal action in humans, according to in vitro studies [68,69].

M. tuberculosis component	Immune cell process inhibited/affected
19 kDa Lipoprotein (LpqH)	MHC class II expression and antigen presentation
Mannose capped lipoarabinomannan	Phagolysosome biogenesis
19 kDa Lipoprotein (LpqH)	Phagosomal processing by MHC class I pathway
Mannose capped lipoarabinomannan	MHC class II expression and antigen presentation
Mannose capped lipoarabinomannan	IL-12 secretion of dentritic cells/macrophages
Mannose capped lipoarabinomannan	Apoptosis of macrophages
Trehalose dimycolate (cord factor)	Phagolysosome biogenesis
Trehalose dimycolate (cord factor)	MHC class II expression and antigen presentation
6-kDa early secreted antigenic target (ESAT-6)	Pathogen containment in phagolysosome/macrophage
NADH dehydrogenase (NuoG)	Apoptosis of macrophages and dendritic cells
ESX-1 secreted proteins	Macrophage proinflammatory cytokine response
Serine/threonine protein kinase G (PknG)	Phagolysosome biogenesis
Lipid phosphatase (SapM)	Phagolysosome biogenesis
Lipoprotein LprA	MHC class II expression and antigen presentation
Lipoprotein LprG	MHC class II expression and antigen presentation
Secretion system SecA2	Apoptosis of macrophages and dendritic cells
Superoxide dismutase (SodA)	Apoptosis of macrophages and dendritic cells

Table 1: Important M. tuberculosis factors that modulate the innate immune response and

promote persistence of the pathogen leading to latent tuberculosis infection.

ROLE OF ADAPTIVE IMMUNE RESPONSE:

The adaptive immune system cells perform the most important role in establishing and maintaining the latency, which will eventually manifest as latent tuberculosis infection without any overt signs or symptoms of active disease. The key cells involved in this process include macrophages, T cells, and various cytokines like IL 12, IL 23, and interferon gamma.

T CELLS:

The cell-mediated immune system is crucial for the treatment of acute Mycobacterium TB infection. The granuloma contains CD-4 and CD-8 T cells, B-cells, macrophages, neutrophils, fibroblasts, and multinucleated giant cells. CD4 T cells are required for the control of both acute and chronic infection, according to murine models [70]. For effective CD8 T cell function, CD4 T cells are crucial as they are the main processor and producers of IFN-gamma, they also help in synthesis of TNF.

This hypothesis has been tested and verified in nonhuman primates with SIV (Simian Immunodeficiency Virus)infection, where the initial decline in CD4 T cell levels was associated with time to reactivation of latent infection, and in patients with HIV infection, where the risk of tuberculosis infection rises with declining CD4 T cell counts[71] [72]. Additionally, it was discovered that the prevalence of extra pulmonary TB, a warning indication for a serious illness, was negatively connected with CD4 T cell concentration [73].

Despite being first thought to be contentious, CD8 T cells are crucial to the immunological response against tuberculosis. According to certain research on murine acute infections, mice missing functioning MHC class-I had larger bacterial burdens than wild type controls [74]. In mice with minimal bacterial loads due to antibiotic treatment, the depletion of CD8 T cells resulted in exacerbation of infection[75].

Although CD8 T cells may produce TNF and IFN-gamma as well as CD4 T cells, at least in mice, they are best recognised for their ability to kill infected cells through cytotoxicity. The ability of CD8 T lymphocytes to secrete perforin enables the creation of pores in infected cells' cellular membranes and facilitates the release of granule-associated proteins like granzymes, which induces apoptosis. In mice, it has been demonstrated that perforin from CD8 T lymphocytes has a significant protective function during acute infection [77]. CD8 T cells are also cytolytic, to generate IFN-gamma, and to make granulysin, which has anti-mycobacterial activity[78]. However, their function in the human tuberculosis infection is still unknown at this time. During acute M. tuberculosis infection, CD8 deletion in rhesus macaques reduced the Bacillus Calmette-Guérin-induced immunological response[80], indicating that CD8 T cells are crucial to the defence against Mycobacterium TB.

CYTOKINES:

A crucial part of the primary and latent infection is played by cytokines. Mice lacking in interleukin-12 (IL-12), which is crucial for the Th1 response, have been shown to have lower survival rates and higher bacterial burdens than controls with IL-12 responses that are normal [81]. Genetic abnormalities in IL-12/IL-23/IFN-gamma axis have been linked to severe disseminated mycobacterial illness in human investigations in the past [82]. Studies on mice have demonstrated that T cell-produced IFN-gamma is crucial for early protection and is necessary for generating NOS2 [83,84]. In reaction to mycobacterial antigens, humans also create IFN-gamma, which serves as the foundation for diagnostic/test known as the Interferon Gamma Release Assay.

In humans, IFN-gamma also stimulates autophagy[85] as a mechanism of decreasing

mycobacterial burden. Genetic defects in IFN-gamma increase the susceptibility to tuberculosis also to other non-tuberculous mycobacteria (NTM)[86]. The relatively inadequate granuloma formation and insufficient macrophage activation seen in that paradigm led to the long-standing recognition that functioning tumour necrosis factor (TNF) is crucial for managing acute and chronic murine infection [84,87]. TNF is crucial for resolving acute infection and preventing reactivation, however recent studies using zebrafish and nonhuman primate models have demonstrated that overall granuloma development is normal in not presence of TNF alfa [88, 89]. There is evidence that higher vulnerability to active TB across the African continent has been linked to genetic variability of the TNF Receptor (TNFR) in humans [90]. TNF's significance is highlighted by the highincidence of TB among individuals using anti-TNF medications for illnesses. Although majority of these instances were first believed to be due to tuberculosis reactivation, but there is concern regarding the possibility of developing active TB now that these medications are accessible in regions with a high TB endemicity, such as India. TNF modifies the expression of chemokines and adhesion molecules, some of which have been shown to be crucial in the early stages of infection [91]. The survival of mycobacteria in macrophages is thought to be harmed by TNF, which is also a modulator of apoptosis. More virulent strains of MTB seem to generate reduced TNF expression in human alveolar macrophages, which will result in self-death of cells as a means of lowering intracellular bacterial population[92]. This suggests that apoptosis of cells is linked to a good outcome of infection. In animal models, an attenuated MTB strain that enhances apoptosis elicited higher CD-8 T cell activation, responses and provides increased protection against virulence [93].

MACROPHAGE ACTIVATION

Mycobacterial eradication depends on macrophage activation. The cytokine IFN-gamma, which is largely produced by T cells, appears to be crucial for activating macrophages. Nitric oxide synthase 2 (NOS2) plays a significant role in the early and chronic stages of infection in mice, and in this model, macrophage cells activation is equivalent to the production of NOS^2. Although NOS2 expression and function have been observed in human samples [96], more research is necessary to determine whether NOS2 plays a role in human tuberculosis. Changes in the NOS2A gene sequence has been linked to higher susceptibility to TB in humans [66].

THE INFLUENCE OF BACILLI AND VARIOUS STRAINS ON LATENCY:

According to the substantially improved knowledge and understanding of the virulence factors linked to M. tuberculosis, the bulk of these virulence factors tend to interfere with and modify host responses. Long believed to have a highly stable genetic makeup was M. tuberculosis. Recent studies have revealed that M. tuberculosis' genome is significantly more plastic than previously believed and that there are important differences across strains and isolates that could influence the severity and progression of an infection. Furthermore, there is evidence that suggests certain clades of M. tuberculosis are connected to populations from particular geographic regions and appear to have coevolved with those populations [97].

The Beijing strain, which makes up around 50% of East Asian TB strains, has emerged in the last ten years as a major source of the disease [98]. There is evidence that Beijing strain infections are linked to more severe disease, and some studies, but not all, have shown that Beijing strain infections are more drug-resistant. In studies done on guinea pigs, some Beijing strains

were found to be more virulent than those with non-Beijing strains, but this was not true universally[99]. According to a study conducted in the Gambia, contacts who were infected with the M. tuberculosis complex member Mycobacterium africanum were more likely to develop latency than those who were infected with other M. tuberculosis strains, such as the Beijing strain, and had a lower risk of developing active TB. In another investigation, strains having a Euro-American ancestry were more likely than other strains to induce pulmonary disease as opposed to meningeal TB. In the same study, it was discovered that the Beijing strains were linked to those who had polymorphisms in the TLR2 gene, which has previously been linked to a higher risk of contracting tuberculosis. Therefore, it's crucial to remember that the bacilli strain that is causing the infection can affect how the infection develops and how it turns out (100). The final result, however, is probably going to be influenced by the host and the bacillus.

THE SPECTRUM OF LATENT INFECTION:

The traditional view of tuberculosis holds that it only manifests as an active illness or a latent infection with no overlap. There is now mounting proof that latent infection likely exists on a spectrum, similar to active tuberculosis (which can appear in a range of severity). Even though the overall mean of IFN- gamma production is higher in patients with active disease than in those with latent disease, a quick review of the interferon gamma release assays (IGRA) results reveals that there is a significant amount of variability within both the latent and active groups, which lowers the assay's predictive value (101). According to a review article written by Barry et al(14), the concept of latent infection is evolving. A latent infection is diagnosed on the basis of a positive tuberculin skin test (TST/Mantoux) or blood test by interferon-gamma

release assays (IGRA) without symptoms and signs (x-ray) of disease, without the need for additional testing, which restricts the capacity to identify subclinical disease A study of 601 culture-positive TB cases found that majority of the individuals were asymptomatic and that 9% had normal chest x-rays. Two-thirds of these subclinical cases—22%—were HIV-positive, which is an established risk factor for abnormal chest x-ray findings. Only 5% of these subclinical cases were HIV-negative (73,102). These findings imply that subclinical illness does exist, albeit with a low prevalence. However, subclinical disease (sputum positive despite normal chest x-ray and lack of disease signs or symptoms with positive TST or blood test) is significantly more common among HIV+ patients who reside in high TB endemic areas and is significantly more common than in the general population (52). Sputum testing has become a common practise for tuberculosis screening in regions with high endemic rates of both HIV and tuberculosis as a result of this. The subclinical instances and rates of tuberculosis among immunological competent people have not been thoroughly and methodically investigated. Anecdotally, recent developments in medical imaging have shown that metabolically active lesions are present in latently infected persons, indicating that latency might be thought of as a dynamic process (103,104).

It stands to reason that a person's relative risk of reactivation will depend on where they fall on the latent infection spectrum. For instance, patients without subclinical disease are probably at a lower risk for reactivation than those with undetected preclinical tuberculosis, who would otherwise be referred to as latently infected and presumably have a high reactivation rate. On the other hand, some patients are unlikely to ever reawaken despite immune suppression; this could be attributable to genuine clearance of the infection or to bacilli residing in a truly latent inactive condition, although separating these states in humans given current technologies is impossible.

To distinguish between infected people for whom immune interventions (such as anti-TNF medication) may be most dangerous and those for whom prevention and antibiotic therapy may be most useful, biomarkers that can discriminate the location on the latency spectrum are urgently needed.

Figure 3 : The spectrum of M. tuberculosis infection outcomes is shown in this figure. In order to better depict the variation in infection in these two categories, the clinical outcomes of active (red line) and latent (blue line) infection are split. Up the spectrum of illness, as indicated by the dashed orange line, the bacterial burden is anticipated to rise. The seesaws show how the granuloma's pro- and anti-inflammatory (P) elements are balanced. These two elements are in equilibrium at the lower end of the latency spectrum, limiting disease while restricting bacterial proliferation. The balance can change as one moves along the spectrum, either with too much pro-inflammatory activity or too much anti-inflammatory activity, which can result in inadequate bacterial control and increasing pathology. The purple line reflects the risk of reactivation in the latent spectrum.

RISK FACTORS FOR REACTIVATION OF LATENT INFECTION:

Mycobacterium tuberculosis can be demonstrated to survive for years inside of a human host, therefore it makes sense to think of latent infection as a dynamic process involving bacterial survival and immune regulation. HIV, malnutrition, tobacco use, indoor air pollution, alcoholism, silicosis, insulin-dependent diabetes, renal failure, malignancy, and immunosuppressive medication such glucocorticoids are established risk factors for reactivating latent infection, according to several epidemiologic research (105,106). The risk factors for reactivation that have received the most attention and study are HIV infection and TNF inhibitor therapy. TNF inhibitors were first made available for the treatment of numerous inflammatory and autoimmune illnesses more than ten years ago. Patients on TNF inhibitors had a higher TB incidence, which is thought to be the result of a latent infection becoming active again (107). According to the literature on human investigations, granuloma structure was entirely normal, in contrast to what was shown in the murine model, and reactivation was also believed to be produced by TNF neutralisation in nonhuman primates with true latent infection (89). Although these findings did not correspond to reactivation instances, studies in patients treated with TNF inhibitors revealed that cells in the blood had decreased T cell activation, IFN-gamma production, and proliferation as well as decreased CD8 memory T cells with lower granulysin (108).

However, sufficient quantities of IFN-gamma were discovered in mediastinal lymph nodes of non-human primates (monkeys) investigations, suggesting that immunologic components in the blood may not always correlate with local illness. TNF neutralisation reduced cellular recruitment (i.e., T cell recruitment) to illness sites, changed the expression of chemokine receptors, and produced an excessive amount of extra pulmonary disease (89).

More importantly,Although a high rate of reactivation (65%) was observed in latently infected monkeys, not all monkeys reactivated after receiving short-term anti-TNF treatment (89), suggesting that TNF is a significant but not always crucial factor in maintaining latent infection and that the range of latent infection likely plays a significant role in the overall risk of reactivation. The most frequent risk factor for TB recurrence is still HIV. HIV's immune suppression has played a major role in the emergence of tuberculosis as a hazard to global health. Compared to non-HIV patients, HIV patients have a nearly 10-fold increased chance of reactivation (109). 85% of cases of tuberculosis had only pulmonary involvement prior to the HIV epidemic. In contrast, advanced HIV-infected patients with tuberculosis had an abnormally high risk of disseminated, extrapulmonary illness. Regardless of antiretroviral medication, the highest risk of tuberculosis was shown to be associated with CD4 T cell counts below 200 cells/ml (71) indicating that CD4 T cells are essential for maintaining latent infection. The risk of TB is still elevated even under ideal circumstances when CD4 T cell numbers increase(110,111), demonstrating that HIV infection causes additional immunological weaknesses independent of CD4 count. When all monkeys suffer reactivation TB either early or late after contracting the SIV infection in latently infected monkeys (112). Following SIV infection, early reactivation was linked to more severe T cell depletion and poor T cell recovery, which is consistent with human data.

The loss of mycobacterial-specific CD4 T cells caused by HIV, increases in HIV load in serum and macrophages brought on by M. tuberculosis, a shift from Th1 to Th2 response via changes in IL-10, regulatory T cells, IL-12, IL-4, and TNF, loss of granuloma integrity, and changes in apoptotic mechanisms are just a few of the immunologic interactions between HIV and M. tuberculosis that (113).

RHEUMATOLOGICAL DISEASES AND LTBI REACTIVATION:

One of the most common autoimmune arthritises, rheumatoid rheumatic (RA) has a prevalence of about 1% worldwide (114), and between 0.50% and 0.75% in India (115,116). The existing treatments for these inflammatory rheumatologic illnesses aim to lessen pain, inflammation, and disease progression. Non-steroidal anti-inflammatory medications, steroids, and disease-modifying anti-rheumatic (DMARDs) medicines are typical therapies . Biologics that target disease-specific inflammatory pathways have transformed the treatment of RDs in patients whose condition is resistant to traditional synthetic (csDMARD) drugs.

Biologic DMARDs (bDMARDs) include monoclonal antibodies that target inflammatory cytokines, such as tumour necrosis factor (TNF) inhibitors such as adalimumab, etanercept, infliximab, certolizumab, and golimumab, interleukin-6 receptor inhibitor tocilizumab, B-cell inhibitor rituximab, and T-cell costimulation inhibitor abat (117). In the treatment of diseases like RA, psoriatic arthritis, ankylosing spondylitis (AS), and psoriasis, anti-TNF biologics have shown effective. Adalimumab, etanercept, infliximab, and golimumab are the anti-TNF biologics that have been given the all-clear in India to treat RDs (Table:2) Biological agents have made great progress in the treatment of rheumatologic illnesses, but the possibility of TB reactivation is a growing source of concern (118–120).

Granulomas the hallmark of tuberculosis, is composed of immune cells and Mycobacterium tuberculosis bacteria. The maintenance of the granuloma structure is very critical for the containment of the bacteria.

TNF and interferon (IFN)-c signaling is important in host defenses against mycobacterial infections. TNF is involved in a number of processes, including the activation of macrophages and the recruitment of cells (such as natural killer cells, granulocytes, fibroblasts, and T cells) to the sites of infection, which results in the formation of granulomas and the containment and eradication of the pathogen. TNF aids in the granuloma's maintenance in patients with latent tuberculosis infection. The physiological TNF-mediated immune-inflammatory responses are interrupted by anti-TNF medication, which disrupts well-formed granulomas and releases live mycobacteria, reactivating TB (121). Nine commonly used biological DMARDs were the subject of a Cochrane review and network meta-analysis, which also revealed a higher risk of TB reactivation (odds ratio [OR] 4.68, 95% CI 1.18-18.60; P = 0.028). (122). Additionally, as compared to the general population, the risk of developing tuberculosis in patients receiving biological treatments is around 56 times higher (123,124). Anti-TNF therapy's potential to reactivate tuberculosis and its link to worsening morbidity and quality of life worry doctors. Therefore, it is strongly advised that this high-risk population be assessed and given the proper prophylactic treatment to lower the risk of TB flare before beginning biologic DMARD medication. The rheumatology guidelines from various international societies, namely: (i) American College of Rheumatology (ACR)2015; (ii) Asia Pacific League of Associations for Rheumatology (APLAR) 2015 for RA; (iii)British Society for Rheumatology (BSR) guidelines for AS; (iv) British Society for Rheumatology and British Health Professionals in Rheumatology (BSR-BHPR) guidelines for psoriatic arthritis; and (v) the Australian Rheumatology Association, all recommend and emphasize the need to strictly screen patients for TB and other infections before biologic DMAEDs are initiated.

Biologics approved in India	Mechanism of Action	Related conditions
Adalimumab	TNF inhibitor, human IgG1 monoclonal antibody	Rheumatoid arthritis ankylosing spondylitis, plaquepsoriasis, psoriatic arthritis, ulcerative colitis
Etanercept	TNF inhibitor, TNF-receptor fusion protein	Rheumatoid arthritis ankylosing spondylitis, psoriasis, juvenile rheumatoidarthritis, psoriatic arthritis
Infliximab	TNF inhibitor, chimeric anti-TNF antibody	rheumatoid arthritis,Ankylosing spondylitis, Crohn'sdisease, psoriasis, psoriatic arthritis, ulcerative colitis
Rituximab	B-cell inhibitor, monoclonal anti-CD20 antibody	Rheumatoid arthritis
Golimumab	Human anti-TNF IgG monoclonal antibody	rheumatoid arthritis Ankylosing spondylitis, psoriaticarthritis.
Tocilizumab	IL-6R inhibitor	Rheumatoid arthritis
Abatacept	Fusion protein (Fc region of IgG1 fused with extracellular domain of CTLA-4)-inhibitor of T cell activation	Juvenile idiopathic arthritis, RA.

IL-interleukin; R-receptor; TNF- tumor necrosis factor; IgG-immunoglobulin G.

Table 2 : Biological intervention for rheumatologic diseases approved in India(125)

<u>ROLE OF SCREENING FOR LTBI IN RHEUMATOLOGIC DISEASES – INDIAN</u>

<u>RHEUMATOLOGY ASSOCIATION RECOMMENDATION:</u>

Reactivation of latent tuberculosis into active illness is now becoming an increasing concern to public health due to the widespread use of biologic drugs for the treatment of rheumatologic diseases, such as anti-TNF therapy and steroids (126). The Indian Rheumatology Association has put up recommendations regarding possible side effects of anti-TNF medication, including tuberculosis testing prior to the initiation of the treatment (126–128). The patient should be screened for tuberculosis with a thorough history of any prior TB, anti-tuberculosis therapy received, if any, and adherence to the therapy, according to the guideline. It also says that a comprehensive physical exam and a chest x-ray should be added to it. To the right patients, prophylaxis may be administered along with a TST and QFT. Prior to initiating anti-TNF therapy, active TB must get appropriate treatment for a minimum of nine months. Anti-TB prophylaxis should be provided to all individuals with a positive TST and a history of tuberculosis. When a patient on anti-TNF therapy exhibits symptoms suggestive of tuberculosis, anti-TNF therapy should be stopped and complete anti-TB chemotherapy administered. All patients starting anti-TNF medicines need to have their TB levels routinely checked. Given that infliximab has a protracted elimination phase during treatment, this must continue for six months after the biological agent is stopped.

DIAGNOSTIC METHODS FOR LATENT TUBERCULOSIS INFECTION:

The diagnosis of latent tuberculosis infection cannot be made with a gold standard test. The available tests include delayed type hypersensitivity reactions to a complex mixture of Mycobacterium tuberculosis antigen known as the PPD(purified protein derivative) and measures of immune response to exposure to Mycobacterium tuberculosis antigen .Two techniques are now accepted for LTBI evaluation. TST (tuberculin skin testing) and IGRA are them (interferon gamma release assays).

TUBERCULIN SKIN TEST:

The Mantoux test, also known as the Pirquet test or PPD test for Purified Protein Derivative, is named after Charles Mantoux, a French physician who developed his test in 1907. Robert Koch initially reported the tuberculin skin test in 1890. (129). The bacillus's glycerol extract, tuberculin, is what is used to make PPD, which was first launched in 1934. Seibert and Glenn created the PPD-S in 1939, and it is now used as the benchmark worldwide. The test from the 19th century that is still extensively used as a crucial test for identifying latent tuberculosis infection is tuberculin skin testing. Despite being widely utilised by doctors around the world, its interpretation is still challenging and controversial. Age, immunological condition, co-existing illnesses, and other factors all affect its results and, consequently, its interpretation (130).

Basis of immunological reaction in TST: The reaction to intra dermally injected tuberculin is the classical example of delayed (cellular) hypersensitivity reaction. The T-cells that are Sensitized by prior infection are recruited to the skin site where they release lymphokines. Theselymphokines induce induration through local vasodilation, edema, fibrin deposition and recruitment of other inflammatory cells to the site of reaction. Features of the reaction include,

1. Its delayed course. The reaction reaches its peak more than 24 hours after injection of the antigen.

2. Its indurated character

3. Its occasional vesiculation and ulceration.

Five tuberculin units (TU) (0.1ml), the normal dose, are injected intradermally and detected 48–72 hours later. The most common tuberculin available in India is PPD RT 23, which is supplied by the Guindy, Chennai-based BCG vaccination laboratory in tween 80 strength of 1 and 2 TU. A detergent called Tween 80 is added to the tuberculin to stop it from adhering to glass or plastic surfaces. An immune response in the skin that contains the bacterial protein is anticipated when a person has been exposed to TB. 5 TU of tuberculin PPD RT 23 is almost always used across the board for the purpose of standardising reading and interpretation of the results. After administration, the Mantoux should be read 48 to 72 hours later. The diameter of the induration should be measured transverse to the long axis of the forearm and documented in millimetres for the sake of standardisation. There have been isolated cases of anaphylaxis and foreign body reaction at the Mantoux test site. Only a very small percentage of people, especially those who have had the BCG vaccine and tuberculosis, run the risk of experiencing a severe reaction to the test, including arm swelling and redness. Rarely do allergic reactions occur.

Live Bacteria are not used in the test so there is no chance of developing TB from the test. Local reactions such as regional lymphangitis and lymphadenitis may occur on rare occasions(128,131,132).

Mantoux testing is not recommended in the following conditions

1) Past reaction more than 15 mm. In this case repeating the test will not provide any new diagnostic information and will create discomfort.

2) Previous TB disease.

3) Infants under 12 weeks old, a positive reaction is very important but a negative reaction may indicate that the child is too young to mount a response and the test willneed to be repeated if exposure had occurred.

4) Pre vaccination Mantoux testing before 12 weeks of age is not recommended and not necessary unless the baby has been exposed to TB.

The person infected with mycobacterium tuberculosis may be identified by tuberculin skintesting six to eight weeks after exposure to bacilli.

SITUATION WHERE MANTOUX TESTING IS NOT RECOMMENDED:

Mantoux testing is not recommended in the following situations:

- Past Mantoux reactions ≥ 15 mm: repeating the test will provide no new diagnostic information and will create discomfort

- Previous TB disease: no useful diagnostic information will be gained and significant discomfort is likely

- Infants under 12 weeks old. A positive reaction is important, but a negative response may indicate that the child is too young to mount a reaction, and the test is to be repeated if exposure has occurred. Pre-vaccination Mantoux testing before 12 weeks of age is not necessary unless the baby has been exposed to TB.

<u>RECENT ADVANCES IN MANTOUX TEST:</u>

The Mantoux test is technically challenging to administer and interpret, therefore if the tester is inexperienced, false readings may happen. It may require four visits by the patient if a two-step test is performed, and compliance with this is sometimes difficult. A test that can be done on a singlepatient visit, such as a blood test, would be easier.

An innovative TB diagnostic test (QuantiFERON-TB GOLD, produced by Cellestis, Inc.) has been given FDA approval. By measuring interferon-gamma (IFN-G) extracted from plasma from whole blood incubated with the M. tuberculosis-specific antigens, ESAT-6(QFT-RD1) and CFP-10, the blood test determines the presence of Mycobacterium tuberculosis (TB) infection. This new immunodiagnostic test has been introduced to help with the LTBI diagnosis. ESAT-6 and CFP10 together were discovered to be extremely sensitive and selective for both in vivo and in vitro diagnosis. In comparison to PPD (7%), the combination demonstrated a substantially greater specificity (93%) and high sensitivity (73%) in people. Particularly in patients with negative microscopy and culture results, the QFT-RD1 test is sensitive for the diagnosis of TB. Despite the fact that antigens like ESAT-6 and CFP10 are not unique to M. tuberculosis, they nonetheless show promise for the precise identification of M. tuberculosis infection and may be a very helpful supplemental tool for the diagnosis of TB.

MANTOUX CONVERSION :

Conversion is the emergence of new or intensified hypersensitivity as a result of infection with tuberculous or non-tuberculous mycobacteria, including BCG vaccination. Boosting is the recall of the hypersensitive response in the absence of new infection.

Mantoux conversion is defined as a change (within a two-year period) of Mantoux reactivitywhich meets either *of* the following criteria:

- a change from a negative to a positive reaction

- an increase of $\geq$ 10 mm.

- Conversion has been linked to an annual TB illness incidence of 6% in contacts of smear-positive individuals or 4% in adolescents.

The amount of time needed for the immunological changes that result in Mantoux conversion after infection is up for discussion. Children experienced positive response between three and seven weeks after inadvertently immunising against M. tuberculosis (the Lubeck disaster).

Other investigations have found clinical disease with a positive tuberculin test 19–57 days (with a mean of 37 days) after exposure. Therefore, when testing TB contacts for conversion, the second tuberculin test is done eight weeks after the date of last contact with the source case. (In the past, the traditional windowperiod, or interval, of 12 weeks was used.)

The [Center for Disease control] published guidelines for using the QuantiFERON test in December 2005. QuantiFERON-role TB's in the diagnosis of LTBI is currently unclear. In the future, it might be conceivable to use this or another in vitro assay in place of the skin test.

IGRA – INTERFERON GAMMA RELEASE ASSAY:

Latent tuberculosis infection can be diagnosed using interferon-gamma release assays (IGRAs) (LTBI). They are cellular immune responses to Mycobacterium tuberculosis and serve as proxy markers of Mycobacterium tuberculosis infection. Latent infection and active tuberculosis (TB) disease are indistinguishable by IGRAs. When BCG vaccine is given beyond infancy or when numerous (booster) BCG vaccinations are given, IGRAs are particularly helpful for evaluating LTBI in BCG-vaccinated persons. When stimulated with M. tuberculosis-derived antigens, white blood cells in most M. tuberculosis-infected patients release interferon-gamma. A blood sample is incubated with antigens and controls to conduct an IGRA test. Different IGRA tests use different antigens, testing procedures, and interpretation standards. Mycobacterium marinum and Mycobacterium kansasii, two NTM that infect humans, have gene sequences that encode for ESAT-6 or CFP-10, the antigens employed in the new IGRAs. In IGRAs using these antigens, infection with either of these NTM has been demonstrated to result in good outcomes. Results from an IGRA can be obtained in 24 to 48 hours.

<u>QUANTIFERON - TB GOLD (QFT G):</u>

QFT G was approved by the FDA in 2005, QFT-G is an enzyme-linked immune sorbent

assay (ELISA) based whole blood test in which blood samples are incubated with the

mycobacterial antigens (ESAT-6 and CFP-10) for 16 to 24 h. If the patient is infected

with Mycobacterium tuberculosis, their white blood cells will release interferon-gamma in

response to contact with the TB antigens which is quantified.

<u>QUANTIFERON TB GOLD In-Tube TEST (QFT-IT):</u>

This test was approved by the FDA in Oct 2007, this ELISA-based assay for quantification

of interferon-gamma uses heparinized whole blood sample drawn directly into specialized

three blood collection tubes with antigens (ESAT-6, CFP-10, and TB7.7 proteins) dried onto

their walls and transferred to an incubator within 16 h of collection. The manufacturers

claims to have specificity of more than 99% in low risk individuals and sensitivity of 92% in

individuals with active disease.

TREATMENT FOR LATENT TUBERCULOSIS:

The following 15 risk groups have evidence of increased prevalence of LTBI, risk of progression from LTBI to active TB disease, and increased incidence of active tuberculosis, according to data from three very well-conducted systematic reviews that sought to identify the at-risk population groups that would be prioritised for latent tuberculosis infection testing and treatment among 24 pre-defined population groups(137):

(i)   Adult and child TB contacts

(ii)   Health-care workers and students

(iii)   People living with HIV

(iv)   Patients receiving dialysis

(v)   Immigrants from high TB burden countries

(vi)   Patients initiating anti-tumor necrosis factor (TNF) treatment

(vii)   Illicit drug users

(viii) Prisoners

(ix)   Homeless people

(x)   Patients receiving organ and hematologic transplantation

(xi)   Patients with silicosis

(xii)   Patients with diabetes

(xiii) People with harmful alcohol use

(xiv)   Tobacco smokers

(xv)   Underweight people.

Identification and treatment with preventive therapy or prophylaxis of LTBI can substantially decrease the risk of development of active tuberculosis disease (by as much as 60%), and also it is an important TB control strategy in low-TB incidence settings where reactivation disease usually accounts for the majority of non-imported tuberculosis disease. For high tuberculosis burden countries such as India, what shouldbe the preferred approach toward the management of latent tuberculosis infection?

This question was answered by World Health Organization (WHO) in 2015 when theypublished its first comprehensive guideline on management of latent tuberculosis infection(138) by giving the following comprehensive algorithm.
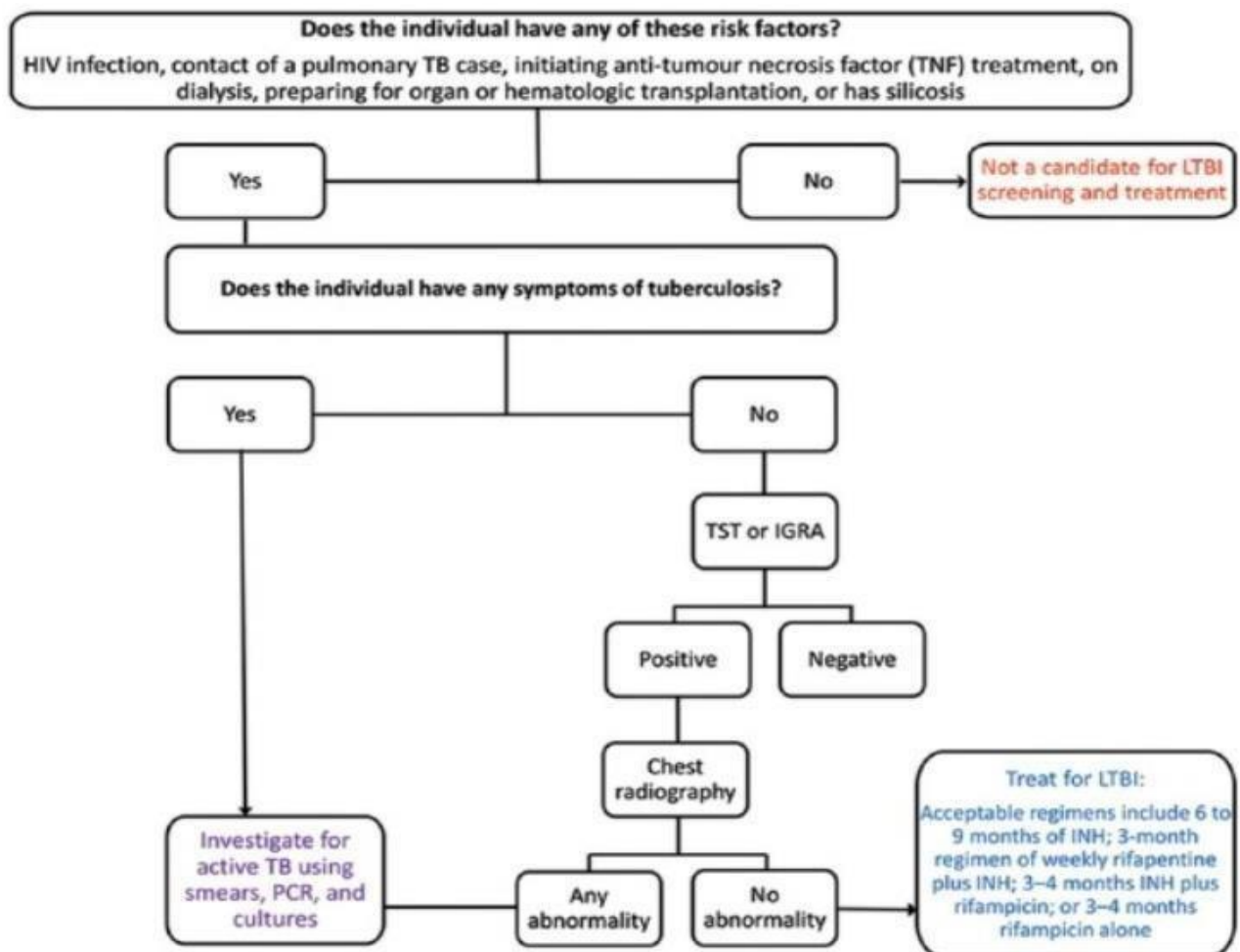
Figure 4: World Health Organization algorithm for latent tuberculosis infectionmanagement. Source: Adapted from WHO, Geneva(138).

TREATMENT OPTIONS AVAILABLE FOR LTBI:

The three drugs isoniazid (INH), rifapentine (RPT), or rifampin are used in the four treatment plans for latent TB infection (LTBI) (RIF). All treatment plans have been found to be efficient. Healthcare professionals are urged to prescribe shorter, more convenient regimens whenever possible because patients are more likely to comply with and complete these shorter treatment plans (139).

Drug Regimen	Dose per Kg body weight	Maximum Dose
Isoniazid alone, daily for 6 or 9 months	Adults, 5 mg Children, 10 mg (range, 7–15 mg)	300 mg
Daily rifampicin alone for 3–4 months	Adults, 10 mg Children, 15 mg (range, 10–20 mg)	600 mg
Daily isoniazid plus rifampicin for 3–4 months	Isoniazid: Adults, 5 mg Children, 10 mg (range, 7–15 mg) Rifampicin Adults, 10 mg Children, 15 mg (range, 10–20 mg)	Isoniazid, 300 mg Rifampicin, 600 mg
Weekly Rifapentine plus isoniazid for 3 months (12 doses)	Individuals aged ≥ 12 years: Isoniazid: 15 mg Individuals aged 2–11 years: isoniazid: 25 mg Rifapentine: 10.0–14.0 kg = 300 mg 14.1–25.0 kg = 450 mg 25.1–32.0 kg = 600 mg 32.1–50.0 kg = 750 mg > 50 kg = 900 mg	Isoniazid, 900 mg Rifapentine, 900 mg

Table 4:WHO recommendation for the treatment of LTBI.

Source- Latent TB –Updated and consolidated guidelines for programmatic management.

EFFECT OF STEROIDS ON MANTOUX AND QUANTIFERON TEST:

The question of whether the use of steroids and biologics affects the sensitivity and specificity of the tests that are now used to detect latent tuberculosis infection is one that has been the subject of numerous research that have been published in the literature. The answer to this query is YES, steroid use decreases TST and IGRA accuracy. According to S Agarwal et alprospective .'s cross-sectional study of 300 patients with rheumatoid arthritis and irritable bowel syndrome who were receiving steroid treatment and had biological agent therapy planned from Lucknow, India, treatment with steroids specifically for those who were receiving steroid doses greater than 20 mg and within the previous three months had significantly decreased TST positivity (140). Steroids, they found, lessen the frequency of TST positive in LTBI. Another prospective multicenter study from Copenhagen University, Denmark, by Belard et al. found that methotrexate, long-acting steroids, and azathioprine did not have the same negative effects on QFT and TST performance as oral steroids (141). The makers of commercial IGRA assert that it has higher sensitivity than TST in this case of steroid exposure by mentioning internal negative and positive controls that raise the test's sensitivity (142).

RHEUMATOID ARTHRITIS:

Rheumatoid arthritis is the most commonly diagnosed systemic inflammatory arthritis. Women, smokers, and those with a family history of the disease are most often affected. Rheumatoid arthritis can be difficult to diagnose in its early stages because the early signs and symptoms mimic those of many other diseases.

DIAGNOSIS AND TREATMENT FOR RHEUMATOID ARTHRITIS:

Historically, the 1987 American College of Rheumatology (ACR) criteria were used to make a RA diagnosis. These criteria were based on how long arthritic symptoms persisted, but this system of classification missed people with early inflammatory arthritis. It is now generally acknowledged that early therapeutic intervention dramatically enhances clinical outcomes and lessens disability and irreparable joint damage. With this in mind, the ACR and the EULAR developed and proposed new classification criteria for early rheumatoid arthritis, which evaluate joint involvement, autoantibody status, acute-phase response, and symptom duration. They also updated criteria for classifying RA in newly presenting patients, those with erosive disease typical of RA, and those with inactive disease with or without treatment (143).The 2010 ACR/EULAR criteria involves 4 domains with maximum possible score of 10 and a score more than 6 is needed to diagnose a patient as RA. The 4 clinical domains involved are

Table – The ACR/EULAR 2010 classification criteria for RA

To be applied to patients: (1) who have ≥ 1 joint with definite synovitis, excluding the DIP joints, first MTP joints, and first CMC joints, and (2) in whom the synovitis cannot be explained by another disease.

Criteria	Score
A. Joint involvement:	
1 large joint	0
2 - 10 large joints[a]	1
1 - 3 small joints (with or without involvement of large joints)	2
4 - 10 small joints[b] (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)	5
B. Serology (at least 1 test result is needed for classification):	
Negative RF and negative anti-CCP antibodies	0
Low-positive RF or low-positive anti-CCP antibodies[c]	2
High-positive RF or high-positive anti-CCP antibodies[d]	3
C. Acute phase reactants:	
Normal CRP level and normal ESR	0
Abnormal CRP level or abnormal ESR	1
D. Duration of symptoms:	
< 6 weeks	0
≥ 6 weeks	1

ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; DIP, distal interphalangeal; MTP, metatarsophalangeal; CMC, carpometacarpal; MCP, metacarpophalangeal; PIP, proximal interphalangeal; IP, interphalangeal; RF, rheumatoid factor; anti-CCP, anti–cyclic citrullinated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

[a] Large joints = shoulders, elbows, hips, knees, ankles.
[b] Small joints = MCPs, PIPs, second - fifth MTPs, thumb IPs, wrists.
[c] Low-positive is ≤ 3 times the upper limit of normal.
[d] High-positive is > 3 times the upper limit of normal.

Table 5: The ACR/EULAR classification criteria for RA

There is no cure for RA. Clinical studies suggest that early initiation of treatment reduces the morbidity and leads to remission of the disease. The treatment options available are as follows

    I.   NSAIDS

   II.   Steroids

  III.   DMARDS – Disease modifying anti-rheumatic drugs

 IV.   Biological therapy.

If the medical therapy fails, there are few surgical modalities that can improve symptoms andreduce morbidity. The surgical modalities practiced for RA(144) are

    I.   Synovectomy

   II.   Tendon repair

  III.   Joint fusion

 IV.   Total joint replacement.

METHODOLOGY:

Inclusion Criteria:

- Patients with Rheumatoid Arthritis who were not receiving Disease Modifying Antirheumatic Drug or anti-TNF-α agents
- Willing to give written consent

Exclusion criteria:

- Patients with diseases associated with nonspecific immunosuppression
- Patients who had received immunosuppressive treatment
- Active tuberculosis, known hypersensitivity to purified protein derivative (PPD) and positive serology for HIV
- Not willing to give written consent

METHOD:

The patients were recruited from the Orthopedics and Pulmonary medicine outpatient facility. All patients who fulfilled entry criteria for participation in the study were included the recruited subjects proceeded to participate after they had provided written informed consent. A detailed past history of tuberculosis, exposure to tuberculosis was obtained. Treatment history for the primary disease, duration of treatment and dose of steroids used in the last 3 months were also obtained. History of vaccination with BCG was alsoobtained and confirmed by checking the induration mark. Detailed history of current symptoms if any pointing towards active tuberculosis infection was obtained. If any symptoms were positive they will be screened for active TB with AFB smear(if symptomatic) and Chest X ray and excluded from the study and if active TB is detectedthe treating physician and concerned unit will be intimated and appropriate treatment will be initiated. Enrolled patients were tested for latent tuberculosis infection withQuantiFERON GOLD (QFT) and tuberculin skin testing (Mantoux).

<u>TUBERCULIN SKIN TEST</u>:

Tuberculin skin test was performed on all recruited patients. TST was performed using 5 TU (0.1 ml) PPD intra dermally using a 26G needle on the left forearm.

How TST was done –

After cleaning the desired site an intra-dermal bleb of about 4-6 mm was raised by a 26 Gneedle using 5TU (0.1 ml) PPD. The site was marked and educated the patient about test and expected skin reaction. The size of induration was marked, measured and recorded on to the proforma sheet after 48 to 72 hours.

<u>TST RESULTS</u>-

$\geq$ 5 mm for RA patients.

$\geq$ 10 mm for controls with clinical suspicion.

$\geq$ 15 mm for control without clinical suspicion.

QuantiFERON GOLD (QFT):

Blood for QFT was collected form all recruited patient who consented for the study.

How QFT was done –

1 ml of blood sample will be drawn into each of 3 blood collection tubes: one containing heparin alone (as negative control), the second containing the T cell mitogen phytohemagglutinin (PHA; as a positive control), and the third with peptides of ESAT-6, CFP-10 and TB 7.7 (TB antigens) dried on the inside of the tube. After mixing, the tubes will be incubated upright for 20 h at 37°C before plasma to be harvested for further analysis. The concentration of IFN-γ present in each plasma sample to be determined using the QFT ELISA. IFN-γ release in the saline control tube (Nil) will be subtracted from the TB antigen and PHA-stimulated samples. Samples with ≥ 0.35 IU/ml IFN-γ following stimulation with M. tuberculosis-specific antigens will be considered positive, while samples < 0.35 IU/ml will be considered negative.

The QFT test result will be considered indeterminate if the concentration of IFN-γ will be <0.35 IU/ml for TB antigens and < 0.5 IU/ml for the positive control.

Harvesting plasma

1 ml of fresh whole blood in NTP culture tubes

↓

Incubate for 22 +/- 2 hrs at 37* C

↓

Harvest the plasma and perform IFN-Gamma assay

IFN GAMMA ASSAY

    Add 20 ul sample dilutiom buffer and add 50 ul sample/standard

       ↓

   Incubate 60 min

       ↓

   Add 50ul HRP- Conjugate

       ↓

   Incubate- 60min

       ↓

   Wash 5 times

       ↓

  50ul A and 50 ul B substrate

       ↓

  Incubate 15 minutes

       ↓

  50ul stop solution

       ↓

  Read the absorbance 450nm Or 450/600-650nm.

IGRA RESULTS-

      Read the absorbance 450nm

       < 0.35 IU/ml will be negative

      ≥ 0.35 IU/ml result positive.

      Result will be indeterminate-if

      <0.35 IU/ml for TB antigens.

      <5 IU/ml for positive control.

Detailed diagrammatic Algorithm of the study

```
                    ┌─────────────────────────────┐
                    │ Patient with RA             │
                    │ orthopedic and Pulmonary    │
                    │ medicine outpatient         │
                    │ department                  │
                    └─────────────────────────────┘
                                  │
                                  ▼
        ┌──────────────────────────────┐   ┌───────────────────────────────┐
        │ Screening for active         │   │ History and clinical          │
        │ tuberculosis                 │   │ examination                   │
        └──────────────────────────────┘   │ X ray and Sputum AFB smears   │
                                            └───────────────────────────────┘
```

Negative	**Positive**

```
        │                                    │
        ▼                                    ▼
┌──────────────────────────┐     ┌──────────────────────────┐
│ Rheumatoid Arthritis (RA)│     │ Treat for active         │
│                          │     │ tuberculosis.            │
└──────────────────────────┘     └──────────────────────────┘
```

Proceed with further evaluation and treatment for primary disease (RA/AS)	Negative ←	**Tuberculin Skin test**	→ Positive	Referred to primary physician to decide on prophylaxis therapy.
	Negative ←	QuantiFERON GOLD blood sampling by venepuncture	→ Positive	

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                    ┌──────────────────────────┐
                    │ Results and statistical  │
                    │ analysis                 │
                    └──────────────────────────┘
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65

Detailed research plan:

Setting:

a. The study was conducted in the outpatient department of the Department of orthopedic in BLDE (DEEMED TO BE UNIVERSITY) Shri BM.Patil medical college, Hospital and Research Centre, Vijayapura. The study was conducted over a period from May 2020 to June 2022, with duration of the study being 18 months.

b. A total of 33 (9 males and 24 females) patients from outpatient Department of Orthopedics in B.L.D.E. (DEEMED TO BE UNIVERSITY) Shri B.M.Patil's Medical College, Hospital and Research Centre, Vijayapura. with with diagnosis of Rheumatoid Arthritis and willing to participate in this study, were included in this study.

c. At recruitment patients was given an information sheet and explained in detail about the study purpose and his/her contribution to the futuregeneration.

d. An informed written consent was obtained at recruitment by the primary investigator.

e. Tuberculin skin test was performed by a trained nursing staff on the left forearm to maintain convention and 10 ml blood was collected by venipuncture for QuantiFERON Gold (QFT).

f. The skin test was read, interpreted and recorded after 48-72 hours.

<u>Sample size Calculation:</u>

With anticipated overall prevalence of LTBI 13% the study was require a sample size of

834 patients with 99% level of confidence and 3% absolute precision.

Of which proportion of Rheumatoid Arthritis was 4%. Hence 4% of 834= 33 patients.

Formula used:

$$n = \frac{z^2 \, p*q}{d^2}$$

Where Z= Z statistic at α level of significance

d^2= Absolute error

P= Proportion rate

q= 100-p

Statistical analysis:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. Normality of the continuous data, was tested by Kolmogorov–Smirnov test and the Shapiro–Wilk test. Graphical representation of data: MS Excel and MS word were used to obtain various types of graphs such as bar diagram, Pie diagram. p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Results:

Table 6: Age distribution of subjects

		Count	%
Age	**<40 years**	**6**	**18.2%**
	41 to 50 years	**14**	**42.4%**
	51 to 60 years	**8**	**24.2%**
	>60 years	**5**	**15.2%**
	Total	**33**	**100.0%**

Mean age of subjects was 47.55 ± 11.116 years. Majority of subjects were in the age group 41 to 50 years (42.4%).
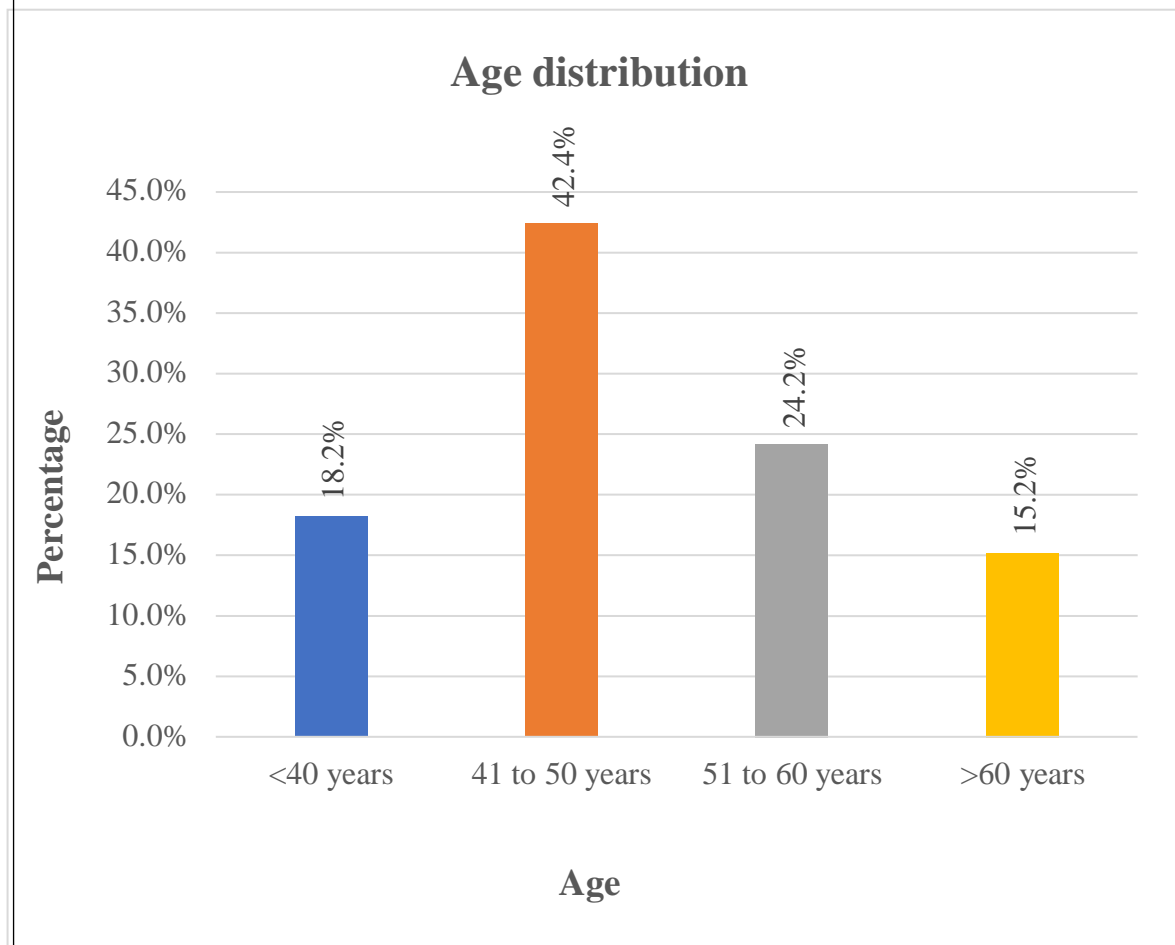
Figure 1: Bar diagram showing Age distribution of subjects

Table 7: Sex distribution of subjects

		Count	%
	Female	24	72.7%
Sex	Male	9	27.3%
	Total	33	100.0%

In the study majority of subjects were females, 72.7% and 27.3% were males.
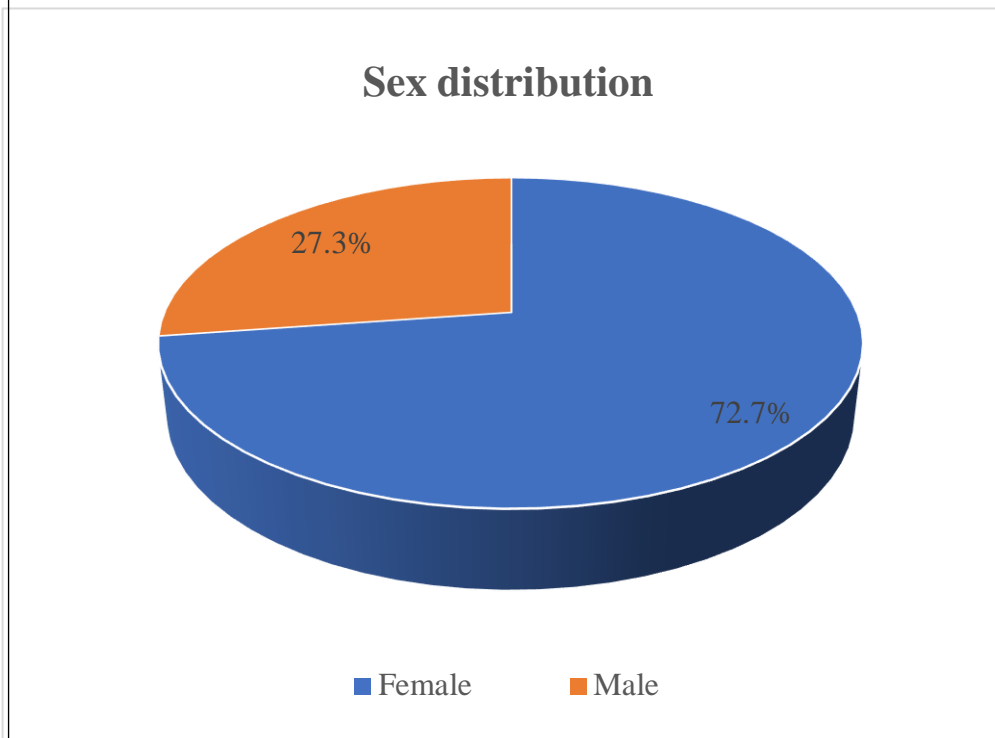
Figure 2: Pie diagram showing Sex distribution

Table 8: Anthropometry measurements distribution

	Height (Cm)	Weight (Kg)	BMI
N	33	33	33
Mean	159.18	63.52	24.98
SD	8.107	13.730	4.71

Mean height of subjects was 159.18 ± 8.107 cms, mean weight was 63.52 ± 13.73 kgs and mean BMI was 24.98 ± 4.71.
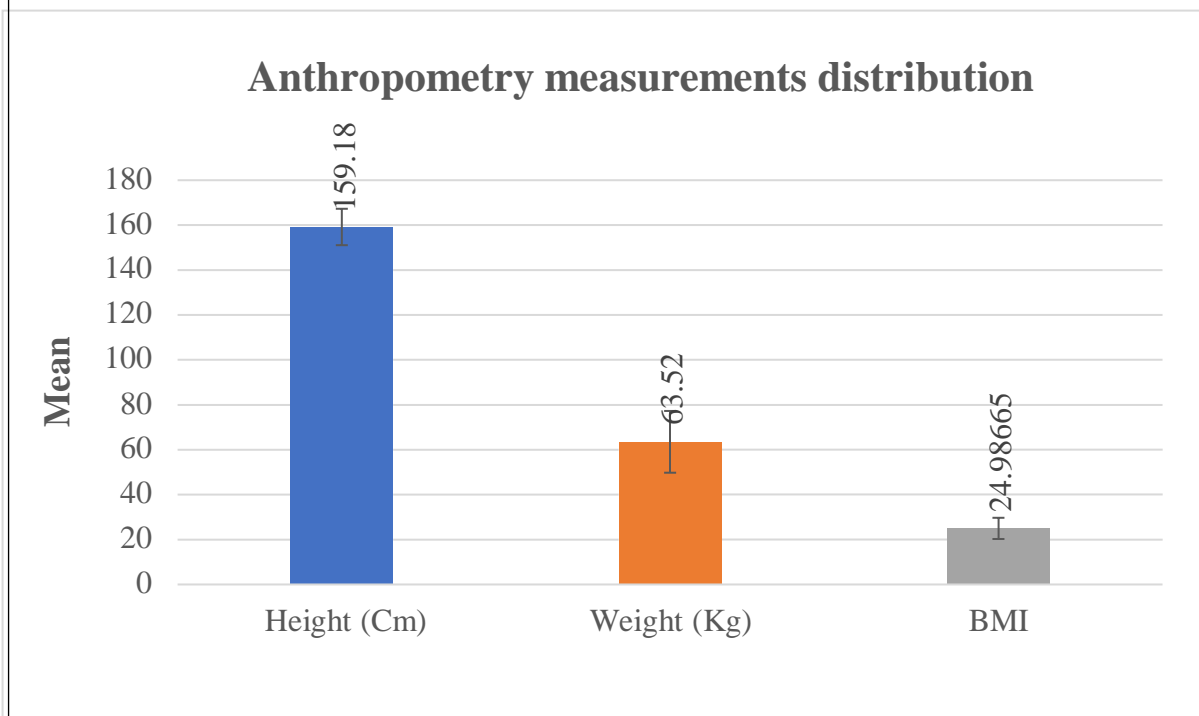
Figure 3: Bar diagram showing Anthropometry measurements distribution

Table 9: BMI distribution

			Count	%
BMI	**<18.5**	**Underweight**	**1**	**3.0%**
	18.5 to 24.9	**Normal**	**18**	**54.5%**
	25 to 29.9	**Overweight**	**10**	**30.3%**
	>30	**Obese**	**4**	**12.1%**
	Total		**33**	**100.0%**

In the study, 3% were underweight, 54.5% had normal BMI, 30.3% were overweight and 12.1% were obese.
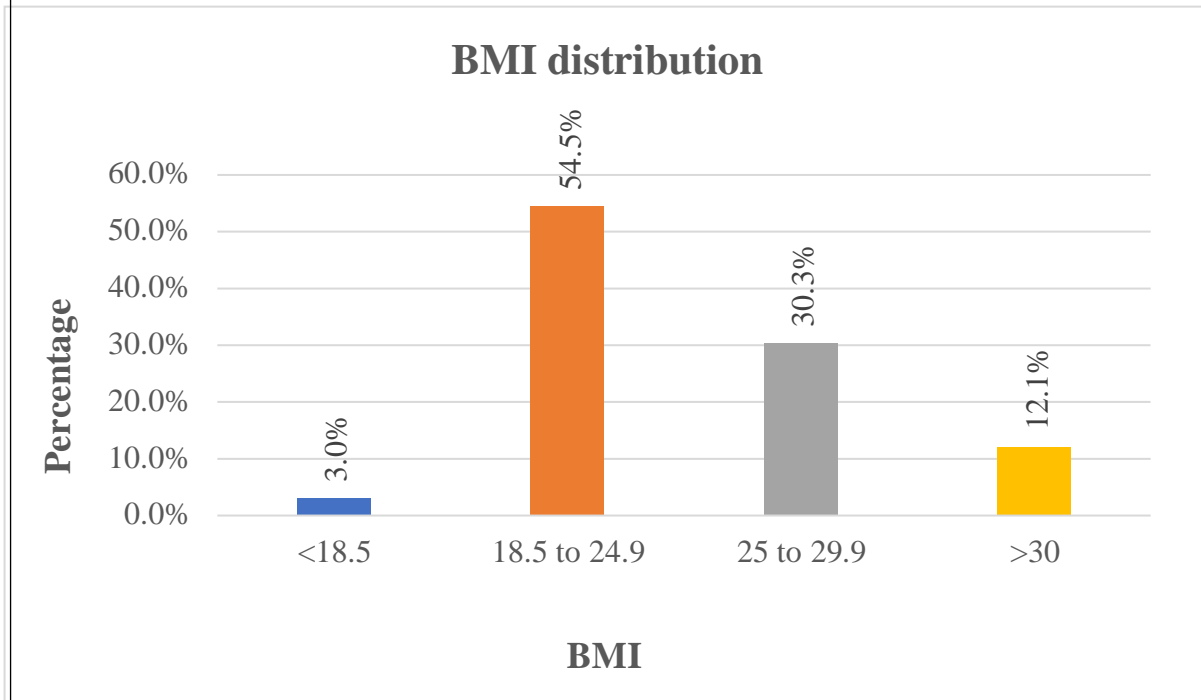
Figure 4: Bar diagram showing BMI distribution

Table10: Diagnosis of Rheumatoid arthritis

		Count	%
RA Factor	**Negative**	**6**	**18.2%**
	Positive	**27**	**81.8%**
Anti CCP	**Not done**	**27**	**81.8%**
	Positive	**6**	**18.2%**

In the study 81.8% were positive for RA factor and 18.2% were negative for RA factor.

Among 6 subjects with negative RA factor, all of them were positive for Anti CCP.
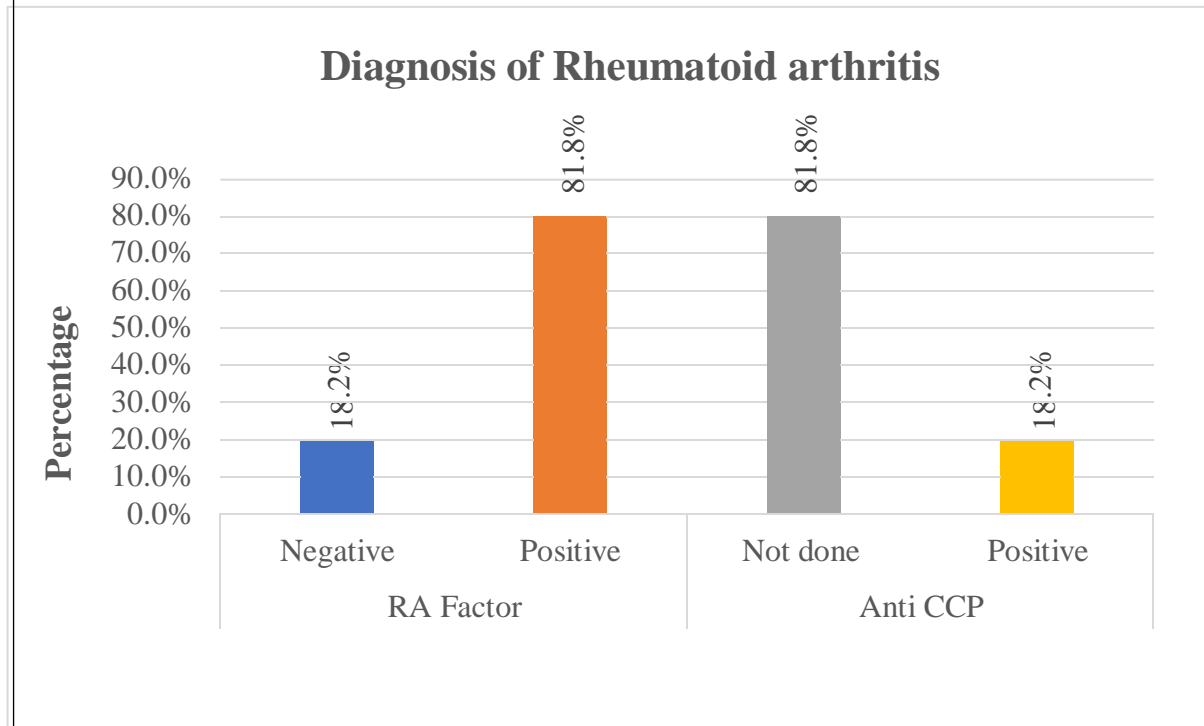
Figure 5: Bar diagram showing Diagnosis of Rheumatoid arthritis

Table 11: QFT Results distribution

		Count	%
QFT Results	**Positive**	**17**	**51.5%**
	Negative	**16**	**48.5%**
	Total	**33**	**100.0%**

In the study proportion of Latent TB based on QFT Results was 51.5%.
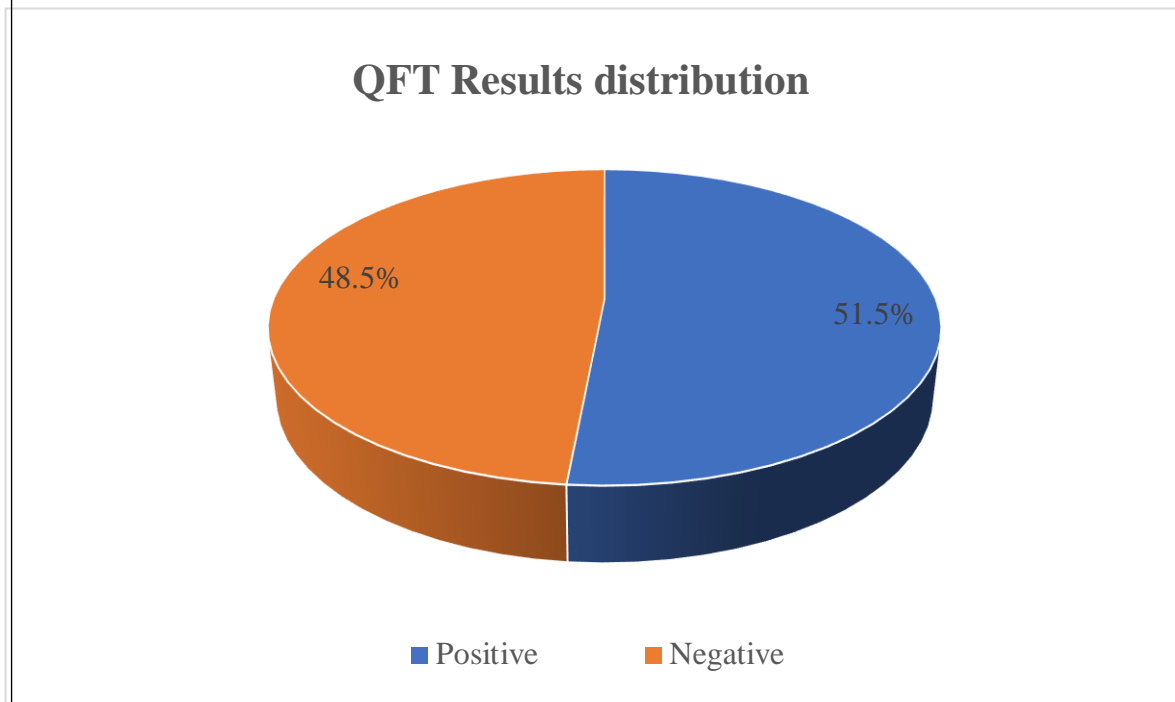
Figure 6: Pie diagram showing QFT Results distribution

Table 12:QFT and TST results

		Count	%
QFT and TST	**Both QFT and TST Positive**	**16**	**48.5%**
	TST Positive	**13**	**39.4%**
	QFT Positive	**1**	**3.0%**
	Both QFT and TST Negative	**3**	**9.1%**
	Total	**33**	**100.0%**
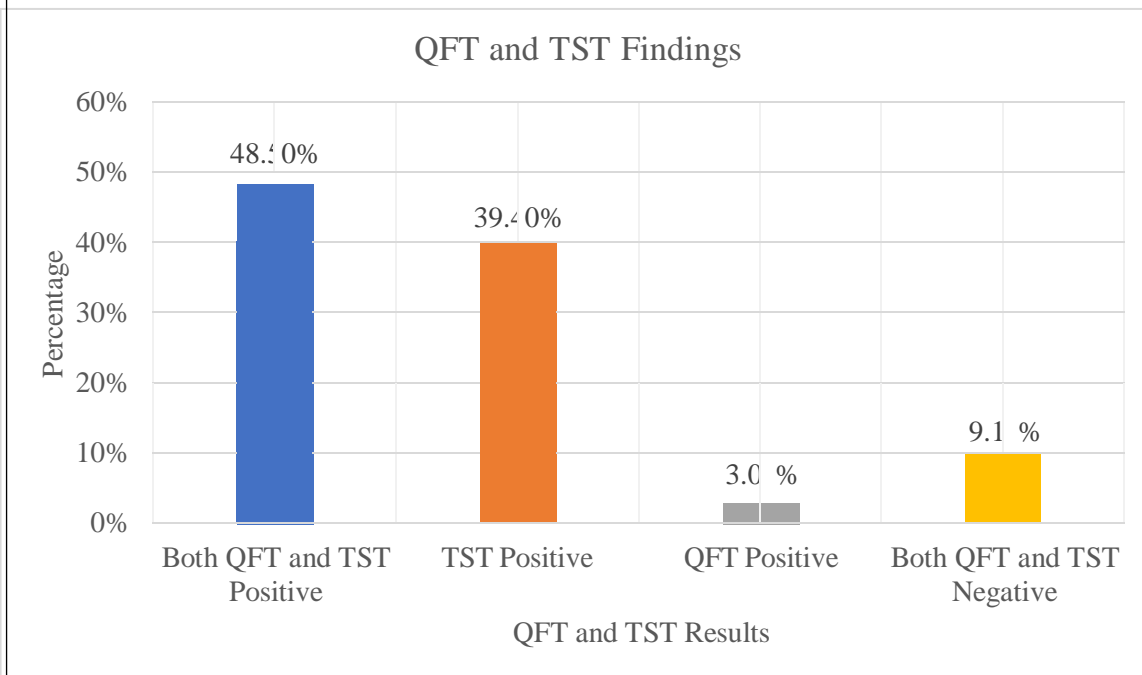
Figure 7: Bar diagram showing QFT and TST results

Table 13: Association of socio demographic profile with respect to QFT results

		QFT Results				P value
		Positive		Negative		
		Count	%	Count	%	
Age	<40 years	1	16.7%	5	83.3%	0.054
	41 to 50 years	7	50.0%	7	50.0%	
	51 to 60 years	4	50.0%	4	50.0%	
	>60 years	5	100.0%	0	0.0%	
Sex	Female	13	54.2%	11	45.8%	0.619
	Male	4	44.4%	5	55.6%	
BMI	<18.5	0	0.0%	1	100.0%	0.755
	18.5 to 24.9	10	55.6%	8	44.4%	
	25 to 29.9	5	50.0%	5	50.0%	
	>30	2	50.0%	2	50.0%	

In the study none of the factors such as age, sex and BMI were associated with Latent Tuberculosis among Rheumatoid arthritis subjects.

Among subjects aged <40 years, 16.7% had LTB, among subjects in the age group 41 to 50 years, 50% had LTB, among subjects in the age group 51 to 60 years, 50% had LTB and among subjects in the age group >60 years, 100% had LTB.

Among females, 54.2% were positive for QFT and among males, 44.4% were positive for QFT.

Among Subjects with underweight, 0% were QFT Positive, subjects with normal BMI, 55.6% were positive for QFT, among overweight subjects, 50% were positive for QFT and among obese subjects, 50% were positive for QFT.

Table 14: History of TB and its characteristics

	Yes		No	
	Count	**Row N %**	**Count**	**Row N %**
Exposure to TB	3	9.1%	30	90.9%
ATT Exposure	1	3.0%	32	97.0%
Biological Exposure	0	0.0%	33	100.0%
Symptoms of TB	1	3.0%	32	97.0%
Chest X ray	1	3.0%	32	97.0%
AFB Staining	1	3.0%	32	97.0%
Smoking	5	15.2%	28	84.8%

In the study 9.1% were Exposed to TB, 3% were given ATT, 0% had Biological Exposure, 3% had Symptoms of TB,

chest X ray findings respectively, 3% were positive in AFB staining and 15.2% were smokers.
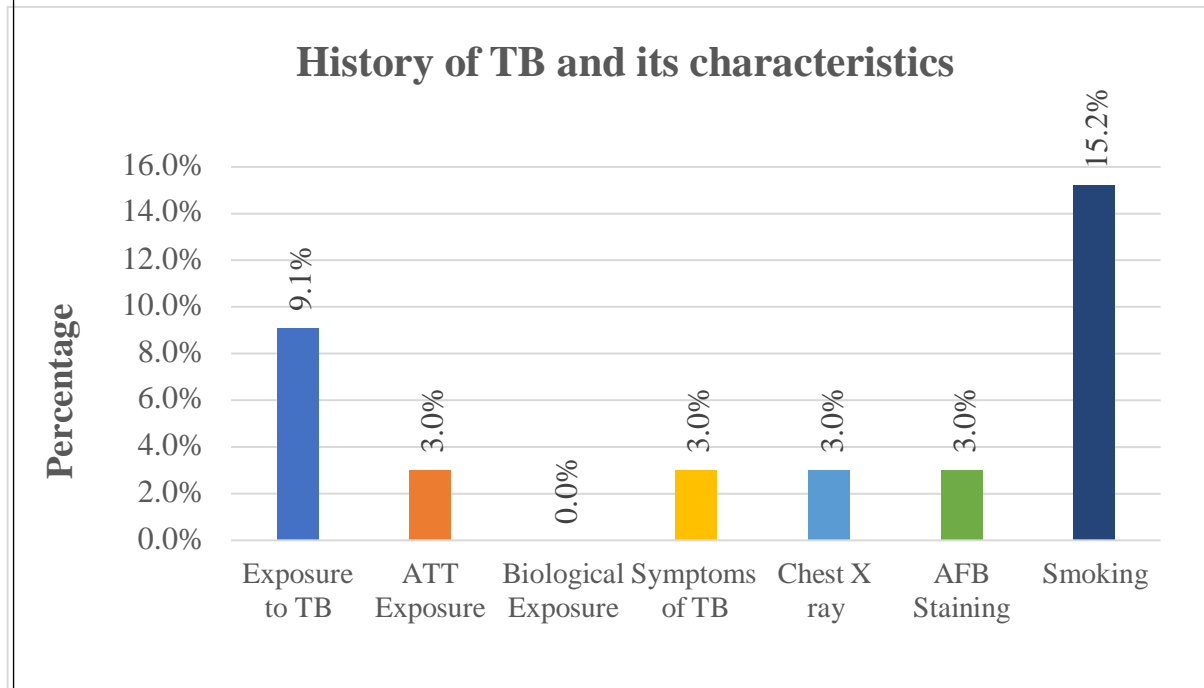
Figure 8: Bar diagram showing History of TB and its characteristics

Table 15: TST and duration from diagnosis distribution

	Mean	SD	Median
TST (mm)	9.52	3.25	9.00
Duration from Diagnosis (Months)	42.24	24.91	36.00

In the study mean TST was 9.52 ± 3.25 mm and mean Duration from Diagnosis was 42.24 ± 24.91 months.
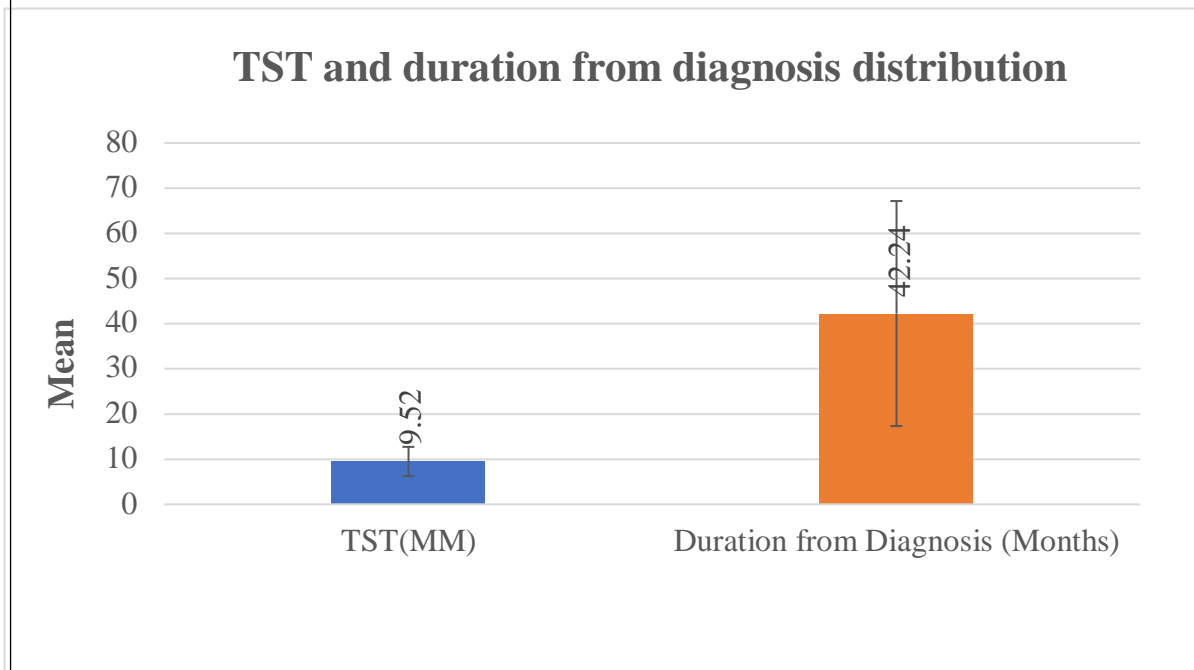
Figure 9: Bar diagram showing TST and duration from diagnosis distribution

Table 16: Steroids Usage among subjects

		Count	%
Steroids Usage	Yes	20	60.6%
	No	13	39.4%
	Total	33	100.0%

In the study 60.6% were using Steroids.
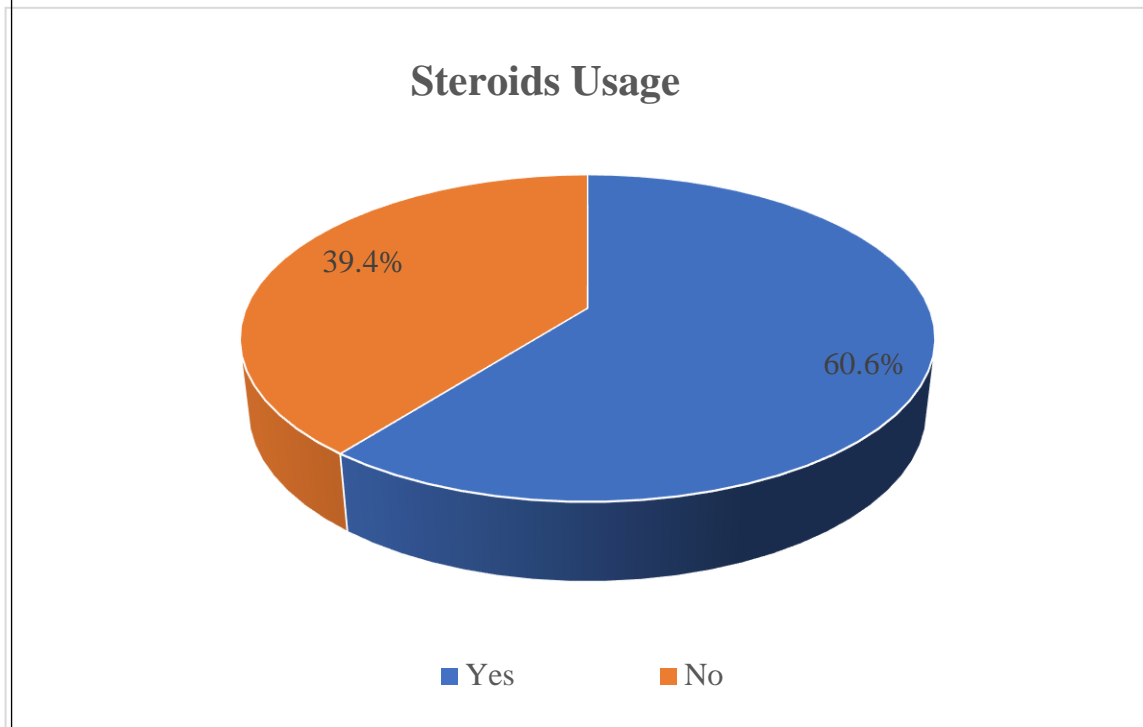
Figure 10: Pie diagram showing Steroids Usage among subjects

Table 17: Steroids dosage among subjects

	Mean	SD	Median
Last Steroid Dose Received (Mg)	**3.88**	**4.05**	**3.00**
Maximum Steroid Dose (Mg)	**5.91**	**6.24**	**6.00**
Duration of Steroid Use (Months)	**5.67**	**6.52**	**4.00**

In the study mean of last Steroid Dose Received was 3.88 ± 4.05 mg, mean Maximum Steroid Dose was 5.91 ± 6.24 mg And mean Duration of Steroid Use was 5.67± 6.52 months.
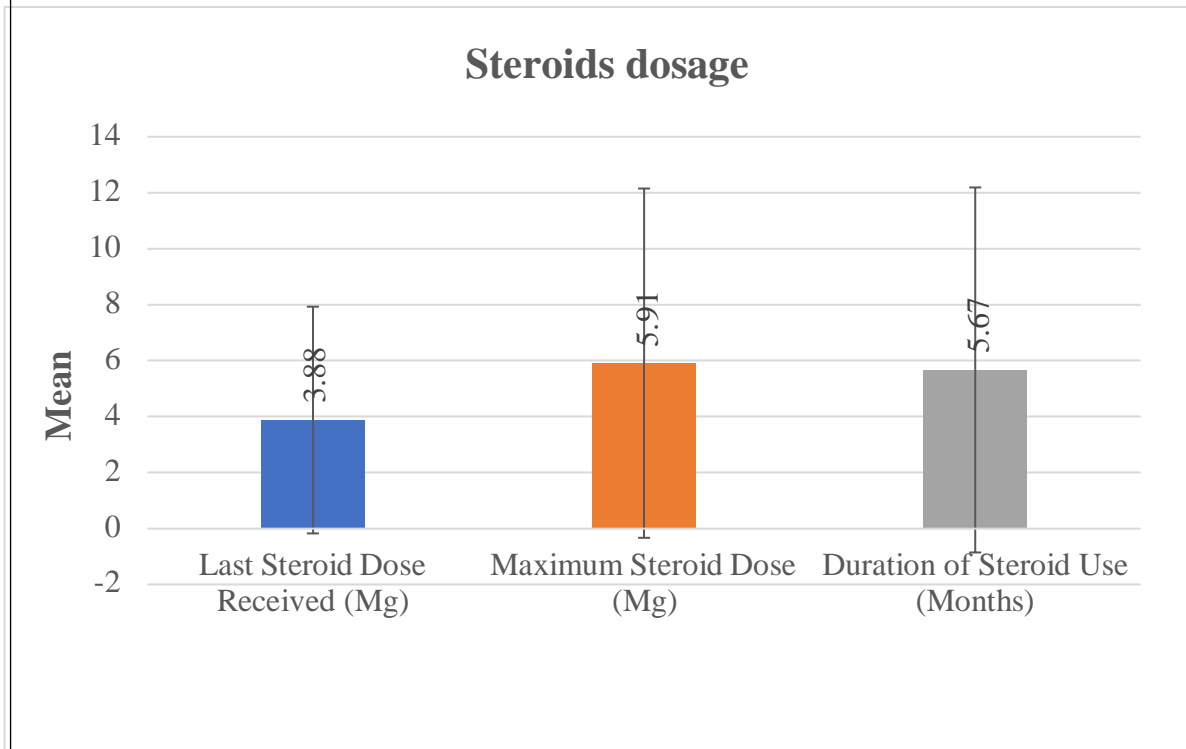
Figure 11: Bar diagram showing Steroids dosage among subjects

Table 18: Association between QFT results and steroids usage

		Steroids Usage			
		Yes		No	
		Count	Row N %	Count	Row N %
QFT Results	Positive	13	76.5%	4	23.5%
	Negative	7	43.8%	9	56.2%

$\chi 2 = 3.696$, df $= 1$, p $= 0.055$ [Chi-square test]

In the study among subjects 17 with LTB, 76.5% were using steroids and 23.5% were not using steroids. Among 16 subjects who were negative for LTB, 43.8% were using steroids usage and 56.2% not using steroids. There was no significant association between Steroids Usage and QFT results.
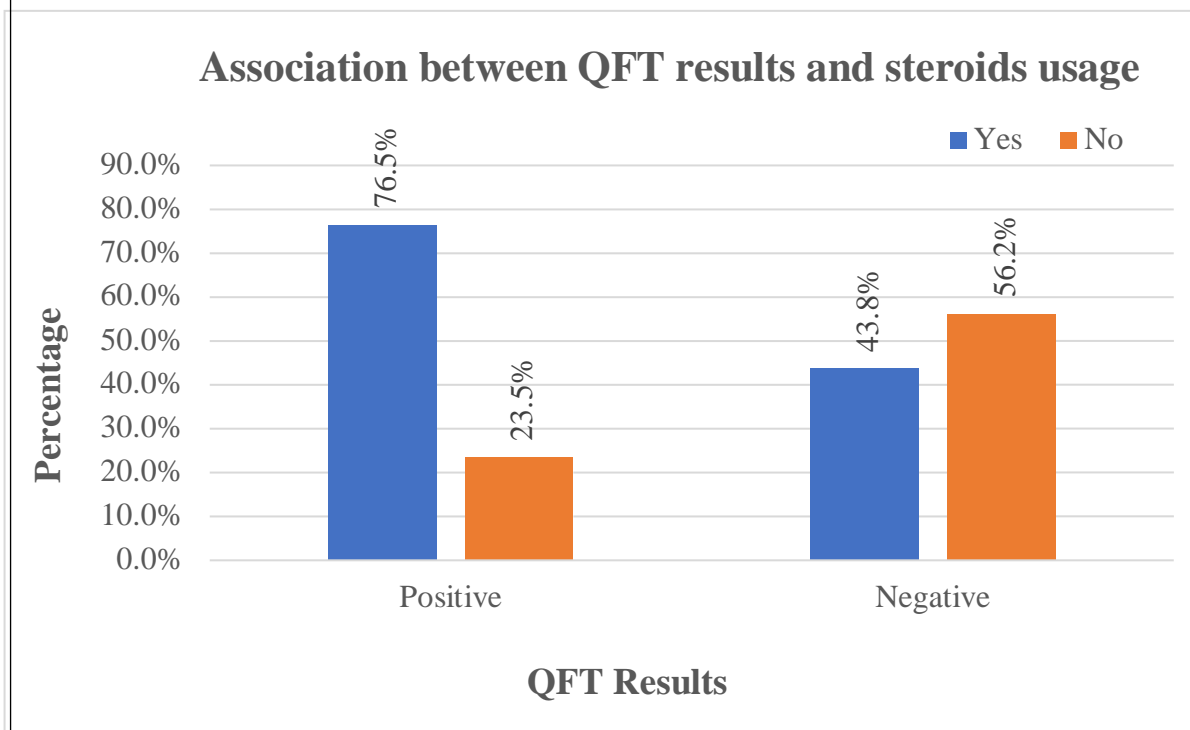
Figure 12: Bar diagram showing Association between QFT results and steroids usage

Table 19: Conversion of latent TB to Fulminant TB among steroids users in subjects with QFT Positive results

		Steroids Usage			
		Yes		No	
		Count	%	Count	%
Conversion to Fulminant TB	No	12	92.3%	4	100.0%
	Yes	1	7.7%	0	0.0%
a. QFT Results = Positive					

$\chi 2 = 0.3269$, df =1, p =0.567[Chi-square test]

In the study among 17 QFT positive subjects, 13 subjects who were using steroids, 7.7% converted to Fulminant TB and 92.3% did not convert to Fulminant TB. Among 4 subjects who were not using Steroids, 0% converted to Fulminant. There was no significant association between Steroid use and conversion to Fulminant TB.
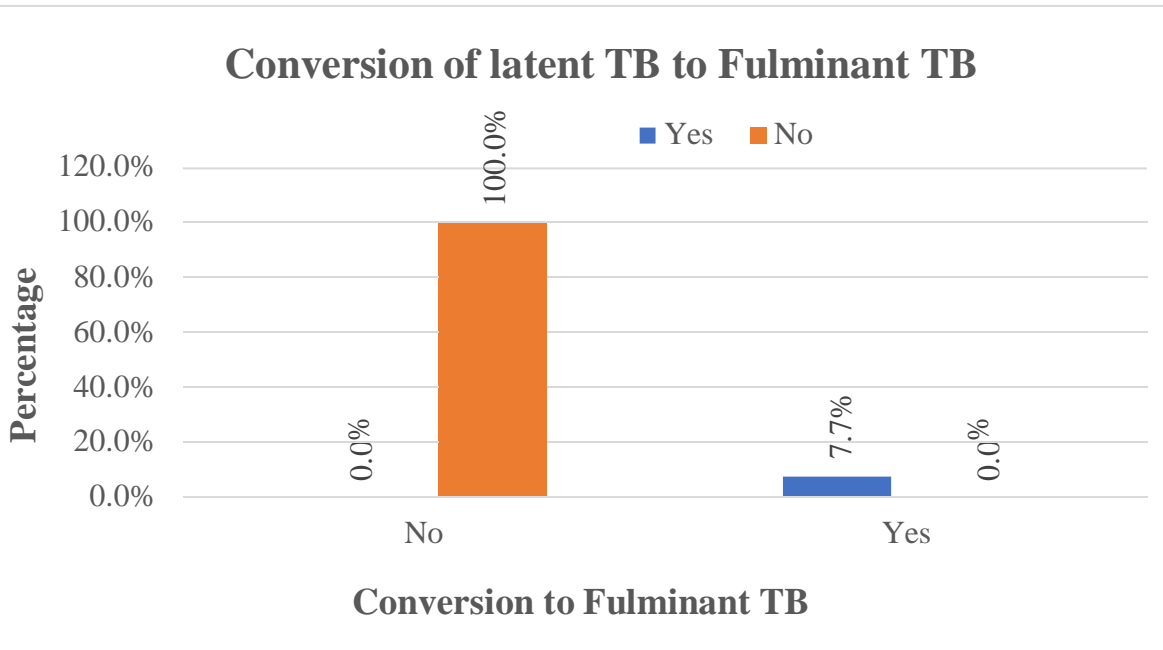
Figure 13: Bar diagram showing Conversion of latent TB to Fulminant TB among steroids users in subjects with QFT Positive results.

DISCUSSION.

Most people who are infected by mycobacterium tuberculosis have no sign or symptoms of disease and according to World Health Organization about 2 to 3 billion people worldwide are infected by Mycobacterium tuberculosis, 5 to 15% of the infected population progress from LTBI to symptomatic disease during their life time. The reactivation of LTBI is responsible for a larger population of active tuberculosis which makes diagnosis and treatment crucial especially in high risk groups(134),

 one of the High risk group being RA.

The correlation between patients with rheumatoid arthritis and latent tuberculosis was well documented by a cohort study conducted by Mehta et al(143).

Moreover, TB screening before starting DMARDs reduces the incidence of reactivation of latent TB infection by up to 85% (144,145)

The objective of our study is to find the proportion of latent tuberculosis infection in patients diagnosed with Rheumatoid Arthritis, by using Interferon Gamma Release Assay (IGRA) and Tuberculin Sensitivity Test (TST).

In our study we have considered TST and IGRA for diagnosing LTBI.

TST has a number of drawbacks; it necessitates two visits to a healthcare facility and is prone to have human errors as one tries to interprets the results. Additionally, it might be affected by a prior BCG vaccination and the immunological condition of the test subject. Due to the immune-mediated pathophysiology of RA or the immunomodulators employed in its treatment, its sensitivity may be reduced in RA patients. Also cross-reactivity with non tuberculous mycobacterial infection could affect its specificity. (146,147)

TST and IGRAs have been compared in numerous studies. Because of the higher sensitivity and lack of the confounding effect of preceding BCG vaccination, some have concluded that IGRAs are more beneficial than TSTs [64].

In the present study, the diagnosis and treatment of LTBI relied on the QFT assay rather than the TST, though TST was performed.

Also, in a study done by Malaviya et al inferred that among the 730 patients (265 rheumatoid arthritis, 400 axial spondyloarthritis ,34 psoriatic arthritis, and 31 others) who were considered for biological DMARDs, 267 (36.6%) were positive for LTBI(151).

In another study by the same investigator prevalence of LTBI was found to be 43% by performing QFT and TST in a cohort of 144 RA patients (152) which was close to the 40% in general population(150).

In our study, we found that the proportion of latent TB based on QFT Results was 51.5% and with TST results was 39.4%.

On either test being positive, the patient was treated with antimicrobial agents according to the protocol for LTBI treatment. There after DMARDs can be initiated when the treating clinician deems appropriate. If both tests return negative, DMARDs can be initiated with repeat test performed yearly during follow-up appointments.

The second objective was to find the risk of latent tuberculosis converting into fulminant tuberculosis by the use of steroids for the treatment of RA.

According to the World Health Organization , 5 to 15% of the infected population progress from LTBI to symptomatic disease during their life time.(134)

follow-up period, one patient who was tested QFT positive i;e 7.7% converted to Fulminant TB, developed into active TB in months after initiation of steroids from the previous hospital prescriptions also was tested sputum positive with changes in the chest xray.

The patient was put on Mtb regimen by the pulmonologist.

The prophylaxis of LTBI plays an important role in the prevention and treatment of TB. IGRAs and the

TST are both used to screen for LTBI, and although some studies in low-TB-prevalence areas reported a higher specificity with IGRAs than with the TST, neither method had a satisfying predictive value for active TB. In the future, a screening method with a better predictive value should be explored. High-risk factors (HIV/AIDs, transplantation, silicosis, TNF-a blockers, close contacts, kidney dialysis) contribute to a significantly increased TB reactivation rate, and for countries with a low TB prevalence, patients with high-risk factors should undergo screening and treatment for LTBI.

Therefore, screening for LTBI is strongly recommended in patients with rheumatic diseases, especially before starting treatment with anti-Tumor Necrosis Factor.

Limitations:

Our research was not without shortcomings. The details about the duration and dosage of the steroid used, or any other exposure to biological disease-modifying treatments received, were not entirely reliable and substantiated. We obtained information about the steroid dose used for treating the primary disease from patients and cross-checked it with the data available in our institution's records. As the data was primarily collected from the subjects and reliable cross-checking of the treatment provided from other medical facilities prior to attending our hospital was not possible, there may be some recall bias.

<u>Conclusion.</u>

Our findings leads us to the conclusion that the proportion of risk population of latent TB with rheumatologic diseases like rheumatoid arthritis is 51.5% and 7.7% converted to Fulminant TB. In the absence of a gold standard test to diagnose latent tuberculosis infection we would recommend testing by tuberculin skin test and IGRA to avoid not diagnosing LTBI which have potential to reactivate to activeTB disease in people with altered immune status like our study population.

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B.L.D.E. (DEEMED TO BE UNIVERSITY) SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

STUDY TITLE: Study of prevalence and Risk of Latent tuberculosis infection in patients with Rheumatoid Arthritis using Interferon Gamma Release Assay and Tuberculin Sensitivity Test – A Hospital Based Study.

Name: OP. No.:

Age: Sex:

1.   I confirm that I have read and understood the information sheet dated…   for the above study and had the opportunity to ask questions. Detailed information about the study, procedure, objectives, advantages & disadvantages, time period and other relevant information has been provided to me in the language I understand.

2.   I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3.   I understand the sponsor of the study, others working on the sponsor's behalf, investigator(s), the Ethics committee and the regulatory authorities will not need my permission to look at my health record both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to his access.

4.   I understand that my identity will not be revealed in any information released to the 3rd party or published.

5.   I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

6.   Every treatment has its own risks including death. I have not been guaranteed about anything. Even after all the precautions sometimes it becomes impossible to prevent complications for which I will not hold the doctor or the hospital responsible.

7.   I have been informed that during the study complicated situations can arise and I give the doctor and his colleagues the authority to deal with them.

8.   I have given my/my patients history of any previous allergies, disease(s) (diabetes, hypertension, asthma, bronchitis, rheumatic arthritis etc), smoking and alcohol consumption to the doctor.

9.   I consent for the use of any blood or blood product, i. v. fluid during the study.

10.   I agree to take part in the study

11.   I am also informed that this study will not affect my treatment.

12.   I have been informed that there will be no payment for participation in the study and that the study is not sponsored by any financial institution.

   Subject/Patient

   Name   Signature/Thumb Impression   Date

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Investigator

Name   Signature   Date

Witness 1

Name   Signature   Date

Witness 2

Name   Signature   Date

ANNEXURE –

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA - 586103
PROFORMA FOR DATA COLLECTION

Sr. No   Date

Name of Patient   Age   Sex

op number…………………….....

Education:

Occupation:

Address of Patient

Any Comorbid condition.

Any other joint problem.

Any immunocompromised stage.

H/O Immunosuppressive therapy.

Family history Of Tuberculosis...

Diagnosis

Rheumatoid Arthritis

Noninflammatory Arthropathy

IGRA Test .

      < 0.35 IU/ml will be negative.

      ≥ 0.35 IU/ml result positive.

      Result will be indetermined-if

      <0.35 IU/ml for TB antigens.

      <5 IU/ml for positive control.

TST Test .

      ≥ 5 mm for RA patients .

      ≥ 10 mm for controls with clinical suspicion.

      ≥ 15 mm for control without clinical suspicion.

SL.NO	NAME	AGE	Age1	SEX	HEIGHT CM	WEIGHT KG	BMI	BMI1	RA FACTOR	ANTICCP	QFTTYPE	QFT RESULTS	TST	QFTandTST	TSTMM	SteroidsUsage	ConversiontoFulminantTB
1	SOMABAI RATHOD	42	2	FEMALE	169	67	23.46	2	POSITIVE		LTB/PLASMA	1	1	1	12	0	0
2	AMBUJA GOVIND BARAJADAR	48	2	MALE	165	75	27.55	3	POSITIVE		LTB/PLASMA	1	1	1	9	1	0
3	MAYAKKA CHAVAN	61	4	FEMALE	145	42	19.98	2	POSITIVE		LTB/PLASMA	1	1	1	6	1	0
4	BANU R	48	2	FEMALE	155	54	22.48	2	POSITIVE		LTB/PLASMA	1	1	1	9	1	0
5	SHRIDEVI DAYANAND	45	2	FEMALE	152	60	25.97	3	POSITIVE		LTB/PLASMA	1	1	1	9	1	0
6	YASTER METRI	45	2	MALE	180	95	29.32	3	NEGATIVE	POSITIVE	LTB/PLASMA	2	1	2	10	0	0
7	KASIYAMMAL	63	4	FEMALE	155	68	28.30	3	POSITIVE		LTB/PLASMA	1	1	1	13	1	1
8	BALASUBAMANIYAM V.	45	2	MALE	159	60	23.73	2	POSITIVE		LTB/PLASMA	1	1	1	14	1	0
9	THULASIAMMA N.	25	1	FEMALE	150	65	28.89	3	NEGATIVE	POSITIVE	LTB/PLASMA	2	2	4	4	1	0
10	RANI R.	41	2	FEMALE	164	62	23.05	2	POSITIVE		LTB/PLASMA	1	2	3	5	0	0
11	PADMA N K	62	4	FEMALE	170	66	22.84	2	POSITIVE		LTB/PLASMA	1	1	1	12	1	0
12	NEELAVATHI K.	54	3	FEMALE	155	70	29.14	3	POSITIVE		LTB/PLASMA	2	1	2	9	0	0
13	SABITA ROY	28	1	FEMALE	154	56	23.61	2	NEGATIVE	POSITIVE	LTB/PLASMA	2	1	2	8	1	0
14	KARUNA J S	55	3	FEMALE	156	75	30.82	4	POSITIVE		LTB/PLASMA	1	1	1	14	0	0
15	BUDDHADEV BERA	46	2	MALE	175	80	26.12	3	NEGATIVE	POSITIVE	LTB/PLASMA	2	1	2	9	0	0
16	AJAY KUMAR SINHA	63	4	MALE	165	81	29.75	3	POSITIVE		LTB/PLASMA	1	1	1	8	0	0
17	VASUGI H A.	46	2	FEMALE	163	67	25.22	3	POSITIVE		LTB/PLASMA	1	1	1	16	1	0
18	ANINDITA ROY	33	1	FEMALE	160	55	21.48	2	POSITIVE		LTB/PLASMA	2	2	4	4	1	0
19	VASANTHA. T	60	3	FEMALE	155	45	18.73	2	POSITIVE		LTB/PLASMA	2	2	4	2	1	0
20	SUFIA BEGUM	42	2	FEMALE	165	58	21.30	2	POSITIVE		LTB/PLASMA	2	1	2	8	0	0
21	MAHBOOB BASHA.	37	1	MALE	165	53	19.47	2	POSITIVE		LTB/PLASMA	1	1	1	14	1	0
22	MALLAVVA V.	64	4	FEMALE	155	55	22.89	2	POSITIVE		LTB/PLASMA	1	1	1	12	1	0
23	MD AASHIM	41	2	MALE	169	59	20.66	2	POSITIVE		LTB/PLASMA	2	1	2	10	1	0
24	KALAWATHI J.	52	3	FEMALE	150	50	22.22	2	POSITIVE		LTB/PLASMA	1	1	1	13	1	0
25	SHAKUNTALA BAI.	56	3	FEMALE	161	83	32.02	4	POSITIVE		LTB/PLASMA	2	1	2	9	1	0
26	KARIAMMA H.	50	2	FEMALE	157	100	40.57	4	POSITIVE		LTB/PLASMA	2	1	2	6	1	0
27	SANTHAVVA G.	52	3	FEMALE	155	73	30.39	4	POSITIVE		LTB/PLASMA	1	1	1	9	1	0
28	SUSHILA R.	35	1	FEMALE	155	56	23.31	2	POSITIVE		LTB/PLASMA	2	1	2	11	0	0
29	DIPAK KUMAR MAITY	55	3	MALE	160	59	23.05	2	POSITIVE		LTB/PLASMA	2	1	2	9	0	0
30	MALATI SHAW	45	2	FEMALE	154	53	22.35	2	NEGATIVE	POSITIVE	LTB/PLASMA	2	1	2	12	0	0
31	PREMA J H.	59	3	FEMALE	145	42	19.98	2	POSITIVE		LTB/PLASMA	1	1	1	12	1	0
32	CHANDA BAI P.	50	2	FEMALE	150	62	27.56	3	NEGATIVE	POSITIVE	LTB/PLASMA	2	1	2	9	0	0
33	SUNIL RAVIDAS	21	1	MALE	165	50	18.37	1	POSITIVE		LTB/PLASMA	2	1	2	7	0	0

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	S	T	U	V	W	X	Y
1	DURATION FROM DIAGNOSIS MONTHS	LAST STEROIDDOSE RECEIVED MG	MAXIMUM STEROID DODE MG	DURATION OF STEROID USE MONTHS	EXPOSURE TO TB	ATT EXPOSURE	BIOLOGICAL EXPOSURE
2	72	0	0	0	NO	NO	NO
3	60	9	9	4	NO	NO	NO
4	82	12	12	8	NO	NO	NO
5	60	12	1	3	NO	NO	NO
6	36	12	1	8	NO	NO	NO
7	6	0	0	0	NO	NO	NO
8	72	6	9	24	YES	NO	NO
9	36	6	12	12	NO	NO	NO
10	4	9	9	3	NO	NO	NO
11	46	0	0	0	NO	NO	NO
12	84	6	6	12	NO	NO	NO
13	34	0	0	0	NO	NO	NO
14	12	6	6	8	NO	NO	NO
15	46	0	0	0	YES	YES	NO
16	8	0	0	0	NO	NO	NO
17	68	0	0	0	NO	NO	NO
18	72	3	9	12	YES	NO	NO
19	12	3	6	3	NO	NO	NO
20	36	6	12	6	NO	NO	NO
21	28	0	0	0	NO	NO	NO
22	24	5	12	8	NO	NO	NO
23	24	5	10	8	NO	NO	NO
24	10	6	24	24	NO	NO	NO
25	48	5	6	5	NO	NO	NO
26	36	6	12	9	NO	NO	NO
27	48	1	15	14	NO	NO	NO
28	72	9	15	10	NO	NO	NO
29	60	0	0	0	NO	NO	NO
30	48	0	0	0	NO	NO	NO
31	9	0	0	0	NO	NO	NO
32	36	1	9	6	NO	NO	NO
33	21	0	0	0	NO	NO	NO
34	84	0	0	0	NO	NO	NO

	Z	AA	AB	AC
1	SYMPTOMS OF TB	CXR	AFB STAINING	SMOKING
2	NO	NO	NO	NO
3	NO	NO	NO	YES
4	NO	NO	NO	NO
5	NO	NO	NO	NO
6	NO	NO	NO	NO
7	NO	NO	NO	NO
8	NO	YES	YES	YES
9	NO	NO	NO	NO
10	NO	NO	NO	NO
11	NO	NO	NO	NO
12	NO	NO	NO	NO
13	NO	NO	NO	NO
14	NO	NO	NO	NO
15	NO	NO	NO	NO
16	NO	NO	NO	NO
17	NO	NO	NO	NO
18	NO	NO	NO	NO
19	NO	NO	NO	YES
20	NO	NO	NO	NO
21	NO	NO	NO	NO
22	NO	NO	NO	NO
23	NO	NO	NO	NO
24	NO	NO	NO	YES
25	NO	NO	NO	YES
26	NO	NO	NO	NO
27	NO	NO	NO	NO
28	NO	NO	NO	NO
29	NO	NO	NO	NO
30	NO	NO	NO	YES
31	NO	NO	NO	NO
32	NO	NO	NO	NO
33	YES	NO	NO	NO
34	NO	NO	NO	NO