TO STUDY THE INFLAMMATORY MARKERS IN ACTIVE PULMONARY TUBERCULOSIS, IT'S CORRELATION WTH DISEASE SEVERITY AND IT'S RESPONSE TO ANTI TUBERCULAR TREATMENT

Dissertation submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the requirements for the degree of

Doctor of Medicine (M.D) in Tuberculosis and Respiratory Diseases

Branch – XVII

GOVERNMENT KILPAUK MEDICAL COLLEGE & HOSPITAL

CHENNAI, TAMIL NADU

APRIL 2019

BONAFIDE CERTIFICATE

This is to certify that the dissertation "To study the inflammatory markers in active pulmonary tuberculosis, it's correlation with disease severity and its response to anti tubercular treatment" is the Bonafide work done by **Dr. K.RAJARAJESWARI** during her **MD (Tuberculosis and Respiratory Diseases)** course from May 2016 to April 2019 at Government Kilpauk Medical College, Chennai.

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DECLARATION BY THE GUIDE

This is to certify that the dissertation titled "To study the inflammatory markers in active pulmonary tuberculosis, it's correlation with disease severity and its response to anti tubercular treatment" is the bonafide work done by **DR.K.RAJARAJESWARI,** during her MD **(Tuberculosis and Respiratory Diseases)** course in the academic years 2016-2019 at Government Kilpauk Medical College, Chennai, under my guidance

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DECLARATION BY THE SCHOLAR

I**, Dr. K.RAJARAJESWARI,** solemnly declare that the dissertation titled "To study the inflammatory markers in active pulmonary tuberculosis, it's correlation with disease severity and its response to anti tubercular treatment" has been prepared by me. This is submitted to "**The Tamil Nadu Dr. M.G.R. Medical University, Chennai"** in partial fulfillment of the requirement for the award of M.D degree examination branch XVII **Tuberculosis and Respiratory Diseases** from May 2016 to April 2019.

Place: CHENNAI **(**Dr. K.Rajarajeswari)

Date: Signature of the candidate

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INTRODUCTION

TUBERCULOSIS thought to be one of the oldest human diseases, the history of TB is atleast as old as the mankind over the years, not only the medical implications but also the social and economic impact of TB has been **TUBERCULOSIS** has been a major cause of suffering and death since times immemorial. (1)

At the dawn of the new millennium, we are still mute witnessess to the silent yet efficient march of this sagacious disease, its myriad manifestationsand above all its unequalled ,vicious killing power through the millennia.TB never ever disappeared from the developing world.(1)

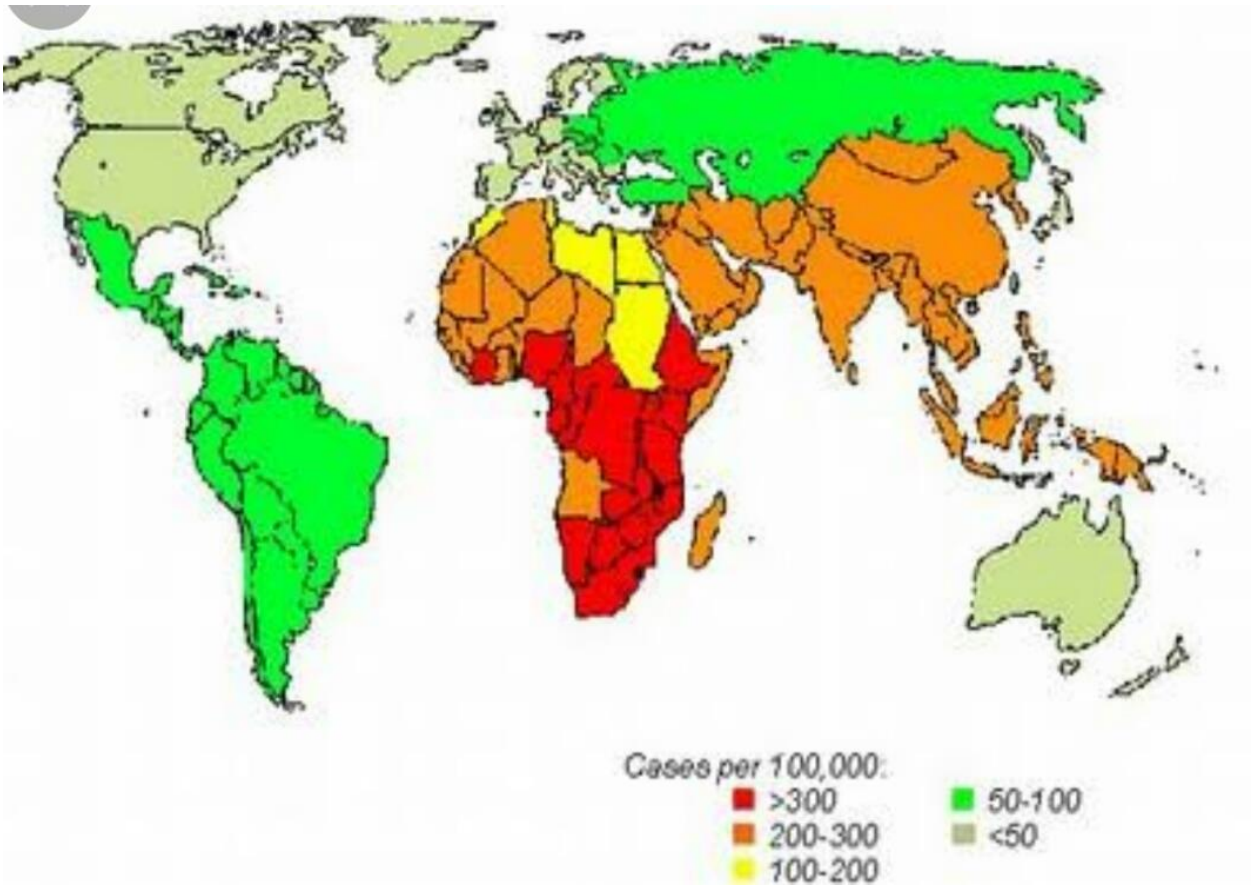
Most technologically advanced world has seen more than a century of declining incidence of tuberculosis but the less developed world has not been so fortunate and in those populous regions TB incidence is increasing. In acquired immunodeficiency region (AIDS) stricken regions – sub Saharan Africa, For example –TB case rates now are 50-100 times those in North America and Europe. Spurred by immune deficiencies resulting from HIV infection, these case rates will continue to increase in coming decades.

The history of Tuberculosis reveals that this disease has swept across large regions of the world in slowly moving epidemic waves with periods measured in centuries. The factors contributing to the rise and decline of these waves are only partially known and are difficult to control in the absence of massive and political changes. There is however, much that we might learn from considering the historical spread of TB and the ways in those countries now favoured with low TB incidences managed their high incidence problems in their region.

In pulmonary tuberculosis patients are dependent on sputum smear/culture based monitoring of treatment, but this will be challenging to use in extra pulmonary TB, and in patients with pauci -bacillary disease such as is seen in HIV-coinfected patients and in children. Both sputum volume and quality decreases in response to treatment and many patients cannot provide sputum samples for culture/smear after a few weeks of treatment. The development of non-sputum-based biomarkers of treatment response would represent an advance for individual monitoring of TB patients.

Hence we proposed that these inflammatory markers will be an indespensible tool in assessement of tuberculosis disease severity and their fall in response to treatment will be a surrogate marker of disease control. We selected the inflammatory marker CRP-c-reactive protein, ESR-erythrocyte sedimentation rate, NLR- neutrophil lymphocyte ratio, RDW-red cell distribution width in our study and proceeded.

BURDEN OF TUBERCULOSIS



The regions shown in red are very high burden countries, that is the incidence rate is >300//1,00,000, they are mostly African countries. India belongs belong to high burden country having incidence rate of> 200-300 per 1 lac people.

India accounts for one fourth of global tuberculosis incidence, 2.2 million out of 9.6 million cases annually. In India more than 40% are infected with tuberculosiswith

mycobacterium tuberculosis (prevalence of infection). It is estimated that there are 2.5 million prevalent cases of all form of tuberculosis. it is also estimated 2.2 lakhs people die of TB annually.

	Incidence	Prevalence	mortality
Global	9.6 million 176/lac year	13 million 227/lac /year	1.1 million 21/lac /year
India	2.2 million 167/lac/year	2.5 million 197/ lac/year	2.2 lakhs 17/ lac/year

- Tuberculosis (TB) is one of the top 10 causes of death worldwide.
- In 2017, 10 million people fell ill with TB, and 1.6 million died from the disease (including 0.3 million among people with HIV).
- Millions of people fall sick with TB each year,there were cases and age groups but overall 90% were adults,9% were those living with HIV and 2/3rd were in eight countries ; **INDIA (27%),**china (9%),Indonesia (8%), the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa amounting to 4-7 %
- In 2017, an estimated 1 million children became ill with TB and 230 000 children died of TB (including children with HIV associated TB).
- TB is a leading killer of HIV-positive people.

- TB is a leading killer of HIV-positive people: in 2016, 40% of HIV deaths were due to TB.
- Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. WHO estimates that there were 5,58 000 new cases with resistance to rifampicin – the most effective first-line drug, of which - 82% had MDR-TB.
- Globally, TB incidence is falling at about 2% per year. This needs to accelerate to a 4–5% annual decline to reach the 2020 milestones of the End TB Strategy
- An estimated 54 million lives were saved through TB diagnosis and treatment between 2000 and 2017.
- Ending the TB epidemic by 2030 is among the health targets of the Sustainable Development Goals [12]

Impact Indicators for the National Strategic Plan 2017 – 2025

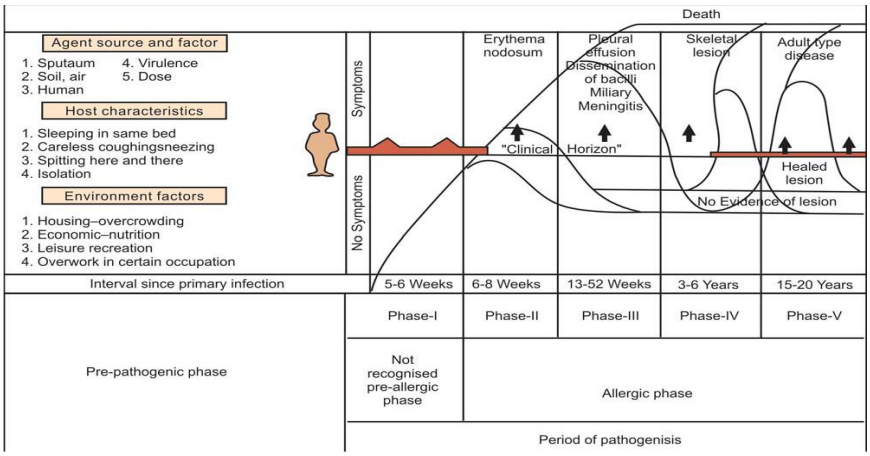
	2015	**2020**
To reduce estimated **TB incidence** (rate per 100,000)	217	142
To reduce estimated **TB prevalence** (rate per 100,000)	320	170
To reduce estimated mortality due to **TB** (per 100,000)	32	15

TUBERCULOSIS IMMUNO PATHOGENESIS

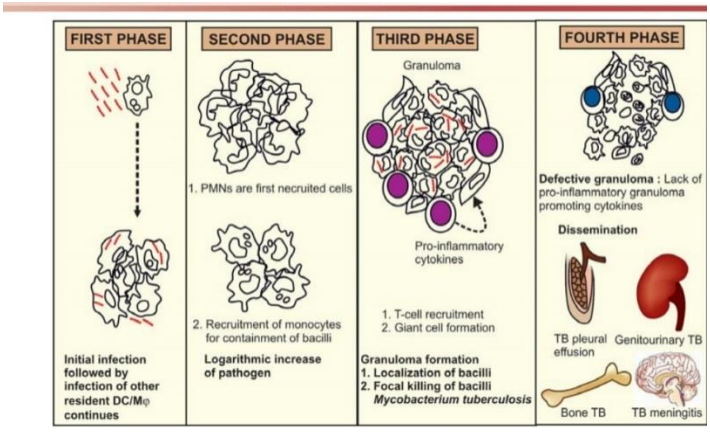
TB is a communicable infectious disease, spread almost exclusively by coughed aerosols carrying pathogens from the mycobacterium tuberculosis complex. TB is characterized pathologically by necrotizing granulomatous inflammation usually in the lung, although any extra pulmonary site can be involved.

Protective immunity and varied clinical manifestations of infection with mycobacterium tuberculosis represent a delicate balance between the bacillus and the type as well as magnitude of immune response elicited by the host.[1]The immune system is extremely complex and some of the intricacies of its function are not completely understood.Essentially tuberculosis is a struggle between the macrophage and bacilli[2]

The epidemiology of TB in a community is the resultantof the interplay between the environmental conditions,socioeconomic state of the population, the host factorsand the agent characteristics. The course of the eventsfollowing infection as observed in humanbeings, followed by that in the community at large.[1]



The above figure shows the natural history of tuberculosis.



EVENTS DURING TB INFECTION

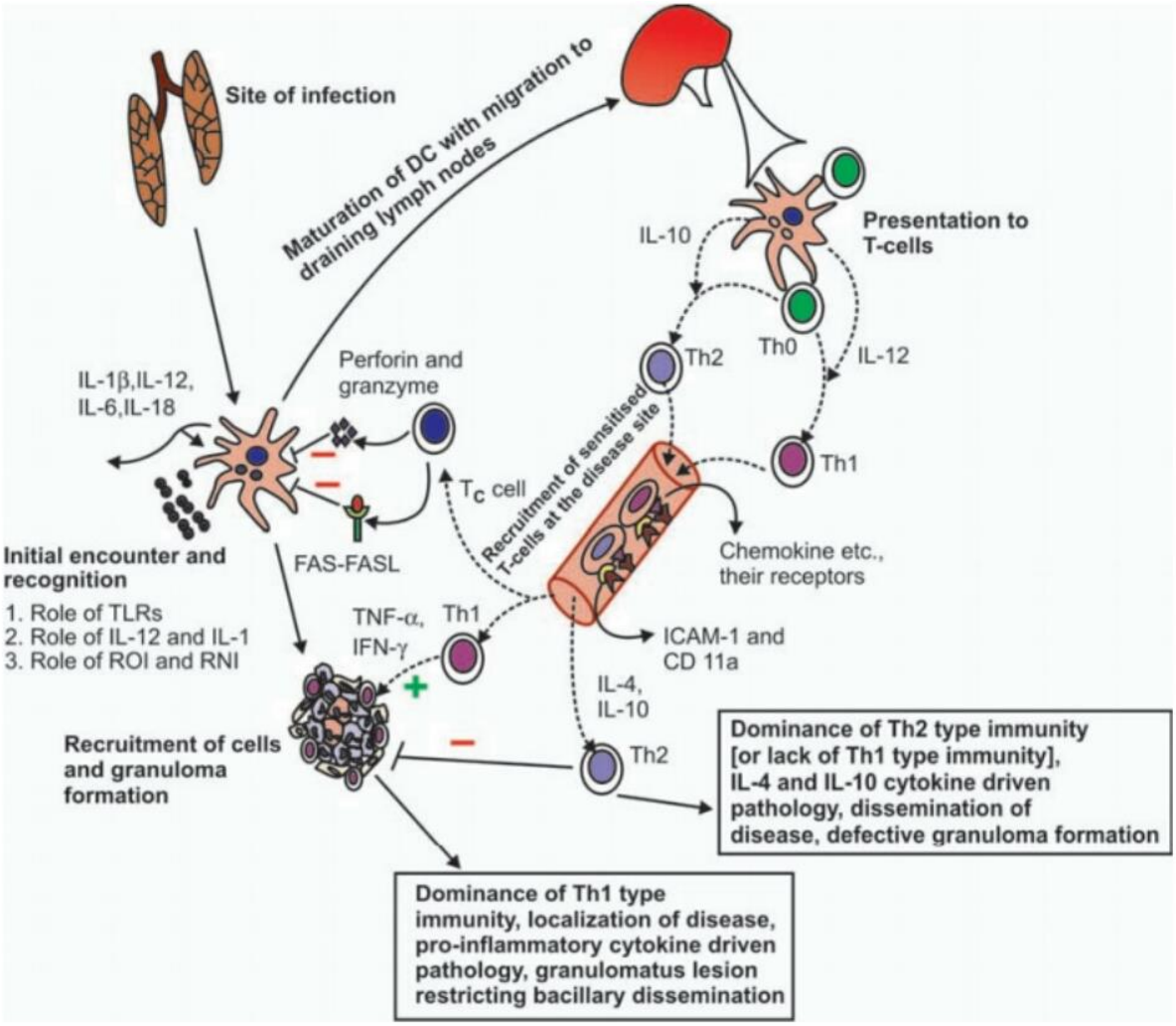
. Broadly, TB infection is divided into four phases: First phase includes an initial establishment of *Mycobacterium tuberculosis* infection in the resident macrophages [alveolar]. This is followed by influx of PMNs, which prevents *Mycobacterium tuberculosis* to escape from the innate immune factors. Subsequently, monocytes are recruited to the site of infection/ pathological site [second phase]. Third phase includes granuloma formation. Core of granuloma is made up of multinucleated giant cells and

elongated epithelioid cells. These are surrounded by T-cells. This is aimed at restricting the bacilli from spreading. The fourth and terminal phase includes dissemination of bacilli. Defective granuloma formation promotes release of bacilli from control of immune system. Organs/loci targeted by bacilli after dissemination are listed

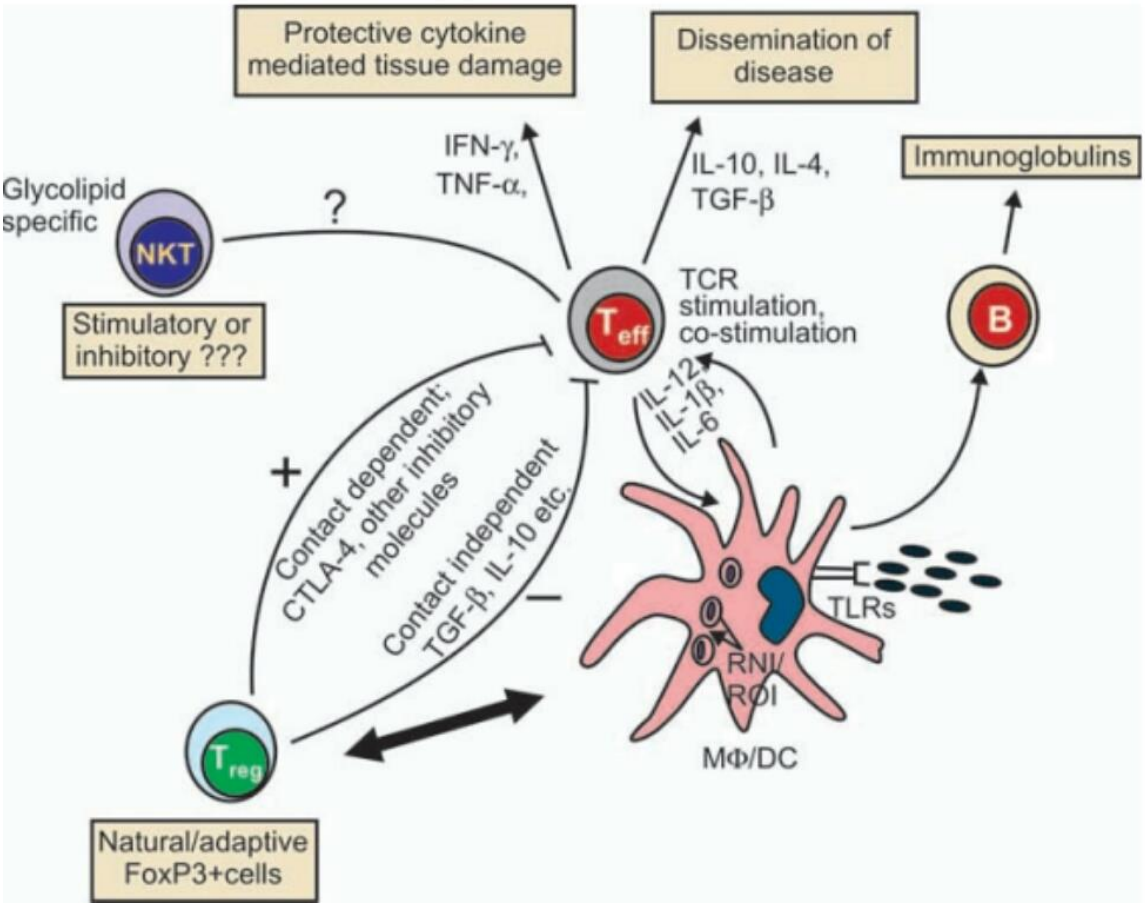
The cytokine network and its interactions are complex in the setting of infection by mycobacterium tuberculosis. Conventional understanding supports a pivotal role for cytokines in the overall immune responses to TB. Although cytokines are produced by the wide variety of cells, primed lymphocytes are the major producers. Particularly two sub populations of CD4+ T lymphocytes - the T helper type 1 (TH) cells that produce IL-2 , IFN –gamma, lympho-toxin alpha,and other proinflammatory cytokines,and the T helper type 2 (TH-2), cells that produce IL-4,IL-5,IL-10,IL-13,and other anti-inflammatory cytokines.(3)

In the immune response,the cytokines produced participate in the regulatory processes as well as in effector functions and are involved in the activation and proliferation of macrophages and T lymphocytes. Thus specific antigens to mycobacteria interact with toll like receptors (TLRs) and other receptors present on surface of macrophages and dendritic cells, thus inducing a cellular immune response predominantly pro-inflammatory.(4)

After infection, innate immune responses try to put check on increasing infection. Meanwhile immature DC s after taking up antigen, move towards the draining lymph nodes and antigen recognition and presentation to t cells occur inside the regional/draining lymphnodes. Recruitment of antigen specific T cells at the pathological sites and production of pro-inflammatory cytokines lead to granuloma formation. Later on depending on the presence of TH1/TH2 skewed response, dissemination or containment of bacilli occurs.



IMMUNE RESPONSE IN TUBERCULOSIS PATHOGENESIS



Orchestration of effector immune component in building up response against *Mycobacterium tuberculosis*. Priming of T-cellsfor effector functions requires recognition by activated macrophages with stimulation by a battery of potentiating cytokines [IL-12, IL-6, IL-1β etc.,] released by the infected macrophages. The response of effector T-cells will decide the extreme/polarity of upcoming disease pathogenesis. Regulatory T-cells [CD4+CD25+FoxP3+ T-cells, natural killer T-cells etc.,] can also modulate the protective effector T cell responses either in a contact dependent/independent way.

The inflammatory reaction is accompanied by a systemic response known as acute phase response (APR).This response is characterized by fever, production of different types of hormones, leukocytosis and protein synthesis in series, which is rapidly regulated mainly in hepatocytes under the control of cytokines arising from the site of the pathology. Thus this acute phase response bring about alteration in hematological indices like leukocytosis, neutrophilia, lymphopenia, thrombocytosis, red cell distribution width, platelet distribution width, haematocrit, plateletocrit, erythrocyte sedimentation rate, etc. and acute protein like c-reactive protein ,serum amyloid A etc. These serum markers of inflammation rise above the range of normal during active infection at par with the severity of infection and will fall when the infection severity comes down.

In pulmonary tuberculosis patients are dependent on sputum smear /culture based monitoring of treatment,but this will be challenging to use in extra pulmonary TB, and in patients with pauci -bacillary disease such as is seen in HIV-coinfected patients and in children. Both sputum volume and quality decreases in response to treatment and many patients cannot provide sputum samples for culture/smear after a few weeks of treatment. The development of non-sputum-based biomarkers of treatment response would represent an advance for individual monitoring of TB patients.

Hence we proposed that these inflammatory markers will be an indespensible tool in assessement of tuberculosis disease severity and their fall in response to treatment will be a surrogate markers of disease control. We selected the inflammatory marker CRP-C-reactive protein, ESR-erythrocyte sedimentation rate, NLR- neutrophil lymphocyte ratio, RDW-red cell distribution width in our study and proceeded.

REVIEW OF LITERATURE

In a study done by Abakay o et al inflammation on 2015…..they aimed to investigate the correlation between red cell distribution width (RDW), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and other inflammatory markers with PTB severity. 70 patients with active PTB were compared with 50 age and gender matched healthy control people without any illness. The mean age of PTB patients in their study were 50.4 ± 21.8 years. There were no significant differences in terms of age, gender, and smoking history between PTB patients and controls. Patients with advanced PTB had a significantly higher white blood cell count, neutrophil count, Red cell distribution width, Neutrophil lymphocyte ratio, and CRP. When compared to patients with mild to moderate PTB. RDW (17.7 versus 15.7 %, p=0.002) and NLR (4.7 versus 3.1, p=0.009) values were higher in patients with advanced PTB as opposed to patients with mild to moderate PTB. NLR and RDW levels may be used as markers of inflammation to help clinically manage patients with TB and to determine disease severity[(9)]

In a study done by Furuhashi k,et al.kakkaku.2012…they studied that Few inflammatory markers closely reflect the activity of tuberculosis and only a few surrogate markers are available. They wanted to clarify the usefulness of measuring the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum amyloid A (SAA), and the association between these markers and T-cell profiles. They enrolled One hundred one patients with active pulmonary tuberculosis. The associations between ESR, CRP,

and SAA values on admission and radiological and microbiological findings and T-cell profiles were assessed. Th1/Th2 and Tc1/Tc2 were determined by analyzing intracellular cytokine staining for IFN-gamma and IL-4 in blood CD4+ and CD8+ T cells using flow cytometry after stimulation with PMA and ionomycin. There found a significant correlations between ESR, CRP, and SAA of which the correlation between CRP and Serum amyloid A was strong (r = 0.88). CRP values significantly correlated with the sputum smear scale and the extent of lesions, and were higher in bilateral lesions. SAA values correlated with the sputum smear scale, whereas all markers were higher in patients with pleural effusion. Both CRP and SAA levels negatively correlated with the ratio of Th1/Th2. In contrast, ESR negatively correlated with the ratio of Tc1/Tc2.C reactive protein reflected the disease severity before treatment. CRP and SAA values were associated with helper T-cell proportions whereas ESR was associated with cytotoxic T-cell proportions, both being type 2 predominant. [(10)]

In a study done by mohd yousoof dar et al ….. they evaluated the immulogical markers namely serum C reactive protein and interferon gamma levels in cases of active pulmonary tuberculosis and the effect of anti -tubercular treatment on the level of these markers.They did a cross sectional study was on total of 54 patients of sputum positive pulmonary tuberculosis and also 10 healthy controls. C reactive protein and INF-¥ was performed on sputum samples and blood by ELISA method. The Mean Serum CRP level in Controls were 2.15±1.3 mg/dl. In cases it was found to be 21.71±6.73 mg/dl at

baseline. The markers Levels decreased further at 2 months to 7.70±4.29 mg/dl and 2.41±1.5 mg/dl at end of 6 months ($p < 0.001$)

The mean Serum Interferon -¥ levels in cases at baseline were 41.47±43.25 pg/ml compared to the controls 1.81±1.6 pg/ml ($p < 0.003$). After treatment at 2 months the serum IFN-¥ levels after was 12.53±8.33 pg/ml and at end of 6 months it was 2.47±1.55 pg/ml ($p < 0.001$). Serum Interferon-¥ levels was comparable to controls at the end of 6 months. Mean sputum IFN-¥ levels at baseline was 24.92±16.53 pg/ml. It decreased after 2 months of treatment to 7.68±5.17 pg/ml and at end of 6 months to 3.86±3.12 pg/ml ($p < 0.001$).thus they concluded that the Patients with pulmonary TB have significantly elevated serum CRP and IFN-¥ levels compared to normal controls and levels of these makers decrease significantly with treatment. Hence they concluded serum CRP and IFN-¥ (both serum and sputum) may be useful in early detection of disease and in monitoring the response.[13]

In a study done by hamida et al…..The study group has 44 men. The mean age was 41 years in the two groups. Tobacco smoking was more common in the group of patients with elevated CRP. In both groups, clinical features were dominated by decreased general status and cough. Cavitary lung lesions were more frequent in patients with elevated CRP (16 cases vs 3 cases) often with an extensive and bilateral pulmonary involvement (15 cases vs 3 cases).Similarly, acid-fast bacilli positivity found in sputum were more frequent in the group of patients with elevated CRP (22 cases vs 6 cases).After excluding patients lost during the treatment, well clinical, bacteriological and radiological

evolutions were noted in the term of six months in the majority of patients with CRP <50 mg / l (11 cases vs 10 cases). In 4 patients with elevated CRP, there was a delay (more than1month) to bacilli become negative. Thus CRP can be used as marker of disease activity and to assess treatment response.[15]

In a study done at Ethiopia..bamalkku et al…..Their aim of the study was to determine hematological profiles of TB patients before and after intensive phase treatment. The hematological profiles of TB patients studied. They showed statistically significant difference in hematocrit (38.5 % versus 35.7 %), hemoglobin (12.7 g/lversus11.8 g/l) and platelet (268×10^3/μlversus239×10^3/μl) values of patients before initiation of treatment and after completion of the intensive phase of tuberculosis treatment, respectively. The red cell distribution width (RDW) of treatment naïve TB patients was by far lower (17)(17.6 ± 7.09 %) than the corresponding RDW (31.9 ± 5.19 %) of intensive phase treatment completed patients. Among TB patients that had high platelet distribution width (PDW) ($n = 11$) before initiation of TB treatment, 10 demonstrated lower PDW values after completion of the intensive phase. There was no significant difference on total white blood cell count among TB patients before and after completion of the 2 month treatment.They concluded that the hematological abnormalities in TB patients to be monitored throughout the treatment. The levels of hemoglobin, hematocrit and platelet count of the TB patients were significantly lowered after completion of the intensive phase of TB treatment. Significant variation of the RDW and PDW were also observed among treatment naïve and after completion of treatment. Hematological abnormalities

resulted from TB treatment should be assessed continuously throughout the course of tuberculosis therapy.[16)]

In a study done by marina oliveria et al…..in patients with active pulmonary tuberculosis they found low hemoglobin levels (mean, 10.86 ± 2.04 g/dL) in 89.2% of patients; low transferrin levels (mean, 177.28 ± 58.71 mg/dL) in 65.3%; and low MCV (mean, 82.00 ± 7.77 fL) in 39.7%. In addition, we found high ferritin levels (mean, 520.68 ± 284.26 ng/mL) in 52.7% of patients; high RDW (mean, 16.36 ± 3.47%) in 55.4%; high CRP levels (mean, 5.84 ± 4.22 mg/dL) in 98.2%; and high ESR (mean, 60.30 ± 39.84 mm/h) in 84.3%. they concluded that the prevalence of anemia gives rise to increased RDW.[(17)]

In a study done by yin y et al at china….they studied pretreatment neutrophil lymphocyte ratio in pulmonary tuberculosis. According to the ROC curve, the best cut-off value of NLR was 2.53, with a sensitivity of 70.6% and a specificity of 45.4%. The NLR ≥ 2.53 before anti-TB treatment was associated with PTB retreatment (OR = 1.994, 95% CI: 1.116-3.564; adjusted OR (AOR) = 2.409, 95% CI: 1.212-4.788). The retreatment rates with NLR ≥ 2.53 and NLR < 2.53 were 27.1% and 15.5%, respectively, with a significant difference (log-rank test; $p = 0.010$). Additionally, cavitation on chest X-ray (OR = 2.922, 95% CI: 1.654-5.411; AOR = 2.482, 95% CI: 1.230-5.007), history of smoking (OR = 2.202, 95% CI: 1.158-3.493; AOR = 2.321, 95% CI: 1.135-4.745) and age ≥ 60 (OR = 3.828, 95% CI: 1.626-9.015; AOR = 2.931, 95% CI: 1.122-7.653) were also associated with PTB retreatment. NLR ≥ 2.53 is predictive of PTB retreatment.[(18)]

In a study done by pyrosila Miranda they studied 134 pulmonary TB patients …they measured ferritin and CRP levels …they found Circulating levels of both ferritin and CRP gradually decreased over time on ATT. At pre-treatment, concentrations of these parameters were unable to distinguish patients with positive from those with negative acid-fast bacilli (AFB) in sputum cultures. However, patients who remained with positive cultures at day 60 of ATT exhibited heightened levels of these inflammatory markers compared to those with negative cultures at that time point.CRP and Ferritin levels in serum may be useful to identify patients with positive cultures at day 60 of ATT [19]

IN a study done by leem ay et al….. …they studied the changes in multiple cytokines and inflammatory markers in active TB patients following anti-TB drug therapy. Twenty-nine patients with active TB were recruited prospectively between December 2010 and July 2017. Blood samples were collected before (T_0), after 2 months (T_2), and at the end of anti-TB treatment (T_{end}). We measured the levels of Interferon (IFN)-γ, interleukin (IL)-2, IL-12, IL-10, IL-13 and tumor necrosis factor (TNF)-α in supernatants collected from the QuantiFERON-TB Gold In-Tube assay (QFT-GIT), as well as the WBC, neutrophil, platelet count and neutrophil to lymphocyte ratio (NLR) in whole blood. Compared with baseline levels, WBC, neutrophil, and platelet counts were significantly lower following treatment. In addition, the NLR after treatment significantly decreased compared with baseline, whereas the IL-2/IFN-γ ratio increased after treatment. In conclusion, the levels

of IL-2/IFN-γ ratios in the supernatant and the NLR might be useful biomarkers to evaluate the effectiveness of drug therapy in active TB patients.[20]

A study was done to explore the claim that anisocytosis as measured by the red blood cell volume distribution width (RDW) is raised in iron-deficiency anemia, but is normal in the anemia of chronic disorders. In a study done by banes et al Measurements were done for 283 normal patients, 22 iron-deficient patients, and 102 tuberculosis patients, using a model S plus Coulter Electronic Counter with standard calibration. Mean (+/- standard deviation values for RDW were as follows: normal patients, 7.36 (+/- 0.57); untreated patients iron deficiency, 10.39 (+/- 1.37); and 15 not treated anemic patients with tuberculosis, 10.44 (+/- 0.63). Mean values remained above 10.4 in 69 patients with tuberculosis during treatment, irrespective of whether or not the patients are anemic, but value ad fallen towards the normal mean (8.49 +/- 0.8) in the 18 subjects in whom it was measured at the end of therapy. By this time the mean corpuscular volume (MCV) and hemoglobin concentrations had risen to normal. It is concluded that Red cell distribution width values in the chronic inflammatory disorder tuberculosis are not significantly different from those occurring in iron-deficiency anemia.[21]

In a Russian study .they studied Two hundred and forty-three patients with different forms of active pulmonary tuberculosis patients. The level of C-reactive protein (CRP) was determined by immune turbidimetry. C reactive protein normal values were taken to be 0-3 mg/l. The increased CRP levels revealed in 80.7% of the patients formed two peaks in the ranges of 4-5 (25.1%) and 21-100 (28.9%) mg/l. The higher values of

CRP with the maximum of 239 mg/l were found in 11.1% of patients. The values of CRP clearly correlated with the degree of intoxication, the presence and rate of bacterial discharge, the extent of the process, the absence or presence of decay. By the end of an intensive phase of chemotherapy (3 months) in case of its efficiency, the level of C reactive protein significantly decreased thus approaching to the upper clinical range (12.1 +/- 1.9 mg/l) whereas in ineffective treatment it was substantially unchanged (22)

In a study done by RAO et al they found that C-reactive protein levels were significantly higher in smear-positive group as compared with the smear-negative group, the values being 37.598 ± 23.195 and 5.40 ± 1.88 respectively ($P < 0.0005$). Among the smear-positive patients, CRP levels were highest in Smear3+ group (60.00 ± 15.69) as compared with the Smear2+ patients (35.83 ± 8.9) and Smear1+ (5 ± 7.86). Statistically, the difference was found to be significant ($P< 0.0005$). Correlation of CRP levels with extent of disease also revealed that these values were significantly higher in stage III disease (52.44 ± 17.78) as compared with stage II (13.19 ± 13.03) and stage I disease (9.5 ± 9.01). Serum CRP levels may have a role in identifying the advanced and extensive disease patients thereby indirectly helping the health workers to pick up delayed convertors/potential defaulters, so as to guide them to put in extra efforts on these groups, in tuberculosis control programs.(23) In a study done by RAO et al they found thatC-reactive protein levels were significantly higher in smear-positive group as compared with the smear-negative group, the values being 37.598 ± 23.195 and 5.40 ± 1.88 respectively ($P < 0.0005$). Among the smear-positive patients, CRP levels were highest in

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This study done by chio et l at Department of Internal Medicine, Armed Forces Capital Hospital, Seoul, Republic of Korea. The physicians are frequently faced with the difficult task of differentiating between pulmonary tuberculosis (PTB) and pneumonia. They evaluated the role of the C-reactive protein test (CRP) for differentiating between TB and pneumonia among military personnel in South Korea. Only immune competent males were eligible. Forty-six patients with Pulmonary tuberculosis and 67 with pneumonia were enrolled prospectively. Median C reactive protein concentration was somewhat lower in patients with TB than in patients with non-tuberculous pneumonia (3.2 mg/dl [range 0.1-15.7 mg/dl] vs. 8.3 mg/dl [range 0.2-33.7 mg/dl], P < 0.001). The sensitivity and specificity for TB of a low CRP concentration (< 11.2 mg/dl) in serum was 93.3% and 40.9%, respectively. CRP concentration measurement might be useful for eliminating the diagnosis of TB.[24]

In a study done by mohammed kalih sekh et al: The present study was conducted to evaluate the C-reactive protein (CRP) in patients with pulmonary tuberculosis at Liaquat University Hospital, Hyderabad, Pakistan. All patients with pulmonary tuberculosis > 12 years of age, of either gender were evaluated for their serum CRP level. During twelve months study period, 127 patients with pulmonary tuberculosis were evaluated for CRP level, of which 76 (60%) were males and 51 (40%) were females. The observed symptoms were cough and expectoration 115 (91%), weakness and constant fatigue 92(72%), weight loss 110 (86%), fever 90(71%), night sweats 85(67%), chest pain 95(75%), coughing up blood 98(77%), loss of appetite 88(69%), headache 102(80%) and combined / mixed symptoms 100(79%). The overall mean CRP in patients with TB was 9.87±4.83 where as it was 11.21±3.32 and 13.82±4.63 in male and female subjects respectively. The mean±SD of normal and raised serum CRP was 2.76±1.34 and 13.26±4.42 (p 0.01). The complications observed in patients with raised serum CRP were pneumothorax 04(4.7%), fibrosis 08(9.3%), miliary TB 06(7%), empyema 10(11.6%), fungal colonization within tuberculous cavity 09(10.5%), bronchiectasis 11(12.8%), more than 01 complication 11(12.8%) and Nil 19(22.1%). The present study detected elevation of CRP in pulmonary tuberculosis and a high CRP is clearly associated with more severe disease.(25)

In a study done at brazileliene et al Circulating levels of cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α) and C-reactive protein (CRP), as well as values of erythrocyte sedimentation rate (ESR) were measured in cryo preserved serum samples obtained from

73 PTB patients at pre-ATT and day 60 of treatment. Changes of the immune profile over time were compared with mycobacterial loads in sputum and culture conversion at day 60 of ATT. Additional analyses tested associations between improvement of chest radiographic lesions at day 60 and pre-treatment status of inflammation and mycobacterial loads.Within the inflammatory parameters evaluated, values of c reactive protein, interlukin-2, Iinterlukin-4, Tumour necrosis factor-α and Erythrocyte sedimentation rate significantly decreased upon treatment initiation. On the converse, IL-10 levels substantially increased at day 60 of ATT, whereas concentrations of IL-6 and IFN-γ remained unchanged. Multidimensional analyses revealed that ESR, IL-2, IL-4 and C reactive protein were the parameters with the highest power to discriminate individuals before and after treatment initiation, they demonstrated that higher bacterial loads in sputum at pre-Anti tuberculous treatment were associated with increased systemic inflammation and higher risk for positive *M. tuberculosis* sputum cultures at day 60 of treatment. Furthermore, we found that pre-ATT mycobacterial loads in sputum and systemic inflammation synergistically associated with the status of radiographic lesions during treatment (Relative risk for chest X-ray improvement: 10.0, 95 % confidence interval: 2.4–40.0, $P = 0.002$). The mycobacterial loads in sputum are directly associated to the status of systemic inflammation and potentially impact the immune profile, the culture conversion and the evolution of lung lesions upon Anti tuberculous treatment initiation [(26)]

In a study done by yoon et al …THEY identified nine unique studies enrolling 1793 adults from out-patient (five studies, 1121 patients) and in-patient settings (five studies, 672 patients), 72% of whom had confirmed HIV infection. Among out-patients, CRP had high sensitivity (93%, 95%CI 88-98) and moderate specificity (60%, 95%CI 40-75) for active PTB. Specificity was lowest among in-patients (21%, 95%CI 6-52) and highest among out-patients undergoing TB screening (range 58-81%). There was no difference in summary estimates by HIV status CRP, which is available as a simple, inexpensive and point-of-care test, can be used to screen PLHIV presenting for routine HIV/AIDS (acquired immune-deficiency syndrome) care for active TB.[27].

In a study at bt yaji han et alA total of 96 patients were enrolled. Seventeen patients (18%) died during hospitalization due to miliary TB, and 9 (9%) died additionally during the 1-year follow-up period. Eighteen patients (19%) were diagnosed with acute respiratory distress syndrome (ARDS). In multiple logistic regression analyses, increased NLR was associated with ARDS [adjusted odds ratio, 1.15; 95% confidence interval (CI), 1.03–1.28]. By multivariate Cox regression analysis with adjustment of known prognostic factors including age, sex, body mass index, serum aspartate aminotransferase (AST), and hemoglobin, NLR was an independent predictor of in-hospital mortality [adjusted hazard ratio (aHR), 1.08; 95% CI, 1.03–1.13] and 1-year mortality (aHR, 1.08; 95% CI, 1.05–1.12) Pre-treatment NLR at admission may be a useful biomarker for mortality and development of ARDS in patients with miliary TB. (28)

In a study done by mahalakshmamma et al at telengana…..they studied erythrocyte Sedimentation Rate (ESR) as a marker for inflammation in the body. It is the initial test carried out in order to detect infection using Westergrens tube in which ante cubital venous blood is mixed with sodium citrate as an anticoagulant. This study was performed in, pulmonary tuberculosis patients who were taking treatment in hospital and normal young healthy individuals were randomly selected as controls. They were divided into 3 Groups, Group-1 normal and pulmonary tuberculosis patients, Group-2 before treatment and after treatment, Group-3 contains BMI for Both Groups, mean SD and p values are statistically studied by using ANOVA. ESR may help to find out the prognosis of the disease. Determination of Erythrocyte sedimentation rate after the treatment with anti tubercular drugs helps in determine the effectiveness of the drugs used and there by estimate the prognosis of the disease From the above observations it can be concluded that the ESR significantly increases in pulmonary tuberculosis and with adequate treatment with anti tubercular agents (drugs), the ESR significantly decrease also. Tuberculosis is a destructive disease as well as a chronic disease and therefore, fibrinogen and gamma globulin levels increase .which will increase the rouleaux formation and thereby ESR. Greater the level higher is the ESR and which may help to find out the gravity of the disease process. Determination of ESR after the treatment with anti-tubercular drugs help determine the effectiveness of the drugs used and thereby the prognosis if it is good or bad. Anti tubercular drugs (streptomycin, INH, rifampicin) that have been used are the drugs that effectively decrease

In a study done by Charles et al….They studied the extent and severity of hematological and biochemical abnormalities which occurred in 265 patients with pulmonary tuberculosis, and records the hematological changes that occur with treatment. Anaemia was present in 60 per cent of patients, which more frequently in males than in females. Leucocytosis with neutrophilia occurred in 40 per cent, lymphopenia in 17 per cent and monocytopenia in 50 per cent. Platelet count and erythrocyte sedimentation rate were elevated in 52 and 80 per cent respectively. Bone marrow aspiration and trephine biopsy were of limited diagnostic value. Ferritin and vitamin B_{12} levels were increased in 94 and 57 per cent of subjects respectively whilst serum and red cell folic acid were within normal limits in 83 per cent. The frequency of the important biochemical changes were hyponatremia (43 per cent) and hypoalbuminemia (72 per cent); alkaline phosphatase, aspartic transaminase and lactic dehydrogenase levels were elevated in approximately a third of patients possibly due to unsuspected dissemination.

There was a close correlation between the acid-fast bacilli in sputum and abnormal values, particularly those of body weight, hemoglobin, platelet count, white cell count and erythrocyte sedimentation rate. Failure of these indices to return to normal was invariably associated with persistent excretion of acid-fast bacilli. They have shown that hematological and biochemical abnormalities in pulmonary tuberculosis are common and may be valuable aids to diagnosis. Some hematological markers also reflect response to treatment.[32]

In a study done by singh kj et al…they felt that Hematological changes associated with tuberculosis have been incompletely investigated, so In the present study, they have compared peripheral blood and bone marrow findings in patients with disseminated/miliary tuberculosis (DTB/MTB) as well as pulmonary tuberculosis (PTB). They have also attempted to assess the effect of anti-tuberculosis therapy on the hematologic abnormalities. Thirty two patients with disseminated/miliary tuberculosis and 23 patients with pulmonary tuberculosis had been prospectively studied to determine the various hematological manifestations in tuberculosis and the effect of anti-tuberculosis therapy. All patients received standard antituberculosis treatment. They were subjected to a detailed hemogram including peripheral blood examination, which was repeated on completion of anti -tuberculous therapy. Bone marrow aspiration and biopsy was also done in all patients before starting ATT

Normocytic normochromic anemia was the most common abnormality observed in all the groups and subgroups (DTB/MTB 84%, PTB 86%). Other hematological abnormalities of the white blood cells include leucopenia (DTB/MTB 25%, PTB 0%; $p < 0.02$), neutropenia (DTB/MTB 22%, PTB 0%; $p < 0.04$), lymphocytopenia, monocytopenia, leukocytosis, neutrophilia, lymphocytosis and monocytosis. Pancytopenia was observed only in patients with disseminated/miliary tuberculosis ($p < 0.05$). Thrombocytopenia was more common in patients with disseminated/miliary tuberculosis ($p < 0.007$), whereas thrombocytosis was more common in patients with pulmonary tuberculosis ($p < 0.04$). The patients of disseminated/miliary tuberculosis with

granulomas in the bone marrow had certain significant differences as compared to patients without granulomas. These patients showed severe anemia, peripheral monocytopenia and bone marrow histiomonocytosis. The hemogram reverted to normal with antituberculosis therapy in these patients.

In view of the varied hematological abnormalities observed in patients with tuberculosis in this part of the world, they concluded that the differential diagnosis of tuberculosis should be entertained in patients with varied hematological disorders. [33]

In a study done at Babylon…. They aimed to study the changes of some hematological parameters in patients affected with pulmonary tuberculosis in Babylon province. 90 patients with PTB (45 males and 45 females) and 40 healthy controls (20 males and 20 females) have included in present study. Patients have been classified into three groups: thus group1 includes newly diagnosed patients, group 2 includes patients after two months from starting treatment and group 3 includes patients after six months from starting treatment. The mean of ages was 44 ± 2 years for patients and 42 ± 2 years for the control. This study found that values of Hb, PCV, platelets and ESR for both sexes were significantly changed in group 1 in comparison with group 2. Values of Hemoglobin, Packed cell volume, platelets and ESR for both sexes were significantly changed in group 1 in comparison with group 3. This study had showed that values of Hb, PCV, platelets and ESR values for both the sexes were significantly changed in group 1 in comparison with healthy controls. Values ofthe platelets and ESR for both sexes and values of PCV for the males were insignificantly changed while the values of

Hemoglobin for both sexes and values of PCV for females were significantly changed in group 2 in comparison with group 3. This study showed that values of PCV for males were insignificantly changed while values of Hemoglobin, platelets and Erythrocyte sedimentation rate for both the sexes and PCV for females were significantly changed in group 2 in comparison with healthy controls. The results has proved that the values of platelets and ESR for both sexes and Hemoglobin for females and Packed cell volume for males were insignificantly changed while values of Hb for males and PCV for females were significantly changed in group 3 in comparison with healthy controls [34]

MATERIALS AND METHODS

PRIMARY OBJECTIVE:

To study the inflammatory markers in new sputum positive tuberculosis and its response to anti tubercular treatment.

SECONDARY OBJECTIVE:

To assess the correlation of mean levels of inflammatory markers with the disease severity.

AIM:

To prove that inflammatory marker levels reflects the disease severity and its decline reflects response to treatment, thus these inflammatory markers can be used as an adjunct in microbiologically confirmed TB cases and as a prime tool in clinically diagnosed cases .

SAMPLE SIZE:

The sample size was determined by using Open Epi software 2.1.3 Version with 95% confidence interval and considering 10% attrition rate sample size of 115 + 11.5 ≈ 127 samples will included in this study.

The estimated sample size was 127.

SUBJECT SELECTION:

Patients attending the thoracic medicine outpatient clinic in government Thiruvotteswarar hospital of thoracic medicine (GTHTM) and government Kilpauk

Medical College (KMC), Government Royapettah Hospital (GRH), with newly detected sputum positive pulmonary tuberculosis.

Symptoms of pulmonary tuberculosis suspect are fever, weight loss, significant weight loss, hemoptysis any abnormalities in chest radiograph as per technical operational guidelines 2016.

Such patients suspected of pulmonary tuberculosis screened with sputum smear examination for acid fast bacilli and new sputum positive patients of pulmonary tuberculosis who are not taken anti tuberculous treatment before are selected for study.

INCLUSION CRITERIA :

- Patients with new sputum positive pulmonary tuberculosis .
- Age >18 < 70 years

EXCLUSION CRITERIA : patients with following co-morbidties

- Previous anti tubercular treatment
- COPD
- Bronchial Asthma
- Ischemic heart disease
- Decompensated liver disease
- Diabetes mellitus

- Renal failure
- Peripheral vascular disease
- Patients with HIV
- Patients with Collagen vascular disease&patients on chronic drugs

STUDY CENTRES:

The study was conducted at three tertiary care institutes

Government Thiruvoteeswarar hospital of thoracic medicine, chennai

Government Kilpauk Medical College, Chennai

Government Royapettah Hospital, Chennai.

STUDY DESIGN:

The study was a prospective cross sectional study.

No specific intervention was carried out.

No controls have been used in study.

SAMPLING:

Simple random sampling

DURATION OF STUDY: 6 months

CONFLICT OF INTEREST: nil

HAZARDS OF STUDY: nil

DATA COLLECTION:

The data of each patient was collected on a proforma

Sl No: Date:

Name:

Age

Gender:

Address

Phone:

BMI: Underweight (< 18.5) Normal (18.5-24.9)

Overweight (25.0-29.9)Obese (>=30)

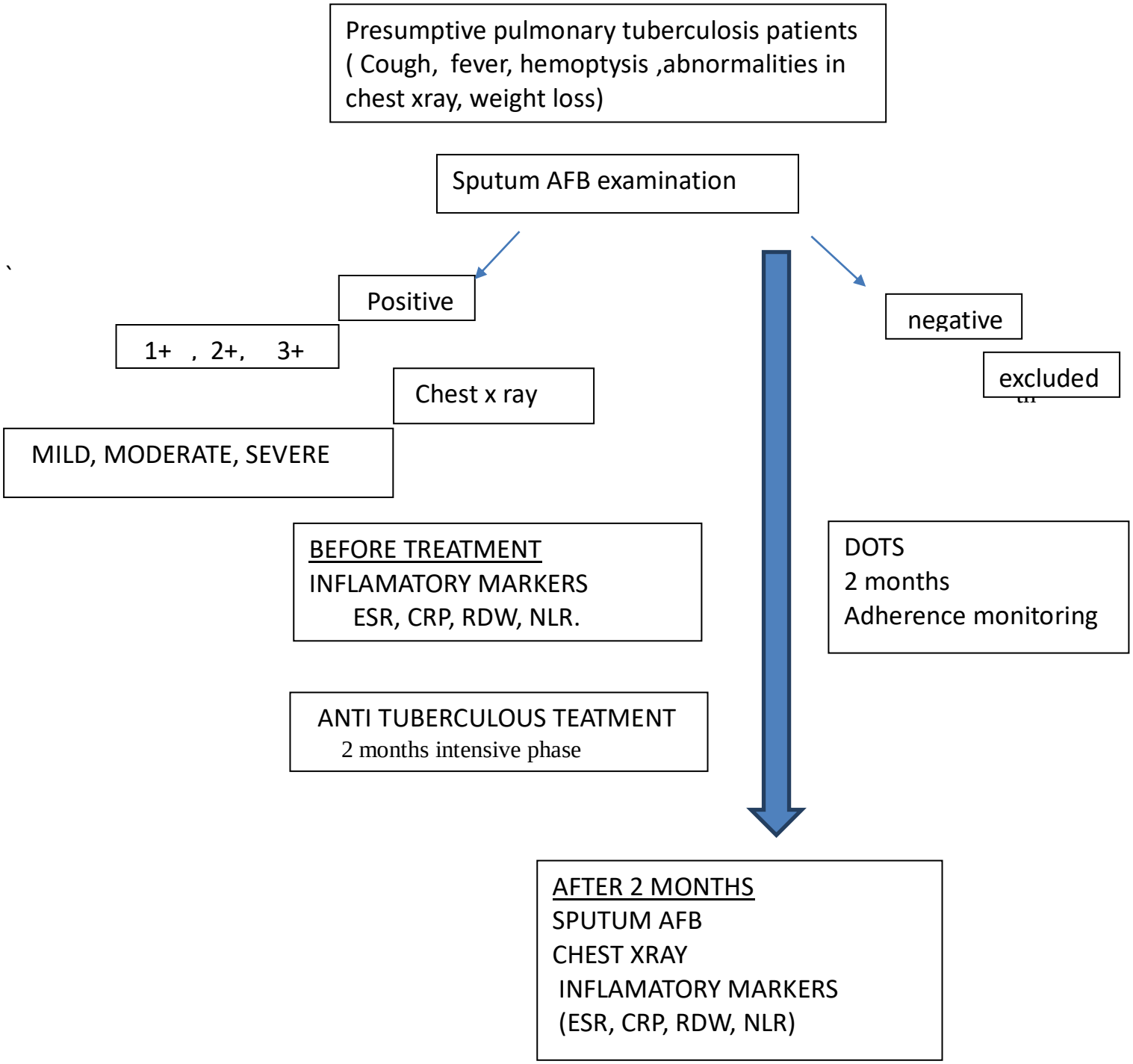
Duration of symptoms:

Smoking history

Treatment	CPR	ESR	NLR	RDW
Before Rx				
At 2months				

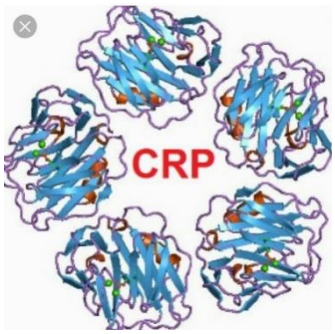
Treatment	Sputum AFB
Before Rx	
At 2months	

METHODOLOGY



INFLAMMATORY MARKERS

C-REACTIVE PROTEIN



C reactive protein is annular (ring shaped) pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute –phase protein of hepatic origin that increases following interlukin-6 secretion by macrophages and T cells. Its physiological role is to bind lyso-phospatidyl choline expressed on surface of dead or dying cells (some types of bacteria) in order to activate the compliment system via c1q[7]

In healthy adults, the normal concentration of CRP varies between 0.8 mg/l to 3.0 mg/l. However, some healthy adults show elevated CRP at 10 mg/l. When there is a stimulus, the CRP level can rise 10,000 fold from less than 50 micro gram /Litre to more than 500 mg/l. Such levels can rise to 5mg/l by 6 hours and peaks at 48 hours. The plasma half life of CRP is 19 hrs. Such half-life is constant in all medical conditions. The rate of CRP production increases with inflammation infection, trauma, necrosis, malignancy and allergic reaction.[8]

Acute phase response occurs as a result of a rise in the concentration of IL-6,which is produced by macrophages as well as adipocytesin response to a wide range of chronic inflammatory conditions such a bacterial, viral or fungal infections. IL-6 stimulates the liver to produce CRP.

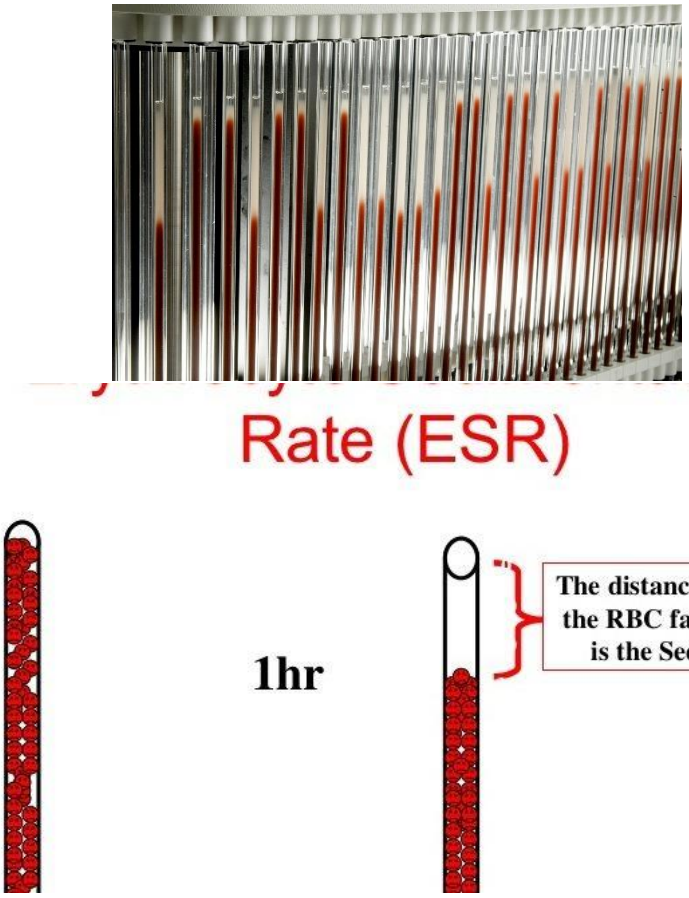
CRP is used as a marker of inflammation apart from liver failure, there are few known factors that interfere with CRP production. Interferon alpha inhibits CRP production from liver cells which may explain the relatively low levels of CRP found during viral infections compared to bacterial infections.[7]

Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. ELISA, immune turbidometry, nephelometry, rapid immunodiffusion and visual agglutination are all methods used to measure CRP.

Normal levels increase with aging. Higher levels are found in late pregnant women, mild inflammation and viral infections 10-40 mg/L). Active inflammation and bacterial infection (40 – 200mg /L),severe bacterial infections and burns (>200mg/L). CRP is a better reflector of the acute phase response than the ESR (erythrocyte sedimentation rate) ESR may be normal while CRP is elevated. CRP returns to normal more quickly than ESR in response to therapy.[7] CRP correlates with the erythrocyte sedimentation rate (ESR) ,however not always directly. This is due to the ESR being largely dependent on elevation of fibrinogen, an acute phase reactant with half life of approximately one week. This protein will therefore remain higher for longer despite

removal of inflammatory stimuli. In contrast CRP (with a half life of 6-8hrs) rises rapidly and can quickly return to within the normal range if treatment is employed. In our study we did CRP for our patients as an inflammatory marker of active pulmonary tuberculosis. The method used is blood CRP by immune turbidometry. Laboratory reference range is less than 5.0 mg /L

ERYTHROCYTE SEDIMENTATION RATE



The erythrocyte sedimentation rate is the rate at which red blood cells sediment in a period of one hour.it is a common hematology test, and is a nonspecific measure of inflammation.To perform the test, anti -coagulated blood was traditionally placed in an

upright tube, known as westergren tube, and rate at which red blood cells fall was measured and reported in mm at the end of one hour.

There are 3 stages in erythrocyte sedimentation

1) Stage 1: Rouleaux formation

2) Stage 2: Sedimentation or settling stage

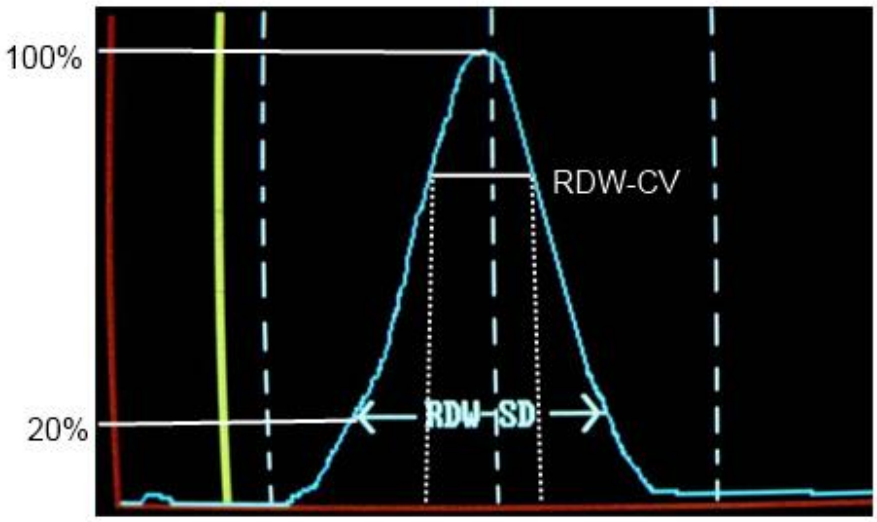
3) Stage 3: Packing stage - 10 minutes (sedimentation slows and cells start to pack at the bottom of the tube)

In normal conditions, the red blood cells are negatively charged. Therefore, negatively charged red blood cells repel each other and do not stack over each other. Besides, if the viscosity of blood is high, red blood cells would be slow to fall to the base, thus lowering the ESR. The rate of erythrocyte sedimentation is affected by both inflammatory and non-inflammatory conditions.

The ESR is governed by balance between pro sedimentation rates, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called rouleaux, which settle faster, due to their increased density. ESR begins to rise at 24 to 48 hours of inflammation, decreases slowly as inflammation resolves, and can take weeks to months to return to normal levels Creactive protein (CRP). Therefore, it is a better marker for acute phase reaction than ESR. While ESR and CRP

generally together correlate with the degree of inflammation, this is not always the case and results may be discordantin 12.5% of the case.The normal range of ESR adults westergrens method men <15 mm /hr, women <20 mm/hr. we did ESR by westergren's method in our study.

RED CELL DISTRIBUTION WIDTH



RDW-CV is a calculation based on both the width of the distribution curve and the mean cell size.

$$\text{RDW-CV} = \frac{1\ \text{SD}}{\text{MCV}} \times 100$$

RDW-SD is an actual measurement of the width of the red cell distribution curve in femtoliters (fL) at the point that is 20% above the baseline.

The width in RDW-CV is sometimes thought to be misleading, because it in fact is a measure of deviation of the *volume* of RBCs, and not directly the diameter. However, "width" refersto the width of the volume curve (distribution width, here presented as the Coefficient of Variation, or CV), not the width of the cells. Thus, it is a reasonably accurate term. RDW is a parameter that measures variation in red blood cell size or red

blood cell volume. RDW is elevated in accordance with variation in red cell size - anisocytosis that is when elevated RDW is reported on complete blood count ,marked anisocytosis (increased variation in red cell size) is expected on peripheral blood smear review.

The reference range of RDW is as follows:

RDW-SD 39-46 Fl

RDW- CV 11.6-14.6 % in adults.

Reference ranges may vary depending on the individual laboratory and also patient's age.

Mathematically, the RDW-CV is calculated with the following formula:

RDW-CV = (Standard deviation of MCV ÷ MCV) × 100.

Depending on the types of hematology analyzer instruments , RDW can be reported statistically as coefficient of variation (CV) and or standard deviation (SD),RDW-CV and or RDW –SD, respectively. RDW –SD (express in fl) is an actual measurement of width of the RBC size distribution histogram as is measured by calculating the width (in fl)at the 20% height level of the RBC size distribution histogram. This parameter is therefore not influenzed by average RBC size.(mean corpuscular volume - MCV)

RDW-CV (express in %) is calculated from standard deviation and MCV as follows

RDW-CV% = 1 standard deviation of RBC volume/MCV * 100%.

Of note since RDW-CV is mathematically derived from MCV, it is there ore affected by the average RBC size (MCV). RDW is useful in determining various types of anemias.

RDW is a recently described novel biomarker that has shown to be predictive of outcomes in pulmonary tuberculosis. although plausible patho-biological mechanisms explaining the relationship of RDW are yet to be elucidated, both inflammation and oxidative stress believed to play a role the molecular basis of the above mentioned association has been mainly attributed to the ability f the RDWs capability to reliably reflect an increase in the levels of circulatory cytokines, such as interlukin-6, tumor necrosis factor alpha and hepcidin. Thus the RDW which is available in the complete blood count serve as inflammatory marker in PTB. In last years the interest in this marker has considerably grown and now a lot of data are available indicating that this simple and inexpensive parameter is a strong and independent risk factor for severity and prognostic indicator in pulmonary tuberculosis. Moreover, several investigations have been performed to investigate the role of RDW in inflammatory and infectious disorders. Generalized, it seems plausible to affirm that RDW can be useful by adding prognostic information in patients with tuberculosis. In our study we collected patient's whole blood by vene puncture in an EDTA tube containing EDTA potassium salt additive as an anticoagulant and analysed in auto analyzer. Thus RDW is analysed as a part of CBC.In our study we have taken red cell distribution width by CV that is RDW-CV as a marker of inflammation.

NEUTROPHIL LYMPHOCYTE RATIO:

Neutrophil lymphocyte ratio is a simple parameter to assess easily the inflammatory status of the subject. It has proven its usefulness in the stratification as a

marker of inflammatory or infectious pathologies. Recently many studies have suggested that neutrophil to lymphocyte ratio may be potential bio marker for PTB severity. Many studies have found a higher NLR in advanced PTB compared to mild to moderate PTB. Neutrophil lymphocyte ratio alert us to the fact that the immune system is dysregulated from inflammation and the patient at high risk of mortality.

Using results from a complete blood count with differential, it is easy to calculate the ratio of absolute neutrophil count to lymphocyte counts. If the neutrophil counts are 4 times greater than lymphocytes (>4:1) then the patient has poorer prognosis than if the ratio were less than 4:1. The normal NLR levels in a healthy adult range between 0.78 and 3.53.

In our study we analysed anticoagulated blood samples of patients in auto analyser. The absolute neutrophil count divided by lymphocyte ratio to calculate NLR.

SPUTUM AFB SMEAR:

In pulmonary tuberculosis, sputum is the specimen of choice. The sputum specimen is collected in a sterile container. It is a common misassumption that mycobacterial specimens are decontaminated before inoculation for culture. Thus the cleanliness of the sputum container is not important. Unsterilized containers may be contaminated with environmental mycobacteria. To facilitate the choice of container, following specifications are recommended for a container: 1) wide mouthed so that the patient can expectorate easily inside the container without contaminating it from outside; 2) volume capacity of approximately 25 ml; 3) made of transparent material in order to

observe specimen volume and quality without opening the container; 4) screw-capped to obtain a water-tight seal, to reduce the risk of leakage during transport; 5)easily-labelledto allow permanent identification; and 6) rigid, to avoid breakage during transit.

An ideal container is the 28 ml universal container, which is a heavy glass, screw-capped bottle. This container is reusable after thorough cleaning and sterilization. The identification number can be permanently on the bottle cap. In TB diagnosis, care must be taken to obtain adequate and satisfactory specimens. Correct collection and transportation of specimens to the laboratory are important to ensure that the results are accurate and reliable.

Grades according to the number of bacilli per high power field seen with fluorescent staining.

No. of bacilli per high power field Grade

Less than 6 per field 1+

6 to 100 bacilli per field 2+

More than 100 per field or large clumps 3+

Grades according to the number of bacilli seenWith Ziehl-Neelsen staining

No. of AFB Fields Report

None per 100 oil immersion fields Negative

1-9 per 100 oil immersion fields Scanty [exact number reported]

10-99 per 100 oil immersion fields 1+

1-10 per oil immersion field 2+[50 fields examined]

Use of microscopy in diagnosis of TB is of paramount importance, as culture takes a long time before the results are ready. Microscopy is also helpful in the detection of open or infectious cases. Stained smears are examined directly from the sputum and after concentration.The tubercle bacilli are Gram positive though they do not take the stain readily. Mycobacteria retain the primary stain even after decolourization with acid alcohol;hence the term “acid-fast”. A counter-stain is employed to highlight the stained organisms for easierrecognition. There are several methods of acid-fast nature of mycobacteria. In the carbol-fuchsin [Ziehl-Neelsen] procedure, acid-fast organisms appear red against a blue background. Acid fastness is based on the integrity of cell wall. Beaded or barred forms are frequently seen in *Mycobacterium tuberculosis* while *Mycobacterium bovis* stains more uniformly. In younger cultures, non -acid-fast rods and granules have been reported. The mycobacterial cell wall is complex in nature. It has high lipid content, which accounts for about 60 per cent of the cell wall weight. The cell wall has several distinct layers. The inner layer, overlying the cell membrane is composed of peptidoglycan [murein]. External to the murein is a layer of arabinogalactan which is covalently linked to a group of long chain fatty acids termed *mycolic acid*. These form a dense pallisade, arranged in rope-like structure, which gives the cell wall its thickness and is largely responsible for acid fastness. Acids termed *mycolic acid*. These form a dense pallisade, arranged in rope-like structure, which gives the cell wall its thickness and is largely responsible for acid fastness. It has been shown that at least 10 000 bacilli per ml of sputum are required for direct microscopy to be positive. The sensitivity can be further improved by examining more than one specimen from a patient. Examination of

two specimens will, on an average, detect more than 90per cent of cases and the addition of a third specimen increases the percentage to approximately 95 to 98 percent. A negative smear, however, does not exclude the diagnosis of TB as some patients harbor fewer numbers of bacilli which cannot be detected by direct microscopy. A poor quality specimen or smear may also produce negative results. New glass slides should be used for making smears as acid-fast bacilli [AFB] are not always removed from the old slides. Only those reagents and diluents should be used which have been shown to be free of environmental mycobacteria to avoid false positive smears. Direct examination is performed by selecting a purulent looking portion of sputum and spreading it thinly on a glass slide with a bacteriological loop or a wooden stick. The watery part of sputum is less likely to contain bacilli. The AFB are seen as bright red rods against the blue, green or yellow background [depending upon the counterstain used in staining]. A negative result does not exclude TB. As recommended by World Health Organization [WHO], before declaring a slide negative it is essential that at least 100 fields are examined taking over at least 10 minutes. Smears can be graded according to the number of bacilli seen, which was described in previous page.

ROENTGENOGRAPHY IN PULMONARY TB:

PRIMARY PULMONARY TB:

Parenchymal consolidation

Tuberculoma

Miliary tuberculosis

Lymphadenopathy

Airway involvement

Pleural effusion

POST PRIMARY TB:

Local exudative / fibro productivelesion

Tuberculoma, Cavitation

Bronchogenic spread, Miliary tuberculosis

Broncho stenosis, Pleural effusion

Grading the disease severity by radiology plays paramount importance regarding the disease extent and progress,but there is no validated system so far grading the severity of chest x ray abnormalities in bacteriologically proven pulmonary tuberculosis. Problems in chest x ray reporting arise from the heterogenous chest xray manifestations of pulmonary tuberculosis and to inaccuracies inherent in chest xray performance and interpretation, including inter observer variation.Despite these short comings, the utility of CXR is well established in tuberculosis diagnosis and monitoring clinically. Many chest x ray scores available for use in adults which predicts the outcome which correlates with bacteriological and clinical severity markers. The scoring systems assess the extent

of involvement of lung fields, percentage of lung involvement,Presence of cavities, presence of pleural effusion etc.

We have taken one such simple scoring system for grading the radiological severity. We divide the study patients into mild, moderate, severe disease according to it we have taken dlugovitzky et al.,criteria for classifying.

MILD: single lobe involvement and no visible cavities or pleural involvement

MODERATE: Unilateral involvement of two or more lobes with a solitary cavity or mild extent plus pleural involvement or effusion

SEVERE: bilateral involvement and multiple cavities OrModerate extent plus pleural involvement or effusion.

STATISTICAL ANALYSIS

Statistical analysis was done using the Microsoft Excel and SPSS software with the help of a statistician. P value is used to assess the significance of correlation between variables. A statistically significant correlation is one in which

Pearson correlation is used to assess the strength of correlation between variables

Pearson correlation:

> 0.5 - Strong correlation

0.3 to 0.5 - Moderate correlation

0.3 - Weak correlation

Chi-square Test:

Chi-square test is performed between two groups and its statistical significance is calculated.

The chi-square ($\chi 2$) test of independence is used to test for a statistically significant relationship between two categorical variables. The term "degrees of freedom" is used to refer to the size of the contingency table on which the value of the Chi Square statistic has been computed

P value is calculated using Excel CHITEST function:

If P value $\leq 0.05 \rightarrow$ statistically significant

If P value $> 0.05 \rightarrow$ statistically in significant

RESULTS

DESCRIPTIVE STATISTICS

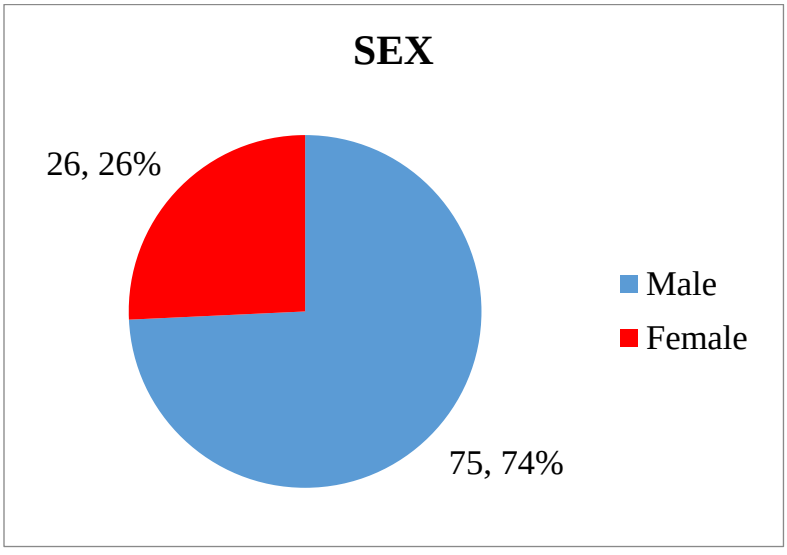
GENDER DISTRIBUTION

In our study males accounted for 74% and females accounted for25 %.

Table 1: Gender wise distribution

Sex	Frequency	percent
Male	75	74.3
Female	26	25.7
Total	101	100

Fig: 1



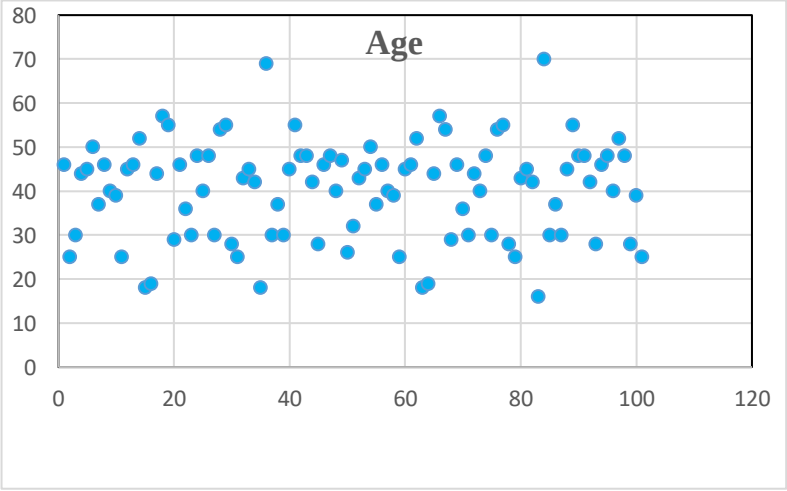
AGE DISTRIBUTION:

In our study the mean age distribution is 40 years with maximum 70 years and minimum age 18 years.

Table: 2 Age distribution among study subjects

Age	
Mean	40.44
Median	43.00
Standard Deviation	11.321
Minimum	18
Maximum	70

Fig:2

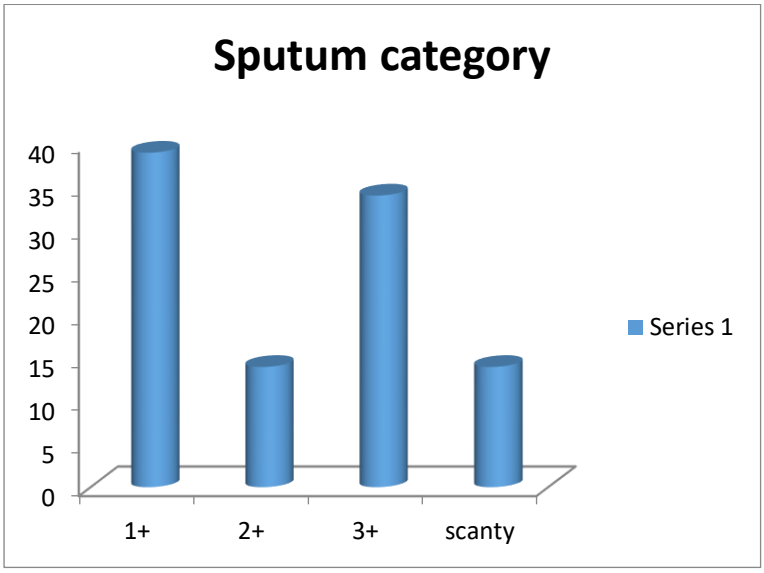


SPUTUM CATEGORY OF PARTICIPANTS

In our study mild disease with 1+ sputum predominated 38.6%followed by sputum 3+ about 33% and scanty and 2+ and scanty accounted for 14% each**.**

Table 3: Sputum Category of study participants

Sputum category	Frequency	Percent
1 +	39	38.6
2 +	14	13.9
3 +	34	33.7
Scanty	14	13.9
total	101	100



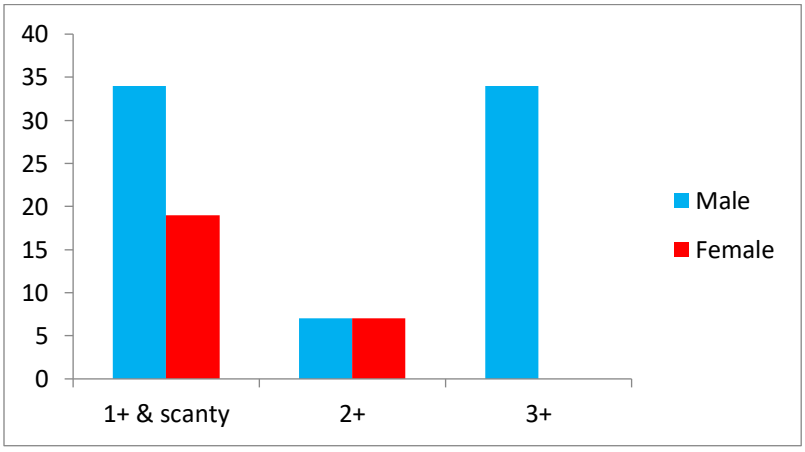
DISTRIBUTION OF DISEASE SEVERITY BY SPUTUM ACCORDING TO GENDER

Table 4: Distribution of disease severity by sputum according to gender

Sputum category	SEX		Total
	Male	Female	
1+	34	19	53
2+	7	7	14
3+	34	0	34
Total	75	26	101

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	18.928[a]	2	.000

Fig: 4

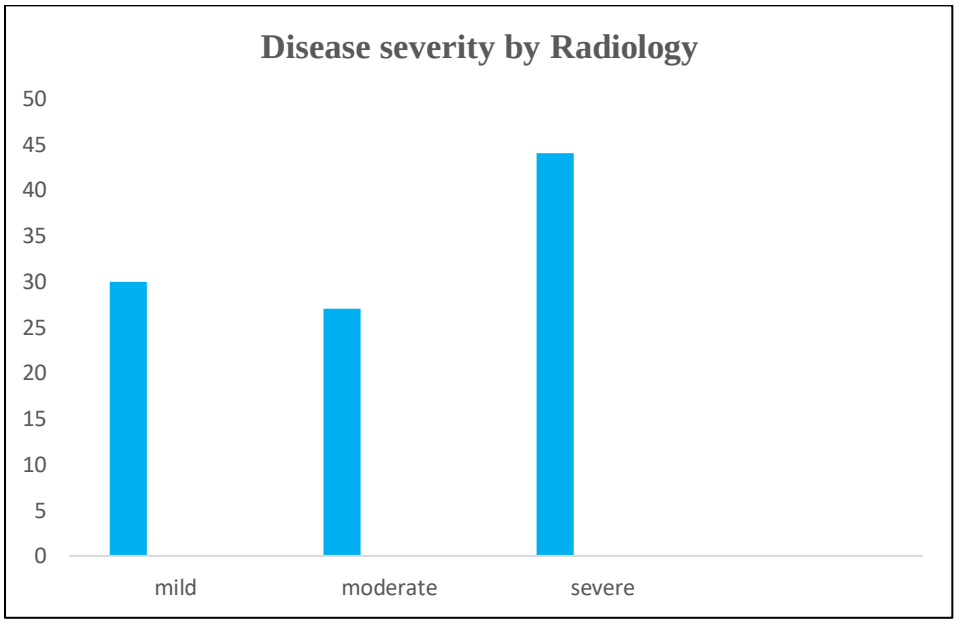


The males were more in number in mild disease and severe disease.males and females are equal in number in moderate diseasein severe disease that is with 3+ sputum there were no females.

Distribution of Severity According To Radiology

Table: 5 Distribution of Severity According To Radiology

Category	Frequency	percent
mild	30	29.7
moderate	27	26.7
severe	44	43.6
total	101	100



In our study the person with mild, moderate, severe disease according to radiological grading are 30%, 27%, and 44% respectively

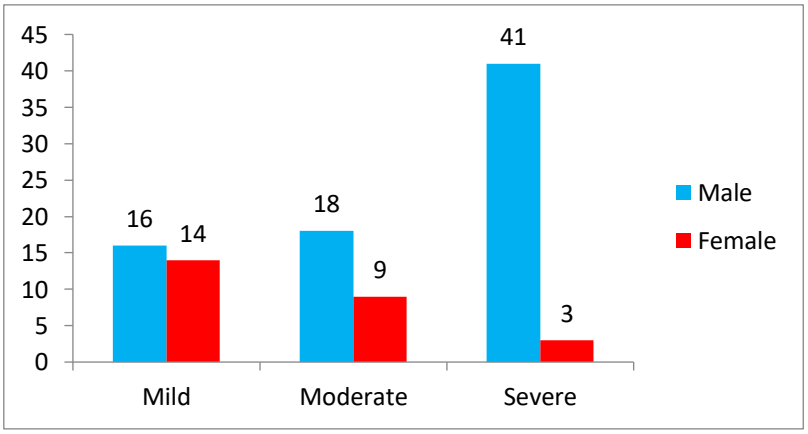
Distribution of disease Severity by Radiology according To Gender

Table: 6 Distribution of Severity by Radiology According To Gender

Severity	SEX		Total
	Male	Female	
1	16	14	30
2	18	9	27
3	41	3	44
Total	75	26	101

	Value	df	P value
Pearson Chi-Square	15.928[a]	2	.000

Fig:6



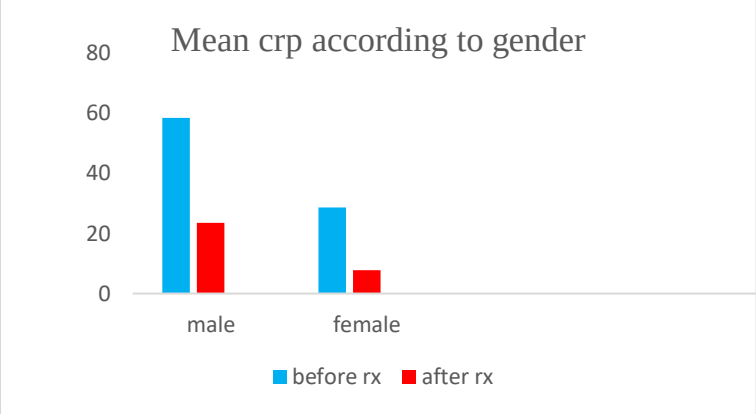
In our study in mild disease both male and females are almost equal n=16 and n=14 respectively. In moderate disease males predominated n=18 when compared to females n=9.in severe disease males were dominant n=41,but only few females n=3

C-REACTIVE PROTEIN

Table: 7 C-REACTIVE PROTEIN

Parameter	Sex	Before rx	After rx
CRP	Male	58.4+/-29.8	23.5+/- 15.3
	female	28.6+/- 23.4	7.8 +/- 10.2

Fig:7



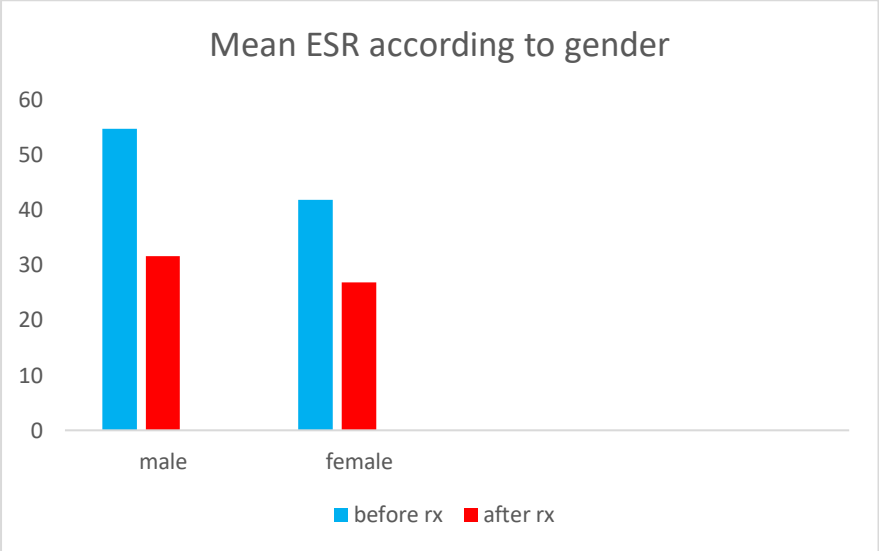
In this study the mean CRP in males before treatment was 58.4 and it decreased after treatment to 25.5 .whereas in females mean CRP was 28.6 before and 7.8 after treatment .so the mean CRP is in Slightly higher range in men both before and after treatment.

ERYTHROCYTE SEDIMENTATION RATE

Table: 8 ERYTHROCYTE SEDIMENTATION RATE

parameter	sex	Before treatment	After treatment
ESR	Male	54.6+/ 24.5	31.6 +/- 7.39
	female	41.8 +/- 10.7	26.85 +/- 5.17

Fig:8



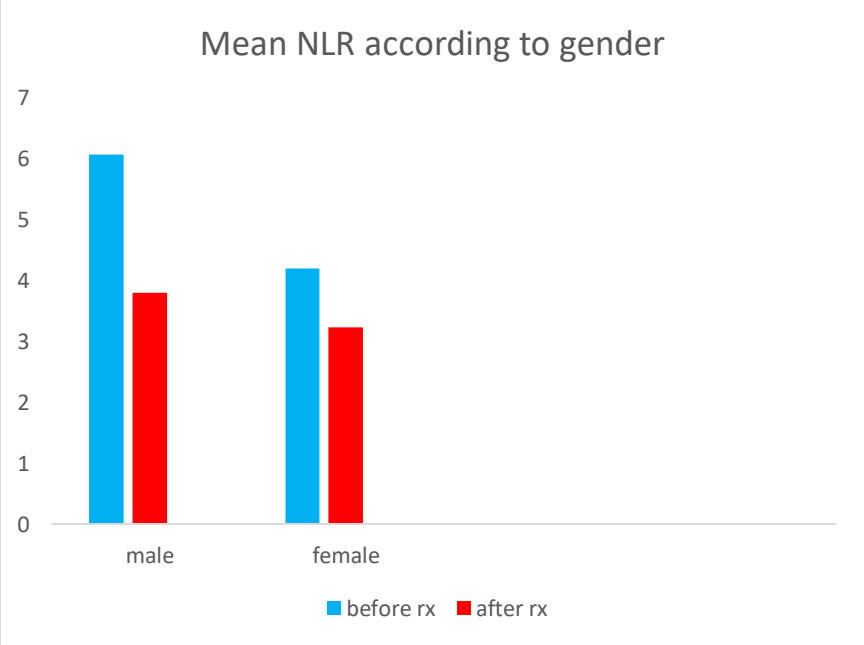
The mean ESR in men is 54.6 mm/hrbefore treatment and that of after treatment is 31.6mm/hr.in females it is 41.8mm/hr and 26.8mm/hr before and after treatment respectively

NEUTROPHIL LYMPHOCYTE RATIO

Table: 9 NEUTROPHIL LYMPHOCYTE RATIO

Parameter	Sex	Before rx	After rx
NLR	Male	6.06+/- 2.3	3.79 +/- 1.2
	Female	4.19 +/- 1.27	3.23 +/- 0.56

Fig :9



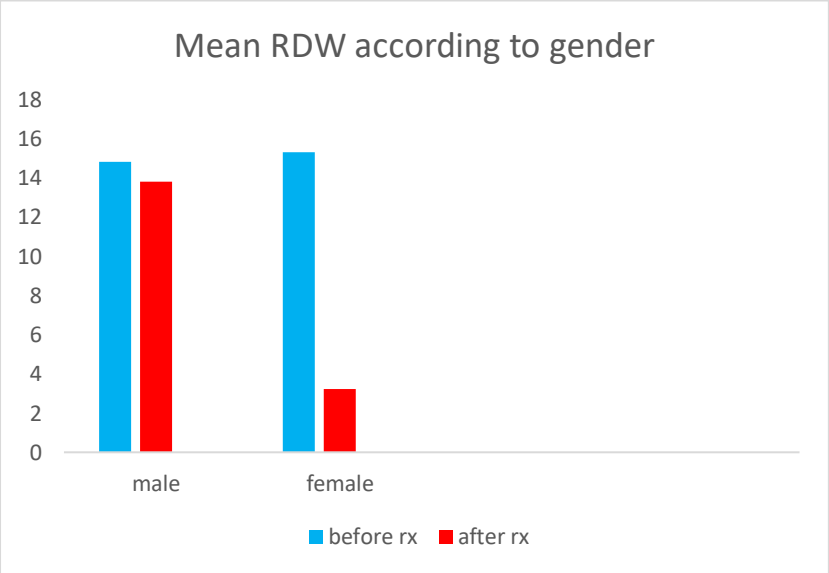
The mean NLR IN male is 6.06 and 3.79 before and after treatment respectively,and that of female is 4.19 and 3.23 before and after treatment respectively

RED CELL DISTRIBUTION WIDTH

Table: 10 RED CELL DISTRIBUTION WIDTH

Parameter	Sex	Before rx	After rx
RDW	Male	14.8+/-1.5	13.8 +/- .98
	female	15.3 +/- 1.7	13.5 +/- 0.72

Fig:10

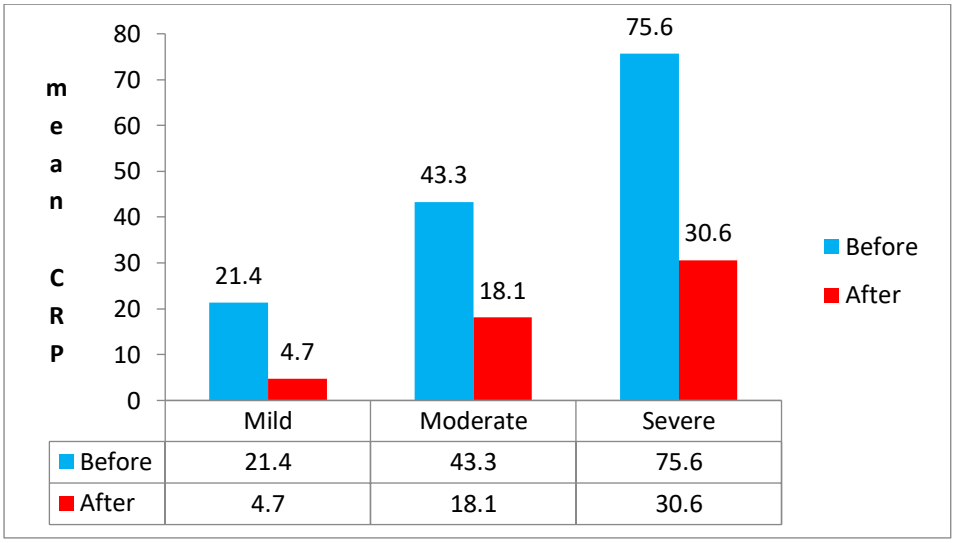


The mean RDW in men are 14.8 and 13.8 before and after treatment respectively, as that of women were 15.3 and 1.5 before and after treatment respectively.

INFLAMMATORY MARKERS ACCORDING TO SEVERITY (RADIOLOGICAL)

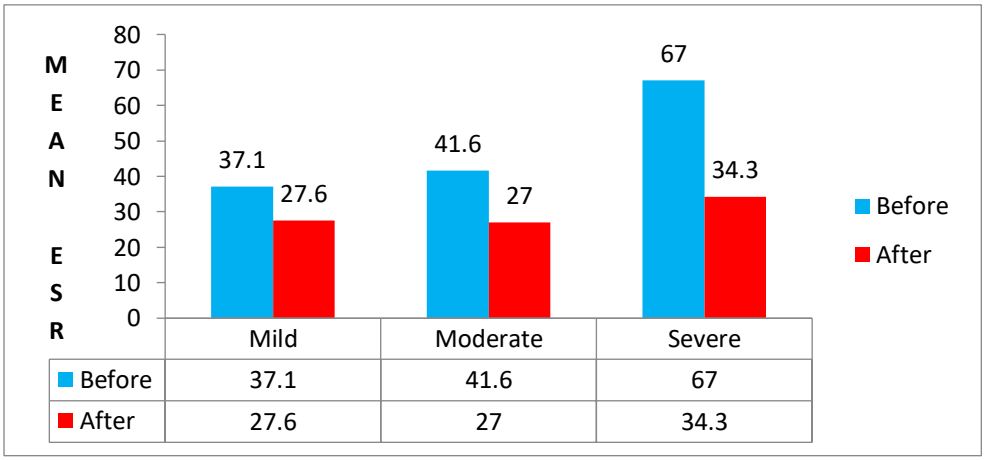
Fig:11Inflammatory markers according to severity (radiological)

C-reactive protein



	Mild	Moderate	Severe
Before	21.4	43.3	75.6
After	4.7	18.1	30.6

Fig:12 Erythrocyte sedimentation rate



	Mild	Moderate	Severe
Before	37.1	41.6	67
After	27.6	27	34.3

When comparing the mean value of CRP and ESR,their levels are highest,higher and high in severe, moderate, mild disease respectively.Thus their mean levels closely reflects the severity. (radiology)

Fig: 13Neutrophil lymphocyte ratio

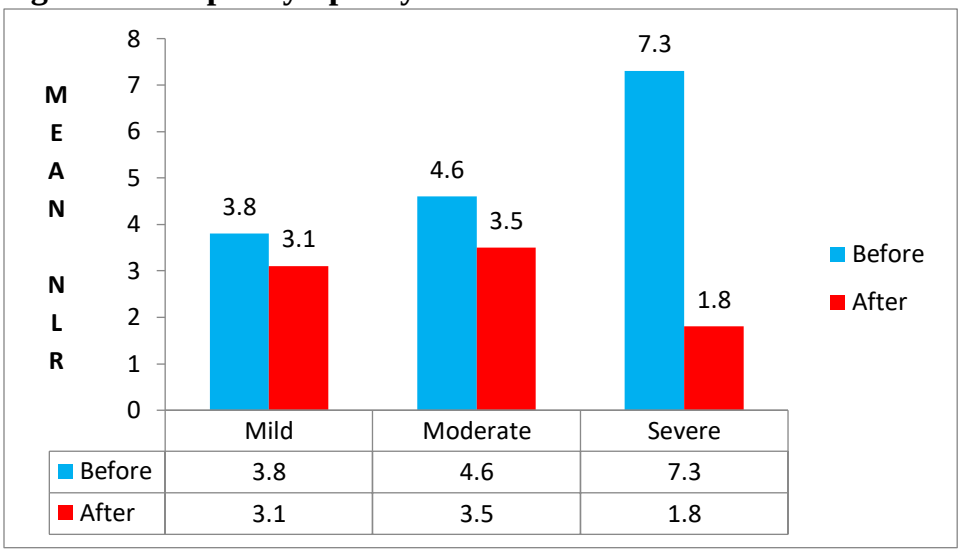
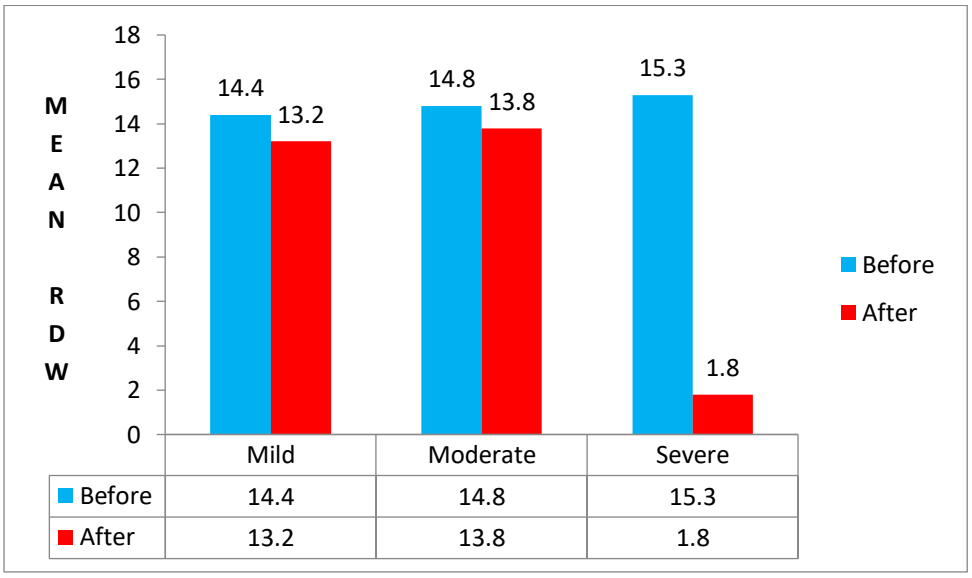


Fig:14 Red cell distribution width



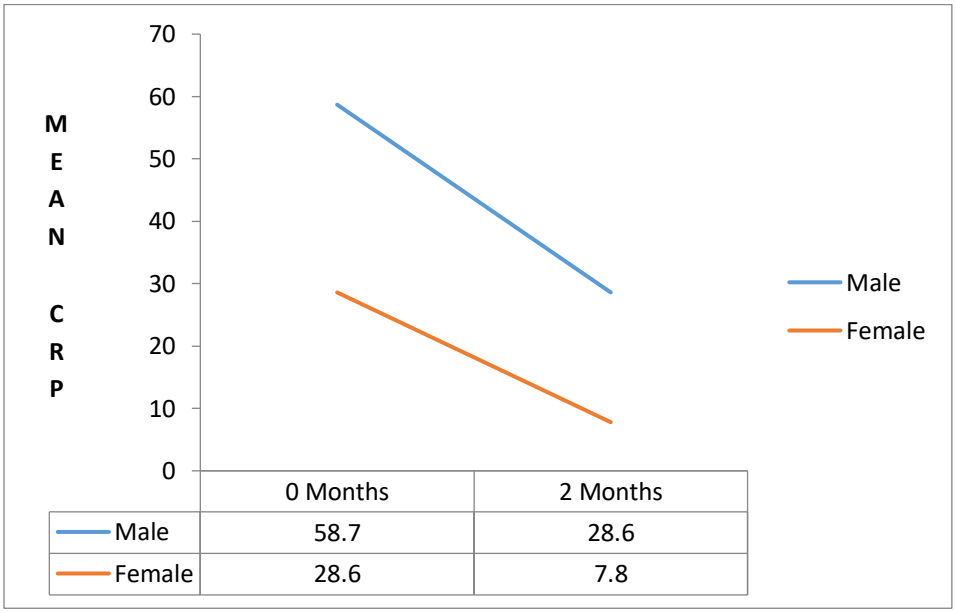
The mean values of the neutrophil lymphocyte ratio and red cell distribution width are highest, higher,high in mild, moderate, severe disease respectively thereby reflecting the severity

RESPONSES OF INFLAMMATORY MARKERS DURINGTREATMENT

Table: 11 RESPONSES OF INFLAMMATORY MARKERS DURING TREATMENT

CRP	Before treatment	After treatment	P value
MALE	58.4 +/- 29.8	23.5 +/-15.3	0.001
FEMALE	28.6 +/- 23.4	7.8 +/- 10.2	

Fig: 14



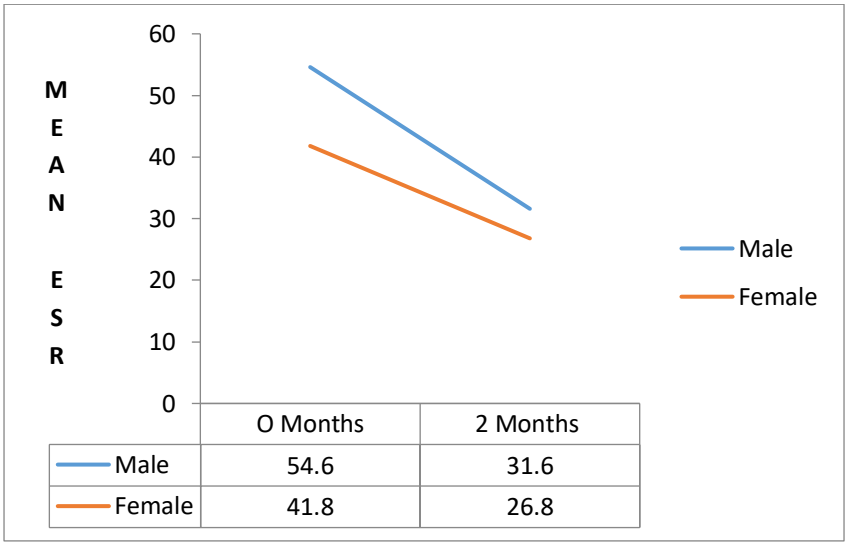
The mean values of CRP fall significantly after treatment for 2months in both males and females.

Table: 12 Erythrocyte sedimentation rate

ESR	Before treatment	After treatment	P value
MALE	54.6 +/- 24.5	31.6+/ 7.39	0.001
FEMALE	41.8 ± 10.07	26.85 ± 5.17	

* Paired T test

Fig: 15

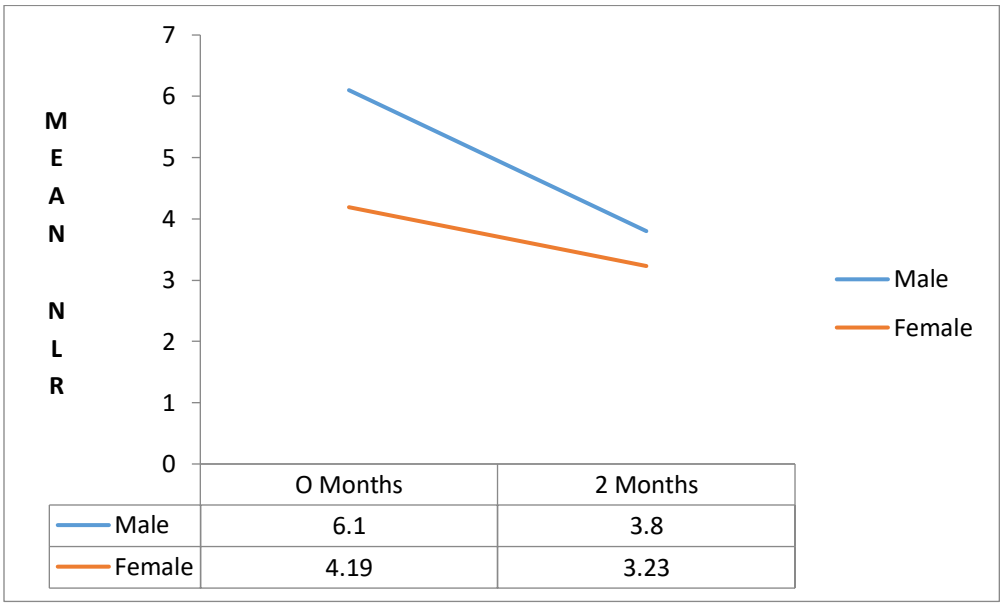


The mean values of ESR fall significantly after treatment for 2months in both males and females.

Table: 13 Neutrophil lymphocyte ratio

NLR	Before treatment	After treatment	P value
MALE	6.06 +/- 2.3	3.79 +/- 1.2	0.004
FEMALE	4.19 ± 1.27	3.23 ± 0.56	0.001

Fig: 16

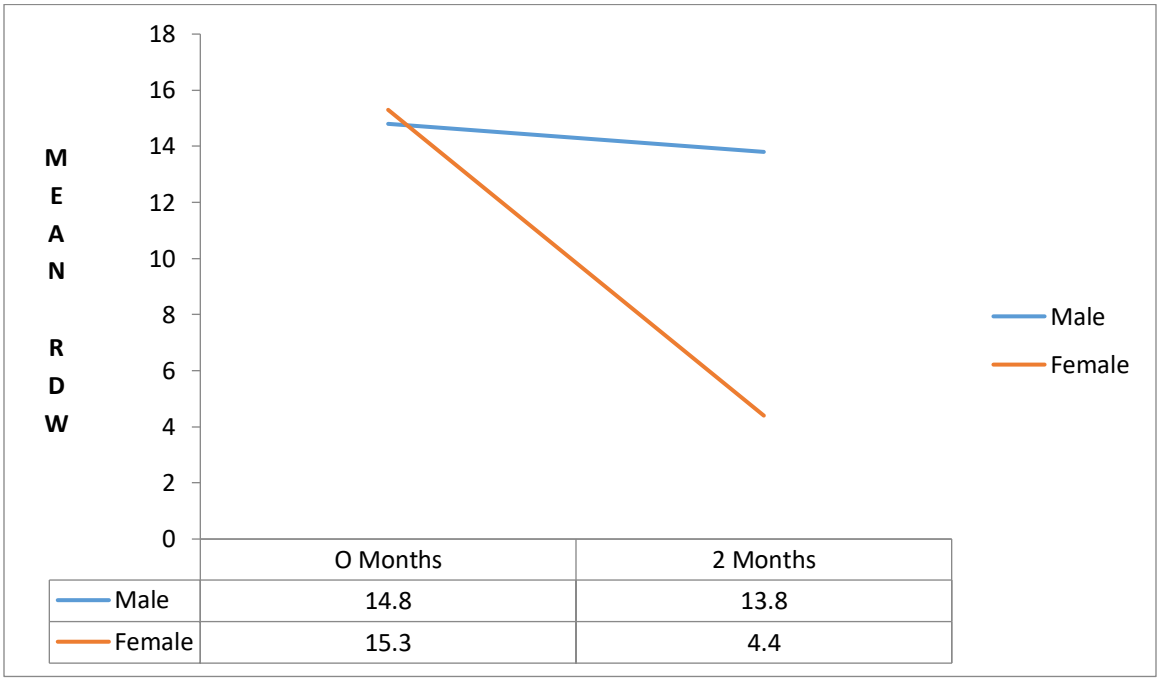


The mean values of NLR fall significantly after treatment for 2months in both males and females.

Table: 14 Red cell distribution width

RDW	Before treatment	After treatment	P value
MALE	14.8 +/- 1.5	13.8 +/- 0.98	0.004
FEMALE	15.3 ± 1.7	13.5 ± 0.72	0.044

Fig: 17



The mean values of RDW fall significantly after treatment for 2months in both males and females.

INFLAMMATORY MARKERS LEVELS ACCORDING TO SPUTUM CATEGORY

Fig: 18

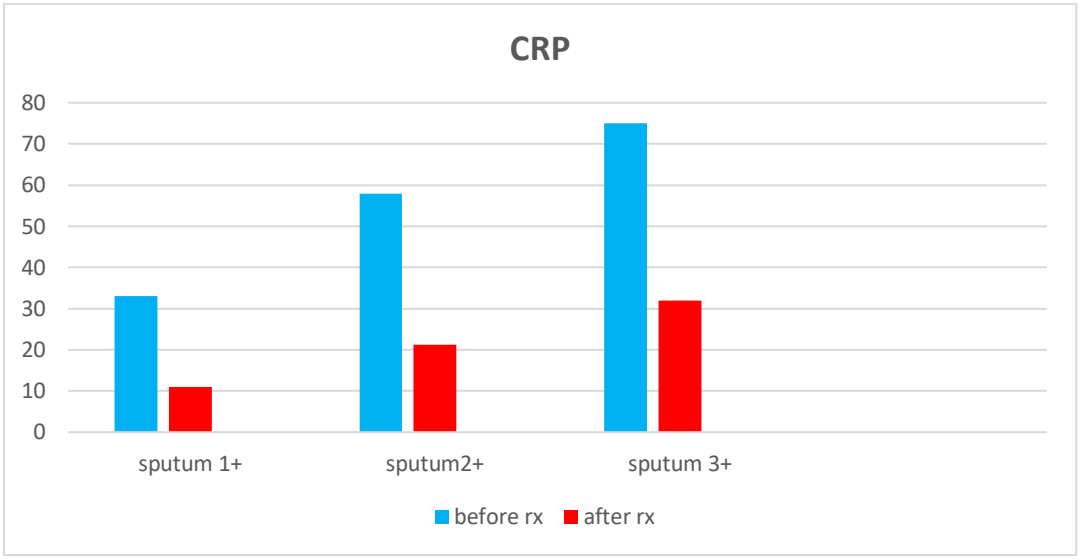
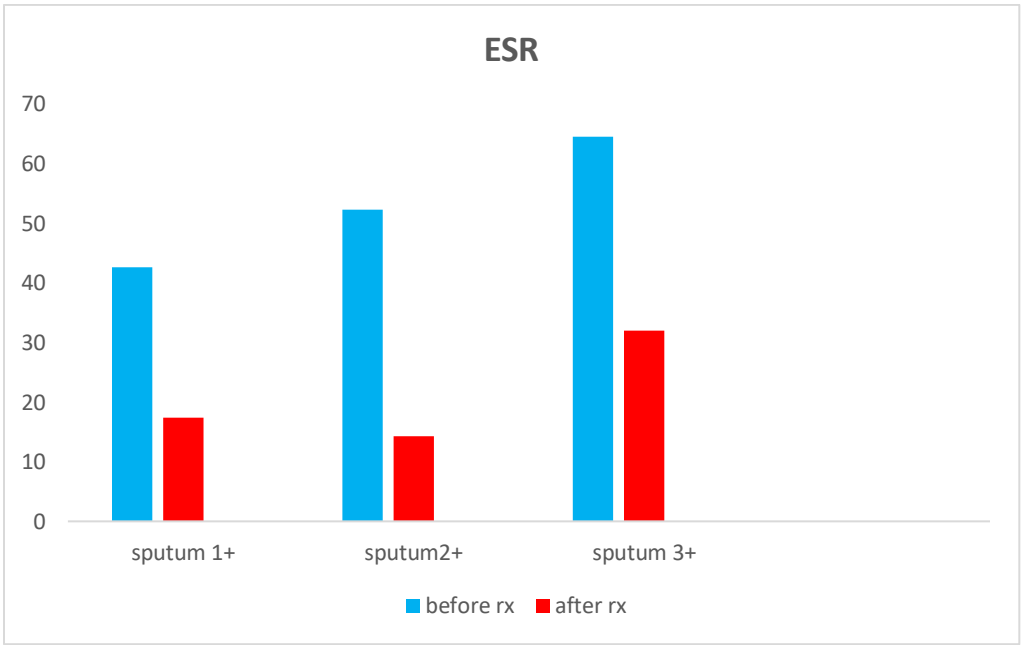


Fig: 19



When comparing the mean value of CRP and ESR, their levels are highest,higher and high in severe, moderate, mild disease respectively.Thus their mean levels closely reflects the severity.(by sputum category)

Fig: 20

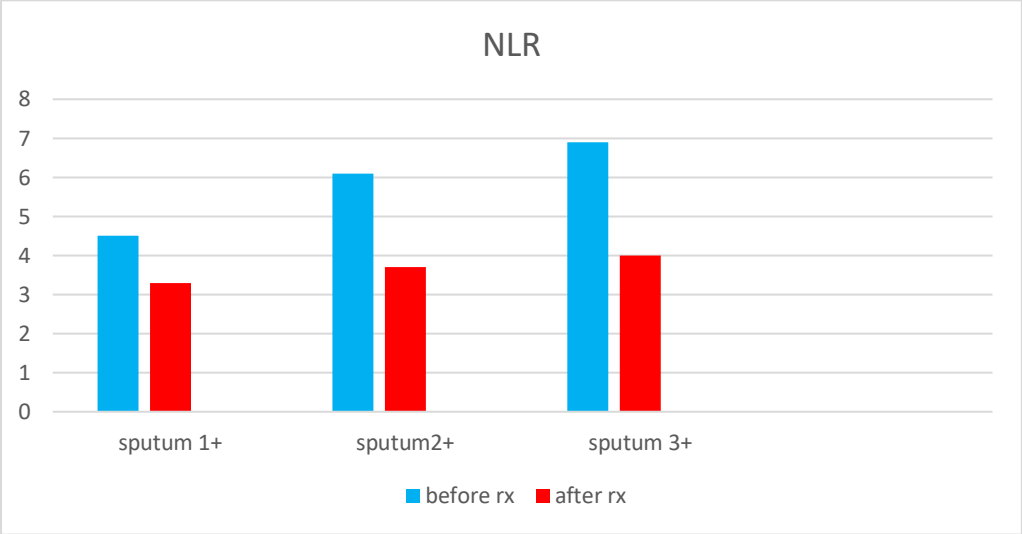
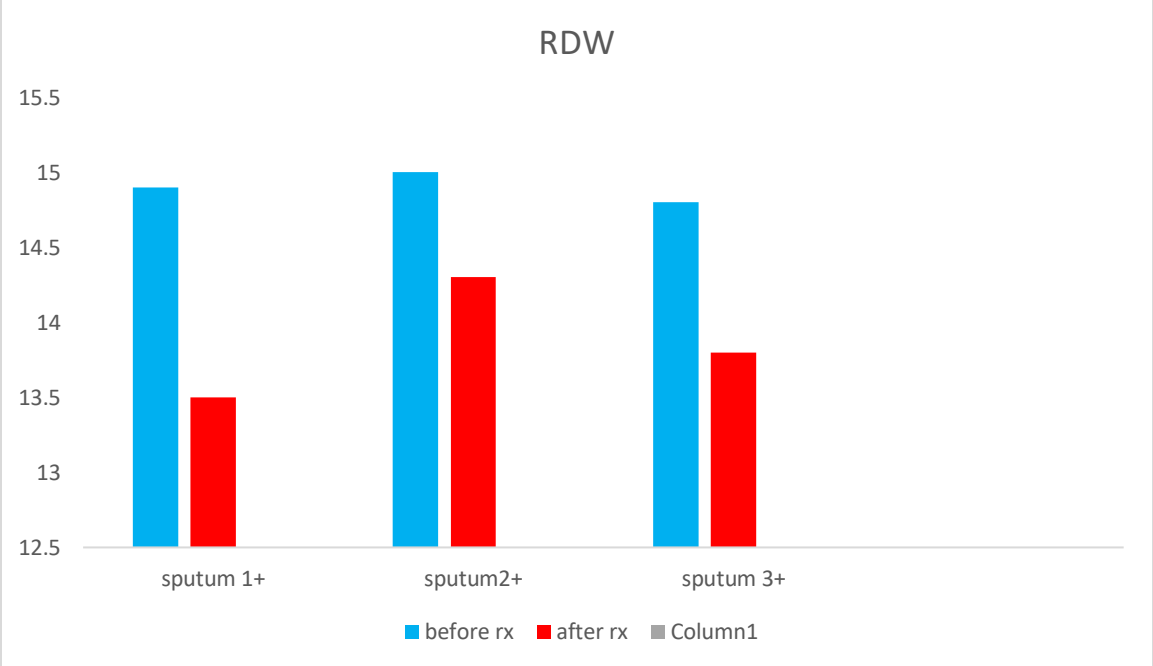


Fig: 21



When comparing the mean value of CRP and ESR, their levels are highest,higher and high in severe, moderate, mild disease respectively.Thus their mean levels closely reflects the severity (by sputum category)

Table: 15 Hematological parameters that demonstrated significant difference based on the sex and severity of TB patients before initiation of anti-TB drugs compared with after completion of the intensive phase of treatment

Parameter	**Sex**	**Before Rx**	**After Rx**	**P Value**
CRP	Male (75)	58.4 ± 29.8	23.5 ± 15.3	0.001*
	Female (26)	28.6 ± 23.4	7.8 ± 10.2	
	Severity			
	Mild (30)	21.04±12.8	4.7 ± 4.8	0.047*
	Moderate (27)	43.3±11.0	18.1 ± 10.7	0.013*
	Severe (44)	75.61±28	30.6 ± 14.5	0.001*
ESR	**Sex**			
	Male	54.6±24.5	31.6 ± 7.39	
	Female	41.8 ± 10.07	26.85 ± 5.17	0.756
	Severity			
	Mild	37.1 ± 8.04	27.63 ± 5.96	0.66
	Moderate	41.6 ± 8.2	27 ± 3.5	0.04*
	Severe	67.0± 25.2	34.3 ± 7.7	0.001*
	Sex			
	Male	6.06 ± 2.3	3.79 ± 1.2	
	Female	4.19 ± 1.27	3.23 ± 0.56	0.004*
	Severity			

NLR	Mild	3.8 ± 0.59	3.16 ± 0.48	0.006*
	Moderate	4.6 ± 1.2	3.5 ± 0.64	0.535
	Severe	7.39 ± 2.2	4.07 ± 1.51	0.105
RDW	**Sex**			
	Male	14.8 ± 1.5	13.8 ± 0.98	
	Female	15.3 ± 1.7	13.5 ± 0.72	0.044*
	Severity			
	Mild	14.4 ± 1.3	13.2 ± 0.52	0.15
	Moderate	14.8 ± 1.5	13.8 ± 0.83	0.05*
	Severe	15.3 ± 1.6	14.04 ± 1.06	0.04 *

Parameter		**Before Rx**	**After Rx**	**P Value**	
CRP	1 +	33.32 20.8	11.06 11.91	0.001	
	2 +	57.9 30.4	21.2 15.3	0.001	
	3 +	75.0 27.7	32 12	0.001	
ESR	1 +	42.6 17.4	28.4 5.42	0.001	
	2 +	52.2 14.3	30.3 6.04	0.032	
	3 +	64.5 25.8	33.4 8.8	0.001	
	1 +	4.5 1.4	3.3 0.69	0.001	
	2 +	6.09 2.4	3.7 0.70	0.001	
NLR	3 +	6.9 2.66	4 1.66	0.186	
	1 +	14.9 1.68	13.5 0.76	0.001	
	2 +	15 1.81	14.3 1.77	0.268	
RDW	3 +	14.8 1.3	13.8 0.53	0.03	

DISCUSSION

Our study sample is 127 of which only 101 patients finally included in the study.Two of the people turned out to be a multi drug resistant tuberculosis,15 people did not come for follow up, 13 patients defaulted treatment, and 2 patients changed to alternative treatment.

GENDER DISTRIBUTION:

In our study population male patients accounted for about 75% and female patients accounted for about 25%.thus males predominated in the study.This probably shows men seek medical care more than women.

AGE DISTRIBUTION:

The mean age of the patients is 40 years, with standard deviation of 11. The maximum age of the patient was 70 years, minimum age was 18 years. This reflects that TB predominantly affects the productive age group which is a major socioeconomic burden for the country.

DISTRIBUTION OF DISEASE SEVERITY ACCORDING TO SPUTUM CATEGORY

According to sputum category the patients were classified into mild, moderate and severe disease with 1+, 2+, 3+ sputum respectively. In this study 53% of the patients hadmild 14 % patients had moderate category and 34% in severe category. Thus mild disease predominated which reflects the integrity of health system and awareness among people. According to gender mild and severe diseases males predominated and in moderate

disease it is in equal distribution.no female patients classified as severe disease which is may probably due to relatively less female study population.

DISTRIBUTION OF DISEASE SEVERITY ACCORDING TO RADIOLOGY

According to radiology 30 % patients are classified into mild disease, 27% into moderate disease,44% into severe disease thus there is discrepancy in classifying the severity between sputum category and radiology. The radiological evaluation detected more number of Moderate disease as compared to sputum grading.

According to gender male and female distribution in mild disease it is almost equal, moderate and severe disease males predominated. Thus males suffer a relatively severe disease.

INFLAMMATORY MARKERS AND DISEASE SEVERITY

We have compared the inflammatory markers levels with disease severity by sputum category and radiological involvement.

The mean CRP levels in mild disease as by radiology and sputum category are 21 mg /l and 33.3 mg/l respectively. The mean ESR level in mild PTB as by radiology and sputum category are 37.1 mm/ hr and 42.6 mm/hr respectively. The mean NLR in mild disease by radiology and sputum are 3.8 and4.5 respectively. The mean RDW in mild disease by radiology and sputum are 14.4 and 14.9 respectively. Thus the inflammatory markers are elevated more in sputum category than in radiological severity as far as mild disease is concerned.

The mean CRP levels in moderate disease as by radiology and sputum category are 43.3 mg% and 57.9 mg % and respectively. The mean ESR level in moderate PTB as byradiology and sputum category are 41.6mm/hr and 52.2 mm/hr respectively. The mean NLR in moderate disease by radiology and sputum are 4.6 and 6.09 respectively. The mean RDW in moderate disease by radiology and sputum are 14.8 and 15 respectively. Thus the inflammatory markers in moderate tuberculosis also are elevated more in sputum category than in radiological severity except for RDW which elevated more in moderate disease as determined by radiology.

The mean CRP levels in severe disease as by radiology and sputum category are 75.6mg% and 75.0mg % and respectively. The mean ESR level in severe PTB as by radiology and sputum category are 67.0mm/hr and 64.5 mm/hr respectively. The mean NLR in severe disease by radiology and sputum are 7.39 and 6.9 respectively. The mean RDW in severe disease by radiology and sputum are 15.3 and 14.8 respectively. Thus the inflammatory markers in severe tuberculosis also are elevated more in sputum category than in radiological severity except for RDW which elevated more in severe disease as determined by radiology

INFLAMMATORY MARKERS RESPONSE TO ATT

The levels of CRP which is elevated during active disease decrease significantly after 2 months of ATT in mild, moderate and severe disease with p value < 0.001. which is in accordance with many previous studies…done by abakay et al. furuhashi et al, mohd yousoof et al..etc

The ESR which is elevated in active disease fall significantly in moderate and severe disease (p value < .001) whereas its fall in mild disease is not statistically significant.(p value 0.66).this is in accordance with studies done by furuhashi et al, mahalakhmamma et al. but in all the reviewed studies all the patients they found statistically significant fall in ESR

The NLR ratio elevated in active disease and fall significantly in mild disease ($p < 0.006$) whereas in moderate and severe disease fall in its level not significant .($p=0.5$).This is in accordance with various literatures examples…studies done by abakay et al, yin y et al,intheir studied they concluded high NLR in severe disease and retreatment cases.

The red cell distribution width(RDW)levels which are elevated in active disease fall in response to active treatment but it is not statistically significant.This is in accordance with studies done by various authors bamalkku et al..,marina oliveria et al, their studies also did not show statistically significant decrease in RDW after treatment.

Thus we find that CRP is a better marker indicating disease severity and prognosis followed by ESR. The RDW and NLR though were good markers of disease severity they are unreliable in assessing the disease prognosis and needs further study to prove its usefulness**.**

CONCLUSION

- Inflammatory markers levels are comparable to degree of severity both by radiological and microbiological criteria. P value < 0.001
- The level of CRP correlates with disease activity.
- Elevated ESR correlates significantly with disease activity only in moderate and severe disease.
- NLR ratio correlates significantly only with mild disease
- These findings can be extrapolated and may be useful in assessing the disease severity and follow up in pediatric TB and extra pulmonary TB where sampling from primary site are not always possible or correlation with other modalities of investigation are questionable.
- Thus the development of non-sputum-based biomarkers of treatment response would represent an advance for individual monitoring of TB patients.

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19) **P**retreatment neutrophil-to-lymphocyte ratio in peripheral blood was associated with pulmonary tuberculosis retreatment.

yin y[1], kuai s[2], liu j[2], zhang y[2], shan z[2], gu l[1], huang q[3], pei h[2], wang .

20)Elevatedlevels of c-reactive protein and ferritin in pulmonary tuberculosis patients remaining culture positive upon treatment initiation pryscila miranda,[#1] leonardo gil-santana,[#2,3,4] marina g. oliveira,[1] eliene d. d.

21) Changes in cytokine responses to tb antigens esat-6, cfp-10 and tb 7.7 and inflammatory markers in peripheral blood during therapy.

leem ay[1], song jh[1], lee eh[1], lee h[2], sim b[2], kim sy[1], chung ks[1], kim ey[1], jung jy[1], park ms[1], kim ys[1], chang j[1], kang ya

21)Red blood cell distribution width in the anemia secondary to tuberculosis.

baynes rd, flax h, bothwell th, bezwoda wr, atkinson p, mendelow b

22) Estimation of serum c-reactive protein values in patients with pulmonary tuberculosis]

article in russian] kaminskaia go, abdullaev riu, komissarova og.

23) C-reactive protein in patients with pulmonary tuberculosis muhammed khalid shaikh, javed akhtar samo, bikha ram devrajani, 1 2 3 syed zulfiquar ali shah, samina shaikh and imran shaikh

24) Serum c-reactive protein in pulmonary tuberculosis: correlation with bacteriological load and extent of disease

rao, sukhesh md[*]; bernhardt, vidya msc, phd[†]

25) Role of the c-reactive protein for the diagnosis of tb among military personnel in south korea. choi cm[1], kang ci, jeung wk, kim dh, lee ch, yim jj

26) C-reactive protein in patients with pulmonary tuberculosis muhammed khalid shaikh, javed akhtar samo, bikha ram devrajani, 1 2 3 syed zulfiquar ali shah, samina shaikh and imran shaikh

27) Associations between systemic inflammation, mycobacterial loads in sputum and radiological improvement after treatment initiation in pulmonary tb patients from brazil: a prospective cohort studyeliene d. d. mesquita,[#1] leonardo gil-santana,[#2,3,4] daniela ramalho,[5] elise tonomura,[6] elisangela c. silva,[5,7]martha m. oliveira,[8] bruno b. andrade,[#2,3,4] afrânio kritski)

28) Diagnostic accuracy of c-reactive protein for active pulmonary tuberculosis: a meta-analysis.

yoon c[1], chaisson lh[1], patel sm[2], allen ie[3], drain pk[4], wilson d[5], cattamanchi a

28)high blood neutrophil-lymphocyte ratio associated with poor outcomes in miliary tuberculosis

yeji han,[1] soo jung kim,[1] su hwan lee,[1] yun su sim,[2] yon ju ryu,[1] jung hyun chang,

29) **R**ed cell distribution width : updated jan 13 2015,author choladda vejabhuti curry md et al.

30) Sedimentation rate values in pulmonary tuberculosis vs normal healthy peoples in khammam region of telangana, india mahalakshmamma. v 1 , eliya raju. a *2 , jhansi. k 3

31) The haematological and biochemical changes in severe pulmonary tuberculosis charles d. w. morris arthur r. bird haylene nell32) The haematological and biochemical changes in severe pulmonary tuberculosis

charles d. w. morris arthur r. bird haylene nell*qjm: an international journal of medicine*, volume 73, issue 3, 1 december 1989 j assoc physicians india. 2001 aug;49:788, 790-4.

33) significance of haematological manifestations in patients with tuberculosis.

singh kj[1], ahluwalia g, sharma sk, saxena r, chaudhary vp, anant m.

34) medical journal of babylon-vol. 8- no. 4 -2011 1122 - studying some hematological changes in patients with pulmonary tuberculosis in babylon governorate muhammad obaid al-muhammadi and hayder gali al-shammery 608

PROFORMA

DATA COLLECTION :

The data of each patient was collected on a proforma

Sl No: Date:

Name:

Age

Gender:

Address

Phone:

BMI :Underweight (< 18.5) Normal (18.5-24.9)

Overweight (25.0-29.9)Obese (>=30)

Duration of symptoms:

Smoking history

Treatment	CPR	ESR	NLR	RDW
Before Rx				
At 2months				

Treatment	Sputum AFB
Before Rx	
At 2months	

ABBREVIATIONS

CRP- Creactive protein

NLR-Neutrophil lymphocyte ratio

RDW-Red cell distribution width

ESR-Erythrocyte sedimentation rate

PTB-Pulmonary tuberculosis

ATT – Anti tuberculosis treatment

SAA – Serum amyloid A

APR – Acute Phase Reactants

MDR-TB – Multi Drug Resistant Tuberculosis

HIV – Human imunodefeciency Virus

TLR – Toll Like Receptor

IFN – Interferon

IL - Interleukin

INSTITUTIONAL ETHICS COMMITTEE
GOVT. KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID. No. 30/2018 Meeting held on 09.01.2018

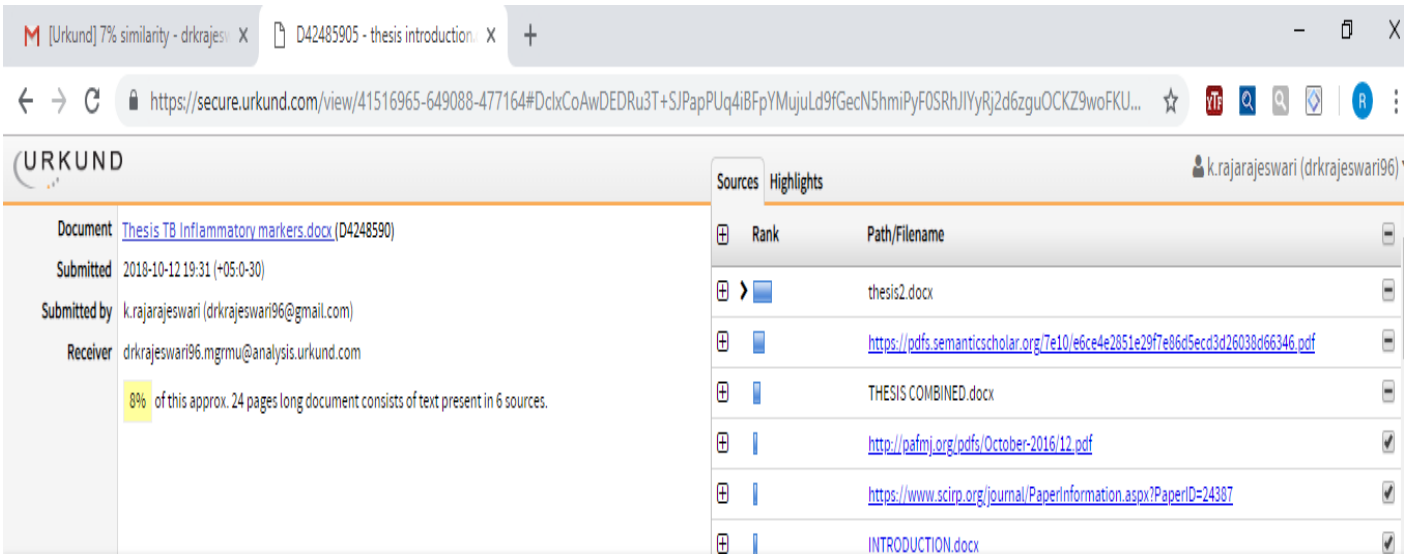
The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval " TO STUDY THE INFLAMMATORY MARKERS IN ACTIVE PULMONARY TUBERCULOSIS, ITS CORRELATION WITH DISEASE SEVERITY AND ITS RESPONSE TO ANTI TUBERCULAR TREATMENT" submitted by Dr.K.RAJARAJAESWARI, Post Graduate in TB and Respiratory diseases, Govt. Kilpauk Medical College, Chennai-10.

The Proposal is **APPROVED.**

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

DEAN
Govt. Kilpauk Medical College,
Chennai-10.

ME I Sec> Ethical Committee



URKUND ORIGINALITY CERTIFICATE

CERTIFICATE - II

This is to certify that this dissertation work titled TO STUDY THE INFLAMMATORY MARKERS IN ACTIVE PULMONARY TUBERCULOSIS, IT'S CORRELATION WTH DISEASE SEVERITY AND IT'S RESPONSE TO ANTI TUBERCULAR TREATMENT of the candidate Dr.K.Rajarajeswari with registration Number 201627252 for the award of M.D in the branch of Tuberculosis and Respiratory diseases . I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 8% percentage of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.