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Autoimmunity in Tuberculosis with Special Reference to Antinuclear Antibody

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Abstract

Introduction: Autoimmune phenomena has been documented in patient with active TB .Growing burden of TB infection alongside its association with autoimmunity increases the need for better preventative and therapeutic means to control this ailment. Autoimmunity is a critical and overlooked process in tuberculosis although various diverse evidence suggests that autoimmunity exacerbates the pathology in TB leading to cavitation and transmission. Sometimes TB patient presents with non-specific symptoms like fever, malaise and weight loss, where we may simultaneously order mycobacterial studies (acid-fast smear and mycobacterial culture) and autoimmune serology. Results of autoimmune serology usually available earlier, so patients are put on systemic corticosteroids rather than anti-TB treatment, rendering the further dissemination of Mycobacterium Tuberculosis bacilli (MTB) and increasing the TB related mortality. Considering all these facts, in our study, we have evaluated the Antinuclear antibody in newly diagnosed both pulmonary and extrapulmonary tuberculosis patients to find out its correlation with autoimmunity.

Materials and Methods: This hospital based observational study was conducted in the Department of Medicine and Department of TB & Chest Disease at Assam Medical College and Hospital, Dibrugarh. All newly diagnosed pulmonary and extrapulmonary tuberculosis patient's ≥ 12 years of age who gave written informed consent were included in the study. We excluded patients who were <12 years, known connective tissue disorders, chronic liver disease, chronic kidney disease, Diabetes mellitus, HIV and who refused to give consent.

Result: Out of total 132 patients of Tuberculosis (Pulmonary and Extrapulmonary) ,the highest number of both male and female patients were from the age group of 20-29 years (49.45% male, 36.59% female) .The mean age and standard deviation was 33.24 ± 14.38 years. Male preponderance of disease (68.94% male while 31.06% female) with male to female ratio of 2.21:1. Pulmonary tuberculosis (65.15%) was more common

than extrapulmonary tuberculosis (34.85%). The most common site of extrapulmonary tuberculosis was pleura in both male and female (35.13% male, 33.33% female), followed by abdomen in male (16.21%) and lymph node in female (22.22%). AFB positivity in sputum smear was 53.7% in male and 56.25% in female. The most common symptoms were cough (86.36%), followed by fever (75.75%) and weight loss (75%). Arthralgia was detected in 4.54% of patient. Polyarthralgia (83.33%) was the most common pattern of joint involvement. Majority of pulmonary tuberculosis patients (39.53%) had symptoms for 1 to 3 month at the time of presentation, while majority of extrapulmonary tuberculosis patients (45.65%) had symptoms for more than 6 months at the time of presentation. ANA was detected in 12.12% of study population. Majority (43.75%) of ANA positive patients were 30-39 years age groups. Homogenous pattern (62.5%) was the most common followed by speckled (18.75%) and rim (12.5%).ANA was detected in 13.95% of pulmonary tuberculosis and 8.69% of Extrapulmonary tuberculosis patients. ANA does not showed statistically significant difference (p value .378) in comparison with pulmonary and extrapulmonary groups. 75% of ANA positive pulmonary tuberculosis patients had sputum smear positive for AFB. Among ANA negative patients, majority (33.33%) of patients had pleural effusion, followed by abdominal Koch's (16.66%), tubercular lymphadenopathy (14.28%), disseminated TB (11.9%), pot's spine (9.52%), tubercular meningitis & tubercular pericardial effusion (4.76% each) and lastly lupus vulgaris & tuberculoma (2.38% each).None of ANA positive patients had rheumatological symptoms, while 5.17% of ANA negative patients had joint pain. After 2 months of treatment with Anti TB drugs, who were found ANA positive before the initiation of Anti TB treatments, 81.25% of patients were found to be ANA negative, while 18.75% of patients still remained ANA positive. **Conclusion:** Tuberculosis induced autoimmunity had positive correlation with active disease process which may be regarded as reactive because the autoimmune marker (ANA) of most of the patients became negative after receiving treatment with anti tubercular drugs.

Keywords: Autoimmunity, antinuclear antibody, tuberculosis.

Introduction

The immune system not only identifies and protects against infection by destroying pathogens but also regulates tissue repair following injury. Dysregulated immune system can lead to autoimmune and auto-inflammatory diseases. Immune defenses are normally categorized into the innate immune response, which provides immediate protection against an invading pathogen and the adaptive immune response, which takes more time to develop but confers exquisite specificity and long lasting protection[1].

Autoimmunity refers to the presence of antibodies or T lymphocytes that react with self-antigens which is present in all individuals. But autoimmune disease occurs only in those individuals in whom the breakdown of one or more of the basic mechanisms regulating immune tolerance results in self-reactivity that can cause tissue damage[2]. Autoimmune diseases are the third leading cause of morbidity and mortality in the industrial world after cancer and heart disease[3]. The pathogenesis of autoimmune diseases encompasses genetic, immunologic, hormonal, and environmental factors[4]. Of all environmental factors with a potential to trigger autoimmunity, perhaps the most important are infections agents, including Tuberculosis[5].

Autoantibody tests are widely used in the diagnosis of rheumatic diseases. Antinuclear antibodies(ANAs) are

directed against one or more components of the cell nucleus, including nucleic acids themselves and the proteins concerned with the processing of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).They occur in many inflammatory rheumatic diseases but are also found at low titre in normal individuals and in other diseases like infection, malignancy etc[6].

Tuberculosis (TB) which is caused by bacteria of the Mycobacterium Tuberculosis complex is one of the top 10 causes of death. Globally, an estimated 1.7 billion people are infected with M. tuberculosis and are thus at risk of developing the disease. TB can be cured with timely diagnosis and treatment with antibiotics. In spite of the availability of new diagnostic technologies many people around the world with TB remain untreated due to the latency of infection and difficulty in diagnosis[7]. Host immune response is vital in the containment of M. tuberculosis bacilli .The ability of M. tuberculosis to become dormant and resist the hostile environment of the phagosome and to develop delayed-type hypersensitivity are the major factors for the chronic nature of infection and granuloma formation. As a result, there is destruction of tissues and immune cells in these granulomas and release of intracellular self-antigens which can provoke the development of autoantibodies[8].

Autoimmune phenomena has been documented in patient with active TB,especially with extrapulmonary disease[9].Growing burden of TB infection alongside its association with autoimmunity increase the need for better preventative and therapeutic means by which to control this ailment[4].Autoimmunity is a critical and overlooked process, driving pathology in tuberculosis. Various diverse evidence suggests that autoimmunity exacerbates the pathology in TB leading to cavitation and transmission[10].There is a rich profile of autoantibodies evident in patients with TB and vice-versa, evidence of anti TB glycolipid antibodies in sera of patient with Systemic lupus erythematosus[11](SLE). Autoantibody titre may decrease or even return to normal, as the infection is controlled, suggests that autoantibodies are reactive to TB instead of being pathognomic and do not required immunosuppressant therapy[12].

Auto-reactive antibodies in tropical infections like TB, malaria etc may result from polyclonal B cell activation or from stimulation of antibodies to certain cross-reactive microbial antigens which may have been modified by the host environment[13]. For a patient in TB endemic areas, sputum samples for acid fast smear and mycobacterial culture should be performed, if a patient has elevated autoantibody levels but no typical or multiple rheumatological symptoms. As the patients with TB presents sometimes with non-specific symptoms like fever, malaise and weight loss, we may simultaneously order mycobacterial studies (acid-fast smear and mycobacterial culture) and autoimmune serology. Results of autoimmune serology usually available earlier, so patients are put on systemic corticosteroids rather than anti-TB treatment, rendering the further dissemination of Mycobacterium Tuberculosis bacilli (MTB) and increasing the TB related mortality[12].

Considering all these facts, in our study, we have evaluated the Antinuclear antibody in newly diagnosed both pulmonary and extrapulmonary tuberculosis patients to find out its correlation with autoimmunity.

Aims and Objectives

Aim: To assess autoimmunity in patients with pulmonary and extrapulmonary tuberculosis.

Objectives: To evaluate Antinuclear Antibody in patients with pulmonary and extrapulmonary tuberculosis.

Materials and Methods

This hospital based observational study was conducted in the department of medicine and department of TB & chest disease at Assam Medical College and Hospital, Dibrugarh after obtaining the clearance from Institutional Ethical Committee in a duration of one year (w.e.f 1st June 2019 to 31st May 2020)

We included newly diagnosed all pulmonary and extrapulmonary tuberculosis patients with age ≥ 12 years who gave written informed consent. Patients with known connective tissue disorders, chronic liver disease, chronic kidney disease, Diabetes mellitus, HIV, age < 12 years and who refused to give consent were Excluded from the study. **Case definition (as per RNTCP 2014 Guidelines):**

New Case of Tuberculosis

A tuberculosis patient who has never had treatment for tuberculosis or has taken anti-tuberculosis drugs for less than one month.

Sample size

Based on prior studies, prevalence of antinuclear antibody in patients with tuberculosis was about 33%.[9]

Hence the required sample size based on formula:

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Description

n = required sample size, t = confidence level at 95% (standard value of 1.96),p = estimated prevalence of ANA in patients with tuberculosis = margin of error at 8% (standard value of 0.08).Calculated Sample size was = 132.

Methodology

Based on prior studies, sample size was calculated as 132. After considering the inclusion and exclusion criteria 132 patients were included in the study. Patients attending Internal Medicine and TB & chest outpatient department (OPD) or admitted in the wards were subjected to detailed history taking, clinical examination and blood investigations like complete blood count (CBC), Renal function tests, Liver function tests, HIV, HBsAg and Anti-HCV. Chest X ray, Sputum / tissue/ fluid AFB, Sputum / tissue/ fluid gene expert analysis was done depending on the diagnosis. Immunological testing for ANA (Antinuclear Antibody) by IFA method was done for all the patients. For those with ANA Positive status, follow-up serum samples were collected after 2months of intensive phase of anti-TB treatment and they were also followed up for development for any Rheumatological symptoms.

Method of collection of data

The data for the purpose of study was collected in a predesigned proforma. The patients were fully informed about the study and their informed consent was taken prior to participation in the study.

History

History was obtained from both patients and attendants. History of cough with expectoration, anorexia, weight loss, fever, hemoptysis, chest pain, breathlessness, arthralgia, early morning stiffness, joint swelling, skin rashes, mucosal ulcers, hair loss were taken, along with duration of illness. Personal history included dietary history (vegetarian/non-vegetarian), whether smoker or non-smoker, alcohol history also taken.

Clinical examination

A through clinical examination was done on presentation. General examination was done to look for pallor, fever, icterus, cyanosis, clubbing, edema, pulse rate, respiratory rate, blood pressure, skin, hair and nail changes etc. All systems were examined thoroughly and noted clinical findings.

Investigations

Bacteriological Investigation: Sputum Smear microscopy was done with the Ziehl-Neelsen technique as per revised national tuberculosis control programme (RNTCP) guidelines. The sputum smears were graded according to the highest number of bacilli visualized per high power field and recorded by the semi-quantitative method (i.e. 1+,2+,3+) as use in tuberculosis control programmes. Cases with scanty AFB on microscopy were recorded as 1+ for grading convenience.

Preparation of slide and staining method

Standard operating procedure was followed.

The results are recorded in the laboratory form and the laboratory register as per the table given below:

Examination	Results	Grading	Number of field to be examined
More than 10 AFB per oil immersion field	Positive	3+	20
1-10 AFB per oil immersion field	Positive	2+	50
10-99 AFB per 100 oil immersion field	Positive	1+	100
1-9 AFB per 100 oil immersion field	Positive	Scanty B+	200
No AFB per 100 oil immersion field	Negative	Negative	100

Extrapulmonary samples for CB-NAAT (under RNTCP)[18,19]

The Cartridge Based nucleic acid amplification technique (CB-NAAT) or Xpert MTB/RIF is a cartridge-based, automated diagnostic test that can identify M tuberculosis (MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification technique (NAAT).The Xpert MTB/RIF detects DNA sequences specific for Mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction (PCR).It is based on the Cepheid Gene Xpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT).

Standard Operative Procedure For Collection, Transport And Processing Of Extra-Pulmonary Specimens[20]

Extra pulmonary specimens were divided in to two groups based on the site and mode of collection and the extent of contamination. Aseptically collected specimens, usually free from other microorganisms (sterile) fluids like spinal, pleural, pericardial, synovial, ascitic, blood, bone marrow, tissues (lymph node, tissue biopsies) and fine needle aspirates (FNAs).Specimens contaminated by normal flora or specimens not collected aseptically (not sterile) like gastric lavage, bronchial washings, urine, pus and stool (in case of disseminated TB in HIV infected patients and infants).

Collection of Extrapulmonary Specimens

Body fluids (spinal, pleural, pericardial, synovial, ascitic) was aseptically collected in a sterile container (falcon tube) by the physician using aspiration techniques or surgical procedures. Specimens were transported to the laboratory as quickly as possible.

Pleural Fluid: The fluid was collected in falcon tube using pleural tap or thoracocentesis with or without help of ultra-sonogram.

Pericardial Fluid: was collected in falcon tube using ultra sonogram.

Tissues: The aseptically collected tissues were placed by the physician in sterile containers (falcon tube) preferably without fixatives or preservatives. The specimen was shipped, it was protected from drying by adding sterile saline and maintaining a temperature of 4 - 15^{o}C. Specimens were transported to the laboratory as quickly as possible.

Pus: was collected in falcon tube with or without help of ultra-sonogram or computed tomography scan under aseptic and antiseptic precaution and transported to the laboratory as quickly as possible.

Ascitic Fluid: The fluid was collected in falcon tube using ascitic tap or paracentesis with or without help of ultra- sonogram.

Cerebrospinal Fluid: The fluid was collected in falcon tube using lumbar puncture under aseptic and antiseptic

precaution and transported to the laboratory as early as possible.

Fine Needle Aspiration Cytopathology (FNAC), Biopsy: In patients with lymph node tuberculosis, FNAC, excision biopsy of the most accessible peripheral lymph node confirms the diagnosis most of the times. For histopathological diagnosis, presence of granulomas, caseation, and demonstration of AFB have been commonly used to define a positive test.In superficial TB lymphadenitis, FNA biopsy of affected lymph nodes is the first-line diagnostic technique. Excisional biopsy has the highest sensitivity, whereas FNA is less invasive and may be useful[21]. Thus, if the FNA examination results were inconclusive, excision biopsy may need to be done.Histopathologic examination requires the specimen was to be placed in formalin, which destroys the mycobacteria and prevents further culture confirmation. Tissue samples were collected in falcon tube with small amount of sterile saline for Xpert MTB/RIF.

Body Fluid Examination

Pleural fluid: The pleural fluid is typically clear or straw coloured, but cloudy or serosanguinous fluid may also be obtained. Pleural TB usually causes an exudative effusion, defined on the basis of Light‘s criteria (pleural fluid/serum protein >0.5; pleural fluid/serum LDH >0.6; pleural fluid LDH > two-thirds the upper limit of serum LDH) with lymphocytic predominance in about 90% of cases. However, polymorphonuclear cells may predominate in patients with symptoms of <2-week duration, though a shift towards lymphocytic predominance is observed at repeat thoracentesis[22].Presence of a large number of mesothelial cells (> 1% of white blood cells) is strong evidence against the diagnosis of tuberculosis. Test for adenosine deaminase activity (ADA) level performed on pleural fluid can help support a diagnosis of pleural TB: > 70 U/L : Highly likely to be pleural TB, 40–70 U/L : Indeterminate level, other risk factors need to be considered. <40 U/L : Low likelihood of pleural TB, investigate for other causes.

Ascitic Fluid: Ascitic fluid in tuberculosis is straw coloured with protein >2.5g/dl, and total cell count of 150-4000/ pi, consisting predominantly of lymphocytes (>70%). The ascites to blood glucose ratio is less than 0.9650 and serum ascites albumin gradient is less than 1.1 g/dl. The yield of organisms on smear and culture is low. Staining for acid fast bacilli is positive in less than 3 per cent of cases. A positive culture is obtained in less than 20 per cent of cases, and it takes 6-8 wk for the mycobacterium colonies to appear. Adenosine deaminase (ADA) is increased in tuberculous ascitic fluid. The lowest cut-off value of ADA is taken as 39 IU/L.

Cerebrospinal Fluid

Clear cerebrospinal fluid (CSF) with moderately raised cells and protein and low glucose constitute the typical CSF picture of TBM. If allowed to stand, a pellicle or cobweb may form, indicating the presence of fibrinogen. The pellicle is highly suggestive but not pathognomonic of tubercular meningitis (TBM).

Pericardial Fluid

Echocardiography, pericardiocentesis and examination of pericardial fluid can help in confirming the diagnosis of pericardial tuberculosis. The raised protein and lactic dehydrogenase values speak for an exudates.

ANA-human epithelial type2 (HEp-2) indirect immunofluorescence assay (ANA-IFA)

Standard Operating Procedure for ANA-HEp-2

It is an indirect immunofluorescence assay to detect nuclear and / or cytoplasmic autoantibodies in human serum.

Principle

HEp-2 human epithelium cell, cultivated from the tissue of a patient suffering from carcinoma of the larynx. The substrate offers a homogenous cell growth and no extra cellular excipient. For the evaluation of samples the mitotic cells are of great importance as nuclear patterns may be defined more exactly. The appropriate end titer is that in which the patient serum shows a simple positive fluorescence.

The evaluation should always be performed with the positive and negative control. Weak fluorescence of the cell nuclei with titres between 1:40 (children) and 1:80 (adults) vagueness with respect to the result should be checked via the controls. In such a case the samples should be collected approximately every 6 weeks and tested in a similar way.

Kit contents & kit storage:- Slides,Conjugate,Positive Control, Negative Control, Mounting Medium, Wash buffer (10x),Sample buffer (1x)

Additional materials required:---Distilled water, test tubes for sample dilution, measuring flask, volumetric pipette,timer,fluorescence microscope with FITC system, (490nm excitation filter, 510nm barrier filter),incubation tray, staining dish, pipette tips, cover slips (24x60 mm),squeeze wash bottle

Sample collection and storage

Preparation of samples: Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Collect blood samples aseptically.Lipemic, icteric, hemolysed or microbially contaminated specimens may case interference. Sera with particles should be low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used during the first $8^{h,}$ respectively storage tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/ -4°F for longer periods. Avoid freezing and thawing.

Precautions: This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend avoiding contact with eyes and skin and wearing disposable gloves. All human source material used for some reagents (controls e.g.) has been tested by approved methods and found negative for HBsAg, Hepatitis C and HIV. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit control and patient samples as if capable of transmitting infectious disease and according to national requirements.

The kit contains material of animal origin (BSA, Immunoglobulin) as stated in the table of contents, handle according to national requirements.

Assay procedure

Prepare prior to pipetting: Allow all components to reach room temperature (20 – 26°C/ 64 – 78,8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test..

Preparation of the wash buffer: Dilute the concentrated buffer 1:10 with distilled water.

Dilution of samples: Dilute patient sera (for screening titer refer to kit procedure section above according to the product references that you are using) with 1x sample buffer. These vary between HEp-2, nDNA, rLKS, EMA etc. kits.

Controls ready to use.

Prepare a protocol: Data interpretation sheets are available in the kit procedure section according to the product references that you are using.

Test procedure

Remove required slide(s) from pouch and mark them. Do not touch the wells. Do not allow the slides to dry out.

Preparation of incubator tray: place a small volume of deionized or distilled water in an incubator tray and place slide(s) on supports in the incubator tray. Incubate slide(s) 30 minutes ± 10 minutes at room temperature in the moist incubator tray. Use consistent incubation times for the conjugate.

First incubation: Pipette an adequate volume of each diluted serum and controls (ready to use) into the appropriate wells, avoid direct contact of pipette with slide surface. Make sure that each well is completely covered with a corresponding serum. It is important to use as much test material as necessary to cover the well completely. But avoid a running between the wells because this may cause incorrect results.

Washing: After incubation remove slides from incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells.

Note: To prevent cross contamination tilt slide first towards one row and, carefully run a stream of wash buffer along the midline of the slide, allowing the wash buffer to run off the lower edge of the slide. Then tilt the slide towards the other row, and repeat this procedure, allowing the wash buffer to run off what is now the lower edge of the slide. Wash slide(s) 10 minutes with wash buffer in a slide staining dish. Avoid direct contact of solid items with the substrate. For optimal results change the buffer solution once after 5 minutes.

Lift slide (s) out of staining dish and carefully remove excess washing buffer.

Note: It is important that slide wells do not dry out during the procedure as this may lead to damage to the substrate. Please do not blot or dry the slide in any manner or allow slide to sit without fluorescent antibody reagent for longer than a few seconds.

Second Incubation: After the washing procedure return slide immediately to incubator tray and cover each well with an adequate volume of FITC – conjugate and make sure that the well is covered completely. Incubate slide(s) 30 minutes ± 10 minutes at room temperature in the dark.

Washing: After incubation remove slides from incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. Wash slide(s) 10 minutes with wash buffer in a slide staining dish. For optimal results, change the buffer solution once after 5 minutes.

Optimal Counter stain: Dilute counter stain (Evans Blue) 1:3000 in wash buffer and mix well. Tilt counter stain into the staining dish and incubate the slides in it. Refer to Kit Procedure section above according to the product reference that you are using for incubation time details. Evans blue covers unspecific background fluorescence. Remove slide(s) after the incubation time and rinse briefly with washing buffer. Remove excess washing buffer. Please do not blot or dry the slide in any manner.

Mounting Medium: Add an adequate volume.

Interpretation

Homogenous nuclear patterns: A uniform diffuse fluorescence covering the entire nucleoplasm sometimes accentuated in the nuclear periphery can be associated with different antibodies, mainly directed to chromosomal components (DNA, histones a, o.). In some cases some more intense staining of the inner edge of the nucleus (nuclear rim) can be seen.

Coarse speckled nuclear pattern: Densely distributed, variously sized speckles, generally associated with larger speckles, throughout the nucleoplasm of interphase cells. Nucleoli are negative, Metaphase and telophase cytoplasm contain speckles with condensation around the chromatin plates which itself is negative. This pattern is most commonly associated with staining of splicesomes.

Fine speckled nuclear pattern: Fine speckled staining in a uniform distribution, sometimes very dense so that an almost homogenous pattern is obtained. Nucleoli may be positive (especially with anti-SSB/La antibodies) or negative. Cytoplasm of metaphase cells shows fine speckles and condensation around the chromatin plates which are negative themselves.

Centromere pattern: Rather uniform discrete speckles located throughout the entire nucleus (40-60 speckles per nucleus).

Nucleolar patterns

Homogenous Nucleolar pattern: This pattern shows a diffuse fluorescence of the entire nucleoli and no staining of chromosome.

Clumsy Nucleolar pattern: Brightly clustered larger granules corresponding to decoration of the fibrillar centres of the nucleoli as well as the coiled bodies. In mitotic cells the metaphase and telophase plate appears to have a fluorescent irregular "fan-like" edge.

Punctuate nucleolar pattern: Small discrete grains mainly in the centre of the nucleoli. In metaphase cells 2-5 discrete speckles are seen within the chromatin body, corresponding to the nucleolar organizing regions.

Cytoplasmic fluorescence patterns:

Fine speckled patterns: Fine granules dispersed throughout the cytoplasm, becoming more evident, towards the periphery of the cell, sometimes producing a stardust-like appearance. No staining of nucleoli or nucleoli.

Diffuse cytoplasm pattern: A very fine dense granular to homogenous staining or cloudy pattern covering part or the whole cytoplasm is observed. No staining of nucleoli, but nucleoli may be homogenously stained if antibodies are directed to ribosomal RNA proteins, precursors of which are found in nucleoli.

Golgi like pattern: Staining of a polar organelle adjacent to the nucleus and composed of irregular large granules. Nuclei and nucleoli are negative. Diffuse staining of the cytoplasm of dividing cells with accentuation around chromosomal material.

Routine blood investigations

Blood Sugar Estimation: by Glucose oxidase and Peroxidase method.

Haemoglobin Estimation: by standard acid haematin method in Hellige's haemoglobinometer. A value less than 10 g/ dl was taken cut off for anaemia.

White Blood Cell Count: By using improved Neuber ruling slide after proper dilution under low power objective.

Differential Leukocyte Count: Peripheral blood film stained with Leishman's stain, examined under oil immersion lens.

ESR: By Westergren's method and reading was taken at the end of the first hour.

HIV: By ELISA.

Renal Function Test: Blood urea and Serum creatinine estimation was done by Beckman Coulter AU 400 analyzer. Blood Urea was measured by Modified Berthelot method. Serum Creatinine was measured by Modified Jaffe's method

Liver Function test

Serum bilirubin: was done using modified Jendrassik and Grof method. Bilirubin reacts with diazotize sulfanilic acid in acid medium to form azo bilirubin, a purple coloured complex whose adsorbance is proportional to bilirubin concentration (normal range 0.2-1 mg/dl).

Serum protein: done using Biuret and Beg Dye Binding method (using total protein and albumin kit). Normal ranges - Protein 6.4-8.2gm/dl, albumin-3.4-5gm/dl, globulin 2.5- 3.5gm/dl.

Aspartate transaminase (AST): estimation was done using Reitman and Frankel Method. AST catalyzes the reaction:

α –ketoglutarate+ L aspartate =glutamate + oxaloacetate

Oxaloacetate so formed is coupled with 2, 4-Dinitrophenyl hydrazine to give corresponding hydrazone, which gives brown color in alkaline medium which is measured colorimetrically. (normal range:15-37 U/L).

Alanine transaminase(ALT): was done using Reitman and Frankel method similar to AST. Here pyruvate reacts with 2, 4 DNPH to give brown color which can be measured colorimetrically. (normal range 12-78 U/L).

Alkaline phosphatase (ALP): was estimated by photometric determination of alkaline phosphatase. (normal range 46-116 U/L).

GGT: was estimated by ɣ- glutamyl transferase method which is an adaptation of the methodology recommended by the International Federation of Clinical Chemistry. (normal range :5-85 U/L).

Radiological investigations:

Chest X-ray PAV: done by Siemens 800mA Machine .Chest x-ray was analysed, noted the presence or absence of cavities, number and location of cavities infiltrates or air space opaicites, consolidation, hilar enlargement, fibrosis, pleural effusion, pneumothorax and for any other findings.

USG Whole Abdomen: Siemens Acuson Antares Ultrasound System.

CT Thorax: Done by Siemen somatom spirit dual slice CT.CT of the chest is currently the best imaging modality to visualise both pleura and lung parenchyma in TB pleural effusions.

Echocardiography: (Suggestive of Tuberculosis=STB Means)Echocardiography is useful for detecting the presence of pericardial fluid .On echocardiography there are patchy deposits 4–8 mm in thickness with fibrinous ‘‘strands criss crossing the pericardial space. The appearance is quite characteristic of TB.

CT Abdomen: (Suggestive of TB=STB Means) Done by Siemens somatom spirit dual slice CT has been found to be very useful in abdominal tuberculosis.

C.T Brain: Done by Siemens somatom spirit dual slice CT .CT scan of the brain may reveal thickening and enhancement of basal meninges, hydrocephalus, infarction, periventricular oedema, and mass lesions due to associated tuberculoma or tuberculosis abscess. Common sites of exudates are basal cistern ambiens, suprasellar cistern and sylvian fissures.

MRI Brain: Done by Siemens magneto avanto 1.5 tesla MRI of the brain may reveal thickening and enhancement of basal meninges, hydrocephalus, infarction, periventricular oedema, and mass lesions due to associated tuberculoma or tuberculosis abscess.

MRI Spine: Done by Siemens magneto avanto 1.5 tesla. MRI is the neuroimaging of choice for spinal tuberculosis. MRI is more sensitive than x-ray and more specific than CT in the diagnosis of spinal tuberculosis.

Statistical Analysis

The data collected was tabulated in Microsoft Excel Worksheet and computer based analysis was performed using the statistical product and service solutions (SPSS) 20.0 software (SPSS, Chicago, Illinois, USA) and Microsoft Excel 2010. The categorical variables were summarized as proportions and percentages. Quantitative variables were estimated using measures of central tendency (mean, median) and measures of dispersion. Chi square test was used to compare frequencies as appropriate. P value was considered significant at a level of < 0.05.

Result and Observation: Total 132 patients of Tuberculosis (Pulmonary and Extrapulmonary) who

fulfilled inclusion and exclusion criteria were included in this study. The following tables and figures illustrate the important features and results of this study.

Table 1: Age distribution of study population

Age groups (in years)	Male		Female	
	Number (n=91)	Percentage (%)	Number (n=41)	Percentage (%)
13 - 19	6	6.59	5	12.20
20 - 29	45	49.45	15	36.59
30 - 39	21	23.08	9	21.95
40 - 49	7	7.69	4	9.76
50 - 59	5	5.49	3	7.32
60 - 69	4	4.40	2	4.88
≥70	3	3.30	3	7.32
Mean Age ± SD	33.24±14.38 years			

Table 1. shows the highest number of both male and female patients were from the age group of 20-29 years (49.45% male, 36.59% female) followed by 30-39 years (23.08% male, 21.95% female). The mean age and standard deviation was 33.24 ± 14.38 years.

Fig 1: Gender distribution of study population

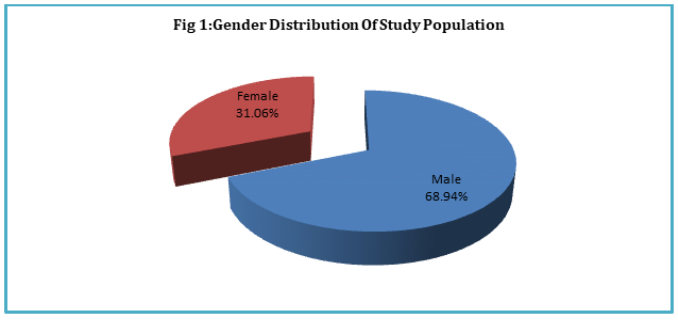

Fig 1 shows that 68.94% of patients were male while 31.06% of patients were of female gender. There was a male preponderance of disease with male to female ratio of 2.21

Fig 2: Sites of Tuberculosis

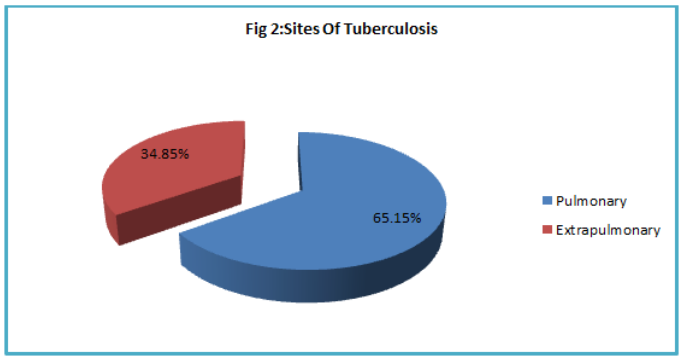

Fig 2 shows majority (65.15%) of patients were having pulmonary tuberculosis, while only 34.85% of patients were having extrapulmonary tuberculosis.

Table 2: Sites of TB (gender wise)

Gender	Pulmonary		Extrapulmonary	
	Number (n=86)	Percentage (%)	Number(n=46)	Percentage (%)
Male	54	62.79	37	80.43
Female	32	37.21	9	19.57

Table 2 shows 62.79% male and 37.21% female were having pulmonary tuberculosis, while 80.43% male and 19.56% female were having extrapulmonary tuberculosis.

Table 3: Distribution of Extrapulmonary TB Sites

Extrapulmonary TB sites	Male		Female	
	Number(n=37)	Percentage (%)	Number(n=9)	Percentage (%)
Pleura	13	35.13	3	33.33
Abdomen	6	16.21	1	11.11
Multiple sites	5	13.51	1	11.11
Lymph node	5	13.51	2	22.22
Spine	3	8.10	1	11.11
Pericardium	2	5.40	0	0
Brain	2	5.40	1	11.11
Skin	1	2.70	0	0

Table 3 shows the most common site was pleura in both male and female (35.13% male, 33.33% female), followed by Abdomen in male (16.21%) and Lymph node in female (22.22%). Skin involvement was rare.

Table 4: Sputum Smear AFB status (in pulmonary tuberculosis)

Sputum AFB	Male		Female	
	Number (n=54)	Percentage (%)	Number (n=32)	Percentage (%)
Positive	29	53.70	18	56.25
Negative	25	46.30	14	43.75

Table 4 shows sputum smear AFB status among pulmonary tuberculosis patients. 53.7% male and 56.25% female were having AFB in sputum smear.

Fig 3: Symptoms at presentation of study population

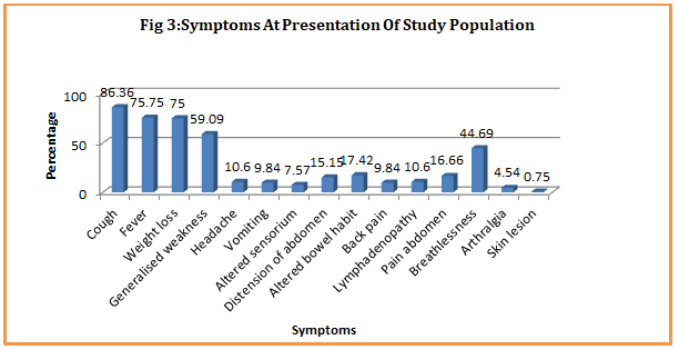

Fig 3 shows the most common symptoms was cough (86.36%), followed by fever (75.75%) and weight loss (75%). Arthralgia was detected in 4.54% of patient.

Table 5: Pattern of joint involvement in study population

Joint involvement	Number (n=6)	Percentage (%)
Monoarticular	1	16.66
Oligoarticular	0	0.00
Polyarticular	5	83.33

Table 5 shows the pattern of joint involvement among Tuberculosis patients. Majority (83.33%) of patients had polyarthralgia.

Table 6: Duration of presenting symptoms of TB

Duration (in months)	Pulmonary		Extrapulmonary	
	Number (n=86)	Percentage (%)	Number (n=46)	Percentage (%)
<1	18	20.93	6	13.04
1-3	34	39.53	8	17.39
4-6	22	25.58	11	23.91
>6	12	13.95	21	45.65
Duration MEAN±SD	4.67 ±2.63 MONTHS			

Table 6 shows majority of pulmonary tuberculosis patients (39.53%) had symptoms for 1 to 3 month at the time of presentation, while majority of extrapulmonary tuberculosis patients (45.65%) had symptoms for more than 6 months at the time of presentation. The mean duration of symptoms and standard deviation was 4.67 ± 2.63 months.

Table 7: ANA status among study population

ANA			
Positive		Negative	
Number (n=132)	Percentage (%)	Number (n=132)	Percentage (%)
16	12.12	116	87.87

Table 7 shows that ANA was detected in 12.12% of study population.

Table 8: Gender wise ANA status of study population

ANA status	Gender			
	Male		Female	
	Number (n=91)	Percentage (%)	Number (n=41)	Percentage (%)
Positive	10	10.98	6	14.63
Negative	81	89.01	35	85.36

Table 8 shows that ANA was detected in 10.98% of male and 14.63% of female among study population.

Table 9: Age Groups and ANA Status

Age groups (in years)	ANA status			
	Positive		Negative	
	Number (n=16)	Percentage (%)	Number (n=116)	Percentage (%)
13 - 19	1	6.25	10	8.62
20 - 29	4	25.00	56	48.27
30 - 39	7	43.75	23	19.83
40 - 49	2	12.50	9	7.76
50 – 59	2	12.50	6	5.17
60 – 69	0	0	6	5.17
≥70	0	0	6	5.17

Table 9 shows that majority (43.75%) of ANA positive patients were belongs to 30-39 age groups, followed by 20-29 age groups (25%).

Fig 4: Pattern of Immunofluorescence Staining

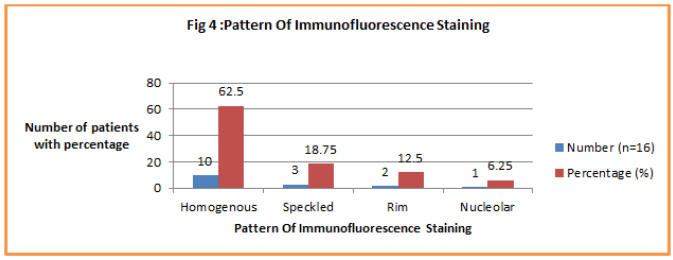

Fig 4 shows homogenous (62.5%) was the most common pattern, followed by speckled (18.75%), rim (12.5%) and nucleolar (6.25%).

Table 10: ANA status and sites of TB

ANA status	SITES OF TB				p value
	Pulmonary		Extrapulmonary		
	Number (n=86)	Percentage (%)	Number (n=46)	Percentage (%)	0.378
Positive	12	13.95	4	8.69	
Negative	74	86.04	42	91.30	

Table 10 shows ANA was detected in 13.95% of pulmonary tuberculosis and 8.69% of extrapulmonary tuberculosis patients. ANA does not showed statistically significant difference (p value .378) in comparison with pulmonary and extrapulmonary groups.

Table 11: Sputum smears AFB status in ANA positive pulmonary tuberculosis cases

Sputum AFB (n=12)			
Positive		Negative	
Number	Percentage (%)	Number	Percentage (%)
9	75.00	3	25.00

Table 11 shows the sputum smear AFB status among ANA positive pulmonary tuberculosis patients. 75% of ANA positive pulmonary tuberculosis patients had sputum smear positive for AFB.

Table 12: Extrapulmonary TB and ANA Status

Extrapulmonary TB	ANA status			
	Positive		Negative	
	Number (n=4)	Percentage (%)	Number (n=42)	Percentage (%)
Pleural effusion	2	50.00	14	33.33
TB meningitis	0	0.00	2	4.76
Disseminated TB	1	25.00	5	11.90
Lupus vulgaris	0	0.00	1	2.38
Pot's spine	0	0.00	4	9.52
Abdominal Koch's	0	0.00	7	16.67
Tubercular lymphadenopathy	1	25.00	6	14.29
Tubercular pericardial effusion	0	0.00	2	4.76
Tuberculoma	0	0.00	1	2.38

Table 12 shows that among ANA positive patients 50% had pleural effusion, 25% each had tubercular lymphadenopathy and disseminated tuberculosis. Among ANA negative patients, majority (33.33%) of patients had pleural effusion, followed by abdominal koch's (16.66%), tubercular lymphadenopathy (14.28%), disseminated TB (11.9%), pot's spine (9.52%), Tubercular meningitis & tubercular pericardial effusion (4.76% each) and lastly lupus vulgaris & tuberculoma (2.38% each).

Table 13: Rheumatological symptoms among ANA positive and negative tuberculosis patients

Rheumatological Symptoms	ANA status			
		Positive	Negative	
	Number (n=16)	Percentage (%)	Number (n=116)	Percentage (%)
Joint pain	0	0	6	5.17
Hair loss	0	0	0	0.00
Oral ulcer	0	0	0	0.00
Skin rash	0	0	0	0.00

Table 13 shows that none of ANA positive patients had Rheumatological symptoms, while 5.17% of ANA negative patients had joint pain.

Table 14: Common clinical characteristics of patients with ANA positive and ANA negative tuberculosis

Symptoms	ANA positive		ANA negative		p value
	Number (n=16)	Percentage (%)	Number (n=116)	Percentage (%)	
Cough	14	87.5	106	91.37	0.6189
Fever	13	81.25	93	80.17	0.8569
Weight loss	13	81.25	92	79.31	0.9191
Generalised weakness	10	62.5	74	63.79	0.9197
Breathlessness	8	50.00	56	48.27	0.8971

Table 14 shows the comparison of common clinical characteristics between patients with ANA positive and negative status. Here the p value is not significant. All clinical characteristics were almost similar between two groups.

Table 15: Radiographical findings among pulmonary TB patients (ANA positive vs negative)

Table 15 shows comparison of radiographical finding among ANA positive and negative pulmonary TB patients. Here the p value is not significant. Radiographical findings were almost similar between two groups.

Table 16: ANA status after treatment with 2 months treatment with Anti TB Drugs

ANA status	After treatment	
	Number (n=16)	Percentage (%)
Positive	3	18.75
Negative	13	81.25

Table 16 shows ANA status among patients after 2 months of treatment with Anti TB drugs, who were found positive before the initiation of Anti

TB treatments. 81.25% of patients were found to be ANA negative, while 18.75% of patients still remained ANA positive.

Discussion

A total 132 patients were included in the study whose serum samples were evaluated for ANTINUCLEAR ANTIBODY. Those who found positive were follow up for development of any Rheumatological symptoms and at the end of Intensive therapy (2months), their serum samples were tested again for ANTI NUCLEAR ANTIBODY. Along with this, clinical radiological as well as sputum/body fluid/tissue results were assessed.

A) Age Distribution

We observed that the highest number of patients was from the age group of 20-29 years (49.45% male, 36.59% female) followed by 30-39 years age group (23.08% male, 21.95% female). The mean age and standard deviation was 33.24 ±14.38 years.

Faris et al (2018)[17] found that tuberculosis was more prevalent in the age group 18 to 35 years of age. B. L. Rapoport et al (1990)[16] found that mean age and standard deviation were 35.1 ± 12.4 years in their study. However, O Elkayamet al (2007)[9]found that it was 52.3 ± 17 years.

We have observed that young adults are most commonly infected with tuberculosis which may be due to frequent and much wider range of social contacts outside household.

B) Sex Distribution

In our study,out of 132 patients, 91 (68.94%) were male and 41 (31.06%) were female, with male-female ratio of 2.21:1.

Faris et al (2018)[17] reported 67.1% males (out of 61 patients). O Elkayam et al (2007)[12] observed male female ratio of 1.38:1 whereas B. L. Rapoport et al (1990)[16] showed male female ratio of 1.9:1.

Our observation shows a male preponderance which is most probably attributed to the increased inhabitants and employees of locales where vulnerable people gather (e.g. occupational exposure, prison etc). It may also be attributed to the fact that in our society women's health problems are not given priority and fewer symptomatic women than men present to health facilities.

C) Sites of Tuberculosis

We encountered Pulmonary Tuberculosis 65.15% and extrapulmonary tuberculosis 34.85%.

In a study by Faris et al (2018)[17] ,pulmonary tuberculosis was 57.3% whereas 42.62% of patients had extrapulmonary tuberculosis. Similarly, a study by Chieh-Yu sheng et al (2013)[12], reported that 96% of patients in their study were having pulmonary tuberculosis.

In our study, pleura (35.13% male, 33.33% female) were the most common extrapulmonary site.Our observation shows that pulmonary tuberculosis is more common than the extrapulmonary tuberculosis. However extrapulmonary tuberculosis is also difficult to diagnose than pulmonary tuberculosis.

D) Sputum smears for AFB positivity

In our study 53.70% of Pulmonary Tuberculosis patients had sputum smear positive for AFB.

Chief-Yu Sheng et al (2013)[12] found that 31% of Pulmonary TB patients were having sputum smear positive for AFB. Faheem et al (2019)[14] found that 67.5%

of Pulmonary TB patients were positive for AFB in sputum smear.

We might get more numbers of sputum positive pulmonary TB cases in ours areas. But as the clinician started Anti TB drugs early, once they diagnosed patient on the basis of clinical and radiological background, we missed many sputum positive cases. Inadequate sample and technical error may be the one of the reason for not getting AFB in sputum smear.

E) Most common symptoms

In our study, cough (86.36%) was the most common symptoms, followed by fever (75.75%) and weight loss (75%).

O Elkayamet al (2007)[9] reported cough in 94% of patients and fever in 73% of patients.

Yogesh Chandler et al (2019)[15] found that 99% of patients had cough and 70% of patients had fever. These two studies are almost correlating with present study.

Chieh- Yu Sheng et al (2013)[12] found that 25% of patients had cough and 14% of patients had fever.

F) Joint involvement

We observed that out of 132 TB patients, only 6 (4.54%) patients had arthralgia and majority of them had polyarthralgia (83.33%).O Elkayamet al (2007)[9] found that 4% of patients had arthralgia. Rheumatological symptoms were rare among TB patients. None of patients had oral ulcer, hair loss, skin rashes etc.

G) Duration of symptoms

In the present study, mean duration of symptoms was 4.67 ± 2.63 months.O Elkayamet al (2007)[9] reported that mean duration of symptoms was 4.4 ± 1.7months among the Diseased study groups, while B. L. Rapoport et al (1990)[16] reported it as 1.9 ± 1.6months, among untreated TB groups. Thus our study is more or less comparable to study done by O Elkayam et al[9].

H) ANA status among Tuberculosis patients

In this present study, out of 132 patients of tuberculosis, we found ANA positivity in 16 patients (12.12%). Out of which 10.98% were male and 14.63% were female. We included both Pulmonary and Extrapulmonary cases. However majority of previous studies on auto antibody in Tuberculosis included mainly active Pulmonary TB cases.

In the present study mean age of ANA positive patient was 33.25 years. A.O. Adebajo et al (1993)[13]found that mean age of patients with ANA positive sera was 37.65 years.

In the present study, we found that 62.5% of ANA positive patients had homogeneous and 18.75% had speckled immunofluorescence staining pattern. A.O. Adebajo et al (1993)[13] found that 35.29% had homogeneous and 64.7% had speckled immunofluorescence staining pattern. B.L. Rapoport et al (1990)[16] found that 33.3% each had homogeneous and speckled staining pattern.In our study 13.95% of Pulmonary TB patients were found to be ANA IIF positive.In a study by Yogesh Chanderet al (2019)[15] it has been reported that out of 89 pulmonary tuberculosis patients, 6 (6.7%) had found ANA IIF positive.

In a study by Faris et al (2018)[17] it has been found that seroprevelance of ANA antibodies among pulmonary tuberculosis patient was 8.8%.O Elkayamet al (2007)[9] observed significantly higher proportion of active pulmonary tuberculosis patients were having ANA IIF positive (33%) whereas A. O. Adebajo et al (1993)[13] found that15% of TB patients were positive for ANA.In a study by B. L. Rapoport et al (1990)[16] it has been found that 6.1% untreated pulmonary TB patients were positive for ANA.Thus the findings of present study almost correlate with study done by A. O. Adebajo et al[13].

In the present study, ANA IIF positivity in Extrapulmonary TB patients were found to be 8.69%. Out of them 50% had Tubercular pleural effusion, 25% each had Disseminated TB and Tubercular lymphadenopathy.

In a study by Faris et al (2018)[17] it has been found that seroprevalence of ANA among peripheral Tuberculosis was 20% and axial Tuberculosis was 6.3%. We were not able to compare ANA status properly among extrapulmonary TB cases. In this present study, among ANA positive Pulmonary TB patients 75% of patients were having sputum smear positive for AFB.In this present study, none of ANA positive TB patients had rheumatological symptoms.

I) Correlation of clinical and radiological characteristics with ANA positivity

There is no difference in clinical and radiological characteristics among tuberculosis patients with ANA positive and ANA negative, in this present study. This finding is consistent with the findings reported by a study done by Chieh-Yu Shen et al (2013)[12], where they found that the presence of autoantibodies neither altered the clinical manifestations and radiographic findings of active TB.Similarly, O Elkayamet al (2007)[9] reported that there was no significant correlation between clinical symptoms and serological findings.

J) ANA status on follow up

In this present study, 81.25% previously ANA positive patients were found negative on 2 months follow up analysis. They were on Anti tubercular therapy. None of them developed any rheumatological symptoms. 18.75% of patients, who were previously ANA positive, they remain positive after 2 months of treatment with anti TB drugs.

Chieh-Yu Shen et al (2013)[12] found that for the 11 patients with elevated anticardiolipin IgG and 6 patients with elevated anti-scl-70 at baseline, follow up serum titres after 3 months of anti- TB treatment, were returned to normal limits in seven and four patients respectively. None of the patients received immune suppressants and DMARDs. Among the five patients with persistently elevated anti-cardiolipin IgG or anti-Scl-70 autoantibodies, none had rheumatological symptoms at the end of 6 month anti- TB treatments.

Thus, from above findings, we can conclude that elevated Antinuclear antibodies in patients with tuberculosis is likely to be reactive as there is lack of clinical correlations and spontaneous regression after anti-TB treatments in most of cases.

B. L. Rapoport et al (1990)[16] found that among untreated TB and treated TB patients, positive ANA test was detected in more numbers among treated TB groups. They found that the appearance of ANA correlated with treatment duration and not with disease duration, suggesting that ANA was induced mainly by anti TB drugs, particularly INH.

So in our study we may consider that, those patients who were remain positive after anti TB treatment, may be due to drug induced autoantibody production.

Limitations of the study

The limitations of our study are single centered, observational study with small sample size in which short term follow up of the study populations were done.

Conclusion

We observed significant number of patients with tuberculosis induced autoimmunity which had positive correlation with active disease process and may be regarded as reactive. This can be explained by the fact that the autoimmune marker (ANA) of most of the patients became negative after receiving treatment with anti tubercular drugs. However, multicentered, large cohort studies are needed to solve this conundrum.

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