



Some Haematological Parameters and Immunoglobulin Levels in *Mycobacterium tuberculosis* Infected Patients in Benin City, Southern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IMO and OEB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CMS managed the analyses of the study. Author OCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Mycobacterium tuberculosis (MTB) infection is a major public health challenge in Nigeria. This research aims at determining the effect of MTB infections on some haematological and immunological parameters in MTB patients in Benin, Nigeria. One hundred and fifty subjects were recruited for the studies. These subjects comprised of 50 MTB positives, 50 MTB positives on direct observation and treatment (DOTS), and 50 controls. Ethical approval was obtained from the Ethics

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Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi campus. Informed consent was obtained from all subjects recruited for the studies. Five milliliters of blood was collected from each subject for HIV test by ELISA method, CD4 count by Flow Cytometric technique, immunoglobulin test by immune-turbidimetric method, estimation of leucocyte profile by automated haematological analyser and ESR by routine method. In addition, sputum was collected from each subject for Ziehl Neelsen technique of *Mycobacterium tuberculosis* examination. Statistical Package for Social Science (version 20) was used for the data analysis. Results obtained from the study showed that the comparison of the WBC of the control subjects and MTB positive subjects was significantly higher in the MTB positive subjects ($p < 0.05$). Also, WBC of *Mycobacterium tuberculosis* (MTB) positive subjects was significantly higher than MTB positives on DOTS. The CD4 count of DOTS administered MTB positives was significantly higher than non-DOTS MTB positive subjects ($p < 0.05$). The mean values of immunoglobulin A, G, M (IgA, IgG and IgM) in MTB positive subjects were significantly higher compared with the values in the controls ($p < 0.05$). Also, IgG value in the DOTS administered MTB positive subjects was significantly higher than the mean value in the control subjects. In same manner, the IgA and IgM value for non-DOTS in each case was higher than MTB on DOTS subjects ($p < 0.05$). The value of Haematocrit (HCT) and Lymphocyte (LYM) count were significantly higher in control subjects when compared with DOTS administered MTB positives and MTB positives in each case ($p < 0.05$). The value of neutrophil for MTB positives was significantly higher than the value in the control subjects ($p < 0.05$). Thus Immunoglobulin G, A, and M could be used as a predictive marker for monitoring MTB infections, alongside haematological parameters and CD4 count.

Keywords: *Mycobacterium tuberculosis*; immunoglobulins; IgG; IgM; IgA.

1. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). It typically affects the lungs (pulmonary TB) but it can also affect other sites (extra-pulmonary TB) as well [1]. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air [2]. The incidence of tuberculosis particularly in Nigeria and the World in general is of major concern to healthcare practitioners. Nigeria has the world's fourth largest tuberculosis (TB) burden, with more than 460,000 estimated new cases in 2007 [3]. Worldwide, TB is one of the most common HIV-related opportunistic infections. It is also the second leading cause of death from an infectious disease worldwide [3]. Infection control of Tuberculosis entails early diagnosis and effective treatment of TB patients [4]. TB treatment may present significant haematological disorders and some anti-tuberculosis drugs also have side effects [5].

Mycobacterium tuberculosis (MTB), one of the oldest known diseases and still a major cause of mortality today, has many manifestations affecting the bone, the central nervous system and many other organ systems. Tuberculosis (TB) is the leading cause of death in HIV patients. The key components of the immune response in TB include T lymphocytes and

alveolar macrophages. The T helper type 1 subclass is the major effectors cell in cellular immunity of TB or the 'policeman' of TB control in the lung resulting in dissemination of *Mycobacterium tuberculosis*. One study hypothesized that TB infection contributes to additional reduction in CD4 cell count in HIV patients presenting with dual infection [6].

On the other hand, Lawn et al. [7] showed that there is selective clonal depletion of *Mycobacterium tuberculosis* specific CD4+ T lymphocytes. Additionally, Swaminathan and Narendran [8] demonstrated the effect of tuberculosis (TB) on CD4 cells and they found that TB develops at any level of CD4 count, though extra-pulmonary and disseminated forms of the disease are more common as immunodeficiency increases. Ukpe and Southern [9] studies in South Africa suggest that active TB is associated mostly with very high ESR values (≥ 100 mm/h) irrespective of HIV status, and that concomitant HIV infection tends to increase the proportion of TB cases with high erythrocyte sedimentation rate (ESR) values. Likewise, Al-Marri and Kirkpatrick, [10], Sarkar et al. [11] indicated that ESR is commonly done as a non-specific test during the initial diagnostic work up for TB, which is a chronic bacterial infection.

Immunoglobulin (Ig) is a large γ -shaped proteins which identifies and neutralize foreign objects such as bacteria and viruses by immune system

[12]. Notably, Kardjito et al. [13] found that tuberculin-positive control subjects had higher levels of antibody than tuberculin-negative controls in the IgG class only. Alongside, Turneer et al. [14] used p32 antigen of *Mycobacterium bovis* BCG to investigate the humoral immune response in human tuberculosis. In their work, immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) were directed against p32 and measured by enzyme-linked immunosorbent assay (ELISA). The mean IgG and IgA antibody levels differed significantly ($p < 0.001$) between active tuberculosis patients and non-active tuberculosis patients. According to them, mean IgG antibody levels, and mean IgA antibody levels were higher ($p < 0.05$) in patients with positive microscopic examination for acid-fast bacilli than in patients with negative microscopic examination. They found a positive relation between mean levels and the extent of disease; but there was no difference in mean IgM antibody levels between patients and controls. According to Turneer et al. [14], only active tuberculosis seemed to induce significant anti-p32 antibody levels and may be associated with positivity. Due to findings from these independent researchers, the effect of tuberculosis infection and therapy in immunoglobulin G, A, and M was explored in this work.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Tuberculosis clinic of the Central Hospital Benin City, Southern Nigeria.

2.2 Study Design

This is a case control study designed to assess some haematological parameters and immunoglobulins in *Mycobacterium tuberculosis* infected subjects.

2.3 Study Population

One hundred and fifty (150) subjects aged 18 – 60 years were recruited from the Tuberculosis clinic, and health subjects of Central Hospital, Benin City as follows;

1. Fifty (50) *Mycobacterium tuberculosis* subjects on DOTS therapy. The amount (gram or milligram) of anti-tuberculosis drugs administered to subjects were dependent on the weight [1].

2. Fifty (50) *Mycobacterium tuberculosis* subjects not on DOTS therapy.
3. Fifty apparently, healthy subjects from the staff and student population of same hospital.

2.4 Ethical Consideration and Informed Consent

Informed consents were obtained from all the participants. The ethical approval for the study was obtained from the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi Campus, Nigeria.

2.5 Inclusion and Exclusion Criteria

Adult male and female subjects aged 18 to 60 years were included in the study, while Pregnant women, HIV positive subjects, subjects with malignancy demanding cytotoxic chemotherapy or radiation therapy and Patients at the extremes of age were excluded since they may naturally have very weakened immune system [13].

2.6 Sample Collection

Six millilitres (6 ml) of blood was collected and dispensed as follows;

Three (3) ml of blood into tripotassium Ethylene diamine tetra-acetic acid (K_3EDTA) vacutainer for CD4 count by flow cytometric method, and determination of haematocrit, haemoglobin level, total white cell count and differential white cell count using an auto-analyser and ESR using standard routine methods.

Three (3) ml into plain vacutainer. This was used for HIV tests by immunochromatographic method and immunoglobulin G, A and M analysis by immunoturbidimetric method, using spectrophotometer.

2.7 Laboratory Methods

2.7.1 Procedure for *Mycobacterium tuberculosis* diagnosis using Ziehl Neelsen staining method

Sputum preparation

The slide was labeled with the aid of Diamond pencil while wire loop was used in making the sputum smear on a clean slide. The smear was passed over Bunsen flame three times to

inactivate the *Mycobacterium* and fix the smear on the slide. The fixed smear thereafter was placed on staining rack.

Sputum staining

The slide was flooded with strong carbol fuchsin, and then the slide and smear- stain was heated with Bunsen flame from the bottom of the slide for about three minutes interruptedly. The slide was flooded with water and blotted afterward. One (1) % acid alcohol was applied to the slide to decolorize it for about 5 minutes. The slide was flooded with Methylene blue for about 30 seconds and the methylene blue was rinsed off with tap water. The slide was allowed to air dry on the bench.

Sputum microscopy

The slide was viewed using the x100 objective and reported as sputum smear positive (using appropriate grading) or sputum smear negative [1].

2.7.2 HIV-1/2 assay as described by Alere Medical Company Limited, Japan

The protective foils were removed from each test strip. Fifty microliter (50 µl) of plasma was dispensed into the specimen pad of the test strip with the aid of precision pipette. Chase buffer was added to the sample pad after one minute waiting. The reaction was allowed for 15 minutes (up to 60 minutes). The appearance of distinct red lines on test region and control region of the kit suggests positive HIV test while one distinct red line in the region of control suggested HIV negative test. Appearance of the distinct red line on the control region validated the HIV result and without that, the kit was assumed to be invalid (i.e. the HIV result cannot be accepted or it is considered invalid).

2.7.3 HIV-1/2 STAT-PAK assay as described by Chembio diagnostic systems incorporated

The Chembio HIV 1/2 STAT-PAK test devices were removed from its pouch and placed on a flat surface. The test device was labeled with the patient's identification number. Five microlitre (5 µl) blood samples were collected with sample loop. The sample loop was held vertically and dispensed into the sample pad. Also 3 drops (about 105 µl) of buffer was added drop wise into the sample well. The reaction was allowed for 15

minutes (up to 60 minutes). The appearance of distinct red lines on test region and control region of the kit suggests positive HIV test while one distinct red line in the region of control suggested HIV negative test. Appearance of the distinct red line on the control region validated the HIV result and without that, the kit was assumed to be invalid (i.e. the HIV result cannot be accepted or it is considered invalid).

2.7.4 Cyflow counter for automated CD4 cell count as described by Partec, Germany

Twenty microliter (20 µl) of K₃EDTA whole blood was collected into Partec test tube (Rohren tube). Twenty microliter (20 µl) of CD4+ antibody was added into the tube. The contents were mixed and incubated in the dark for 15 minutes at room temperature. A 800 µl of CD4 buffer was gently added into the mixture and mixed gently. The mixture in the Partec tube was read in the Flow cytometer machine. The content of the Partec tube was displayed as peaks and interpreted {i.e. the results of each test will be read by the cyflow counter as CD4+ cells (cell/µl) on the liquid crystal display}.

2.7.5 Determination of immunoglobulins G, A, and M using immunoturbidimetric method as described by linear chemicals, Spain

The immunoglobulin reagent and the Chemwell autoanalyser were pre-warmed to 37°C. Ten microlitre (10 µl) of reagent was added for IgM while 7 µl was added for IgG and IgA respectively to 1 ml of sample. The computer interphase attached to the Chemwell autoanalyser was programmed to suite our analytical requirement and the process for analysis of IgA, IgG and IgM were undertaken according to predefined program. The results obtained were recorded in mg/dl.

2.7.6 Determination of full blood count using automated analyzer (as described by Sysmex, Germany)

The automatic voltage regulator, the uninterrupted power supply unit and the power button on the Sysmex KX-21N Haematology analyzer were turned on. The "ready" for analysis was allowed to appear and there was a temporary wait for the analyzer to come to "ready state". Then, the sample numbers of the subjects were entered into the device. Five millilitres (5 ml) of blood collected in K₃EDTA vacutainer was

mixed gently. The stopper of the vacuutainer was opened to start analysis. The tube was set to the sample probe and the start switch was pressed so that the device can aspirate the required volume. The tube was held against the sample probe until the buzzer sounds twice. At the end of each analysis, the device displays and prints a hard copy of the report for onward documentation. After the printing, it goes to ready state so that it can analyze another sample.

2.7.7 Erythrocyte sedimentation rate (as described by Dacie and Lewis, 2001) [15]

Trisodium citrate solution (0.4 ml) was dispensed into a tube with the help of a pipette. 1.6 ml of K₃EDTA blood was dispensed into the tube containing the trisodium citrate solution. The blood and trisodium citrate solution was mixed properly. Then, a clean and dry Westergren (ESR) tube was inserted into the plastic tube containing the mixture of blood and trisodium citrate until it gets to the zero (0) mark. The tube was placed firmly on the tube holder. The timer was set for 60 minutes before reading.

2.8 Statistical Analysis

Statistical Package for Social Sciences (IBM SPSS version 20) was used for the statistical analysis. The variables were expressed in means and standard deviation. Comparison among groups was done using ANOVA, while comparison between two group was done using student t-test. $P < 0.05$ was considered statistically significant.

3. RESULTS

The mean \pm SD value of ESR (mm/hr) of MTB positive on DOTS and MTB positive in each case were significantly higher than control subjects ($p < 0.05$). Also, the mean \pm SD value of HCT (%) and LYM (%) were significantly higher in control subject when compared with DOTS administered MTB positives and MTB positives in each case ($p < 0.05$). Similarly, the mean \pm SD value of MXD (%) and LYM (%) was significantly higher in MTB positives on DOTS compared with MTB positives ($p < 0.05$). Moreover, the mean \pm SD value of NEUT (%) on DOTS administered MTB positives and MTB positives in each case were significantly higher when compared with control subjects ($p < 0.05$). While HGB was significantly decreased in MTB positives compared with the MTB on DOTS and control subjects. (Table 1)

The WBC and NEUT was significantly higher and LYM significantly lower in MTB positive subjects compared to MTB subjects on DOTS and control subjects respectively ($p < 0.05$). Moreover CD4 count was significantly higher in MTB subjects on DOTS than MTB positives, while MXD was significantly higher in MTB positives compared to MTB positives on DOTS ($P > 0.05$). (Table 2)

The mean \pm SD of IgA (mg/dl) and IgM (mg/dl) of MTB positive subject was significantly higher when compared with MTB positive subjects on DOTS and control subjects ($p < 0.05$). Also, the mean \pm SD of IgG (mg/dl) of MTB positive subject and MTB on DOTS were significantly higher in each case when compared with control subjects ($p < 0.05$). (Table 3)

The mean \pm SD value of parameter compared between male and female *Mycobacterium tuberculosis* positive subjects were not statistically significant ($p > 0.05$).

The mean of CD4 cells (cells/ μ l) of MTB on DOTS was significantly higher in female than male ($p < 0.05$) while the other parameters showed no significant difference ($p > 0.05$).

The mean \pm SD value of ESR (mm/hr) and CD4 cells (cells/ μ l) for female controls were significantly higher than male ($p < 0.05$). Conversely, HGB (g/dl) and HCT (%) of male were significantly higher than female ($p < 0.05$). However, other parameters compared between male and female control subjects were found not significantly different ($p > 0.05$).

4. DISCUSSION

According to Al-Marri and Kirkpatrick [8], Sarkar et al. [11], ESR is commonly done as an unspecific test during the initial diagnostic work-up for TB. In this study, the mean \pm SD of ESR in MTB positives on DOTS and MTB positive subjects were higher than the control groups. This could be as a result of the inflammation that results from TB infection as ESR has been shown to be raised in infections and inflammatory conditions. This increase in ESR could be attributed to increased production of acute phase proteins often observed in chronic infections and release of proteins by the causative organism (*Mycobacterium tuberculosis*) into the circulation. This finding also agrees with earlier research that TB is associated mostly with very high ESR values [9].

Table 1. Mean \pm SD of parameter in control subjects, MTB positives on DOTS and MTB positives

Groups	ESR (mm/hr)	HGB (g/dl)	HCT (%)	LYM (%)	MXD (%)	NEUT (%)
(1) Control (n = 50)	22.70 \pm 12.60	12.33 \pm 2.07	39.02 \pm 5.65	44.45 \pm 12.04	13.73 \pm 6.73	41.82 \pm 12.29
(2) MTB positive on DOTS (n=50)	42.63 \pm 29.77	11.33 \pm 2.09	34.96 \pm 7.15	37.42 \pm 12.12	14.16 \pm 7.07	48.42 \pm 13.91
(3) MTB positive (n = 50)	42.13 \pm 22.27	10.21 \pm 1.63	34.28 \pm 4.17	16.25 \pm 6.10	10.76 \pm 4.35	72.99 \pm 9.46
F (p) value	10.06 (0.00)	11.89(0.00)	7.82 (0.00)	78.58 (0.00)	3.58 (0.03)	74.64(0.00)
1 vs 2: p-value	0.00	0.09	0.02	0.03	0.96	0.07
1 vs 3: p-value	0.00	0.00	0.00	0.00	0.06	0.00
2 vs 3: p-value	0.99	0.03	0.86	0.00	0.03	0.00

Key: $p < 0.05$ is significant, while $p > 0.05$ is not significant.

F (p) value = mean \pm SD of the parameters in control subjects, Mycobacterium tuberculosis positive subjects on DOTS and Mycobacterium tuberculosis subjects compared (using ANOVA).

p value of 1 vs 2 = mean \pm SD of parameters between control and Mycobacterium tuberculosis positive subjects on DOTS compared (using t test).

p value of 1 vs 3 = mean \pm SD of parameters between control and Mycobacterium tuberculosis positive subjects compared (using t test).

p value of 2 vs 3 = mean \pm SD of parameters between Mycobacterium tuberculosis positive subjects on DOTS and Mycobacterium tuberculosis positive subjects compared (using t test)

Table 2. Mean \pm SD of parameter in control subjects, MTB positives on DOTS and MTB positives

Parameter	WBC ($\times 10^3/\mu\text{l}$)	CD4 (cells/ μl)	LYM ($\times 10^3/\mu\text{l}$)	MXD ($\times 10^3/\mu\text{l}$)	NEUT ($\times 10^3/\mu\text{l}$)
(1) Control subjects (n = 50)	5.72 \pm 1.51	605.73 \pm 406.13	2.49 \pm 0.86	0.76 \pm 0.36	2.60 \pm 1.36
(2) MTB positive subjects on DOTS (n = 50)	6.19 \pm 2.44	578.85 \pm 295.79	2.23 \pm 0.93	0.83 \pm 0.41	3.13 \pm 2.11
(3) MTB positive subjects (n = 50)	11.07 \pm 4.18	526.80 \pm 125.46	1.65 \pm 0.40	1.07 \pm 0.51	10.17 \pm 4.71
F (p) value	40.90 (0.00)	0.72 (0.49)	12.79 (0.00)	5.39 (0.01)	75.36 (0.00)
p value of 1 vs 2	0.57	0.94	0.30	0.96	0.07
p value of 1 vs 3	0.00	0.47	0.00	0.06	0.00
p value of 2 vs 3	0.00	0.00	0.00	0.03	0.00

Key: p value (level of significance): $p < 0.05$ is significant, while $p > 0.05$ is not significant.

F (p) value = mean \pm SD of the parameters in control subjects, Mycobacterium tuberculosis positive subjects on DOTS and Mycobacterium tuberculosis subjects compared (using ANOVA).

p value of 1 vs 2 = mean \pm SD of parameters between control and Mycobacterium tuberculosis positive subjects on DOTS compared (using t test).

p value of 1 vs 3 = mean \pm SD of parameters between control and Mycobacterium tuberculosis positive subjects compared (using t test).

p value of 2 vs 3 = mean \pm SD of parameters between Mycobacterium tuberculosis positive subjects on DOTS and Mycobacterium tuberculosis positive subjects compared (using t test)

Table 3. Mean \pm SD comparison of parameters in control, MTB positive on DOTS and MTB positive subjects

Parameter	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
(1) Control subjects (n = 50)	350.25 \pm 107.45	1853.48 \pm 666.10	196.80 \pm 87.02
(2) MTB positive subjects on DOTS (n = 50)	346.13 \pm 163.71	2559.50 \pm 529.95	189.28 \pm 98.43
(3) MTB positive subjects (n = 50)	498.40 \pm 117.02	2687.70 \pm 248.04	273.30 \pm 112.59
F (p) value	17.37 (0.00)	3081 (0.00)	8.67 (0.00)
p value of 1 vs 2	0.99	0.00	0.93
p value of 1 vs 3	0.00	0.00	0.03
p value of 2 vs 3	0.00	0.36	0.02

Key: p value (level of significance); $p < 0.05$ is significant, while $p > 0.05$ is not significant.

F (p) of value = mean \pm SD of the parameters in control subjects, Mycobacterium tuberculosis positive subjects on DOTS and Mycobacterium tuberculosis subjects compared (using ANOVA).

p value of 1 vs 2 = mean \pm SD of parameters between control and Mycobacterium tuberculosis positive subjects on DOTS compared (using t test).

p value of 1 vs 3 = mean \pm SD of parameters between control and Mycobacterium tuberculosis positive subjects compared (using t test).

p value of 2 vs 3 = mean \pm SD of parameters between Mycobacterium tuberculosis positive subjects on DOTS and Mycobacterium tuberculosis positive subjects compared (using t test)

The HGB (g/dl) and HCT (%) in control groups were higher than MTB positive and MTB positive subjects on DOTS. This could be as a result of chronic assault of MTB on the system of the subjects with a negative impact of tubercle bacilli on multi-potential stem cells. The report of previous research work [16] observed that chronic infections including TB can cause anemia and various pathogenesis are associated with TB anemia, but most studies have shown suppression of erythropoiesis by inflammatory mediators as a cause of anemia. This finding is in agreement with the work of Akpan et al. [17], which found that the mean \pm SD value of HGB and HCT were significantly higher in control subject when compared with DOTS administered MTB positives subjects. They also discovered that the haemoglobin concentration for pulmonary tuberculosis patients was significantly lower than that of control subjects. They moreover discovered that a group of subjects on anti-tuberculosis therapy had a mean \pm SD of haemoglobin concentration and packed cell volume (haematocrit) lower than control.

Accordingly, LYM (%) of control subjects were significantly higher than the MTB positive subjects on DOTS and MTB positives in each case. This is suggestive of the risk of Lymphopenia in Tuberculosis infected subjects.

This research also found that the mean \pm SD value of MXD (%) was significantly higher in MTB positives on DOTS in each case compared with

control subject and MTB positives. Moreover, the mean \pm SD value of NEUT (%) on DOTS administered MTB positives and MTB positives in each case were significantly higher when compared with control subjects.

Moreso, the comparison of the ESR (mm/hr) in DOTS administered MTB positives with control indicated that MTB positive subjects on DOTS had higher values, but HCT (%) and LYM (%) showed lower values in MTB positive on DOTS compared to control subjects.

This research also found that the mean \pm SD value of WBC ($\times 10^3/\mu\text{l}$) of MTB positives and DOTS administered MTB positive subjects in each case were significantly higher when compared with control subject this may be due to inflammation resulting from TB infection as leukocytosis has been shown to be caused by inflammatory conditions. The mean \pm SD value of CD4 cells (cell/ μl) were significantly higher in MTB on DOTS when compared with MTB positive subjects. This might be attributed to the positive effect of DOTS administration on the CD4 count of MTB subjects. This agrees with Lawn et al. [7] that there is selective clonal depletion of Mycobacterium tuberculosis specific CD4+ T lymphocytes.

The mean of IgG, IgA and IgM was significantly increased in MTB positive subjects compared to the control subjects. This finding agrees with the work of Turner et al. [14] they reported that

mean IgG antibody levels and mean IgA antibody levels were higher in patients with positive microscopic examination for acid-fast bacilli than in patients with negative microscopic examination. This is also in line with the discovery of Hamid et al. [18] also that the mean levels of IgG, IgA and IgM were significantly higher in patients with pulmonary tuberculosis when compared with control groups. According to Amilo et al. [19], PTB infection with or without HIV co-infection significantly affects the immune system response. This might also be associated with the elevated level of IgG, IgA and IgM observed in this study. This work however disagrees with Turneer et al. [14], which found that there was no difference in mean IgM antibody levels between TB infected subjects and controls. We also found that IgA and IgM were also significantly higher in MTB positive subjects when compared with MTB subjects on DOTS.

5. CONCLUSION

In this study, the following conclusions were reached:

The mean values of WBC and NEUT are significantly increased and LYM significantly decreased in MTB positive subjects compared to the control subjects and MTB subjects on DOTS, while CD4 count is significantly increased in MTB subjects on DOTS compared to MTB positive subjects.

The mean IgG, IgA and IgM are significantly increases in MTB positive subjects than the control subjects.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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