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Some haematological parameters and immunoglobulin levels in *mycobacterium tuberculosis* infected patients in Benin city, Southern Nigeria.

ABSTRACT

Mycobacterium tuberculosis 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. These parameters could be employed as predictive marker to monitor progress in MTB treatment. One hundred and fifty subjects were recruited for the studies (50 MTB positives, 50 MTB positives on DOTS, and 50 controls). Informed consent was obtained from all subjects in the studies. Ethical approval was obtained from the Ethics Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi campus. Five millilitre of blood was collected from each subject for HIV test by ELISA, CD4 count by Cyflow technique, immunoglobulin test by immune-turbidimetric method, estimation of leucocyte profile by automated analyser and ESR by routine approach. In addition, sputum was collected from each subject for Ziehl Neelsen technique. Statistical Package for Social Science (version 20) was used for the data analysis. Results obtained from the study shows that the comparison of the WBC of control and MTB positive subjects indicated that the latter was significantly higher ($p < 0.05$). Likewise, WBC of *Mycobacterium tuberculosis* (MTB) positive subjects were significantly higher than MTB positives on DOTS. The CD4 count of DOTS administered MTB positives was significantly higher than non-DOTS subjects ($p < 0.05$). The mean value of IgA, IgG and IgM for MTB positives subjects in each case was significantly raised than control ($p < 0.05$). Also, IgG value for DOTS administered MTB positives was higher than control. In same manner, the IgA and IgM value for non-DOTS in each case was higher than DOTS subjects ($p < 0.05$). The value of HCT and LYM were significantly higher in control subject when compared with DOTS administered MTB positives and MTB positives in each case ($p < 0.05$). The value of neutrophil for MTB positives was significantly higher than control ($p < 0.05$). Thus Immunoglobulin G, A, and M could be used as a predictive marker for monitoring MTB infections, alongside haematological parameters and CD4 count.

Key words: Mycobacterium tuberculosis, immunoglobulins, IgG, IgM, IgA,

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).

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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 (4).

On the other hand, Lawn *et al* (5) showed that there is selective clonal depletion of *Mycobacterium tuberculosis* specific CD4+ T lymphocytes. Additionally, Swaminathan and Narendran (6) 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 (7) studies in South Africa suggest that active TB is associated mostly with very high ESR values (≥100mm/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, (8), Sarkar *et al* (9) 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 protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses (10).

Notably, Kardjito *et al* (11) found that tuberculin-positive control subjects had higher levels of antibody than tuberculin-negative controls in the IgG class only. Alongside, Turneer *et al* (12) 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

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IgM antibody levels between patients and controls. According to Turneer *et al* (12), 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 on immunoglobulin G, A, and M was explored in this work.

MATERIALS AND METHODS

Study Area

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

Study design

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

Study population

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

1. Fifty (50) Mycobacterium tuberculosis subjects on DOTS therapy. (They were either on category I directly observed treatment short-course {rifampicin (150mg), isoniazid (75mg), pyrazinamide (400mg) and ethambutol hydrochloride (275mg)} taken once daily, category II directly observed treatment short-course {rifampicin (150mg), isoniazid (75mg), pyrazinamide (400mg), ethambutol hydrochloride (275mg) and streptomycin (1g)} taken once daily or category III {ethambutol hydrochloride (400mg) and isoniazid (150mg)}). 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.

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.

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)

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Sample collection

Six millilitres (6ml) 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 for HIV tests by immunochromatographic methods and immunoglobulin G, A and M analysis by immunoturbidimetric method, using spectrophotometer.

LABORATORY METHODS

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: Strong carbol fuchsin was used in flooding the slide, and then Bunsen flame was used in heating the smear-stain combination from the bottom of the slide for about three minutes interruptedly. The slide was flooded with water and blotted afterward. 1 % acid alcohol was applied to the slide to decolorize it for about 5 minutes. Methylene blue was used in flooding the slide for about 30 seconds. Tap water was used in rinsing off the methylene blue. 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).

HIV-1/2 ASSAY AS DESCRIBED BY ALERE MEDICAL COMPANY LIMITED, JAPAN.

PROCEDURE

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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).

HIV-1/2 STAT-PAK ASSAY AS DESCRIBED BY CHEMBIO DIAGNOSTIC SYSTEMS INCORPORATED

PROCEDURE

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) sample loop was used to collect the specimen. The sample loop was held vertically and; used to touch the sample pad in the sample well and dispensed. 3 drops (about 105 µl) of the running buffer bottle was added drop wise into the sample well.

UNI-GOLD HIV TEST (AS DESCRIBED BY TRINITY BIOTECH PLC, IRELAND)

PROCEDURE

The kit was taken to room temperature by allowing it to stand for 20 minutes on the work bench. The Uni-gold HIV test devices from their protective wrappers was removed and labeled with appropriate identification number. The disposable pipette was used to transfer the plasma from the bottle. The pipette was held over the sample port and two drops of sample (about 60 µl) was added carefully. 10 minutes interval was allowed from the time of wash reagent addition for reaction to occur. The result was read at the end of 10 minutes incubation. Result should not be read after 20 minutes following sample addition.

CYFLOW COUNTER FOR AUTOMATED CD4 CELL COUNT (AS DESCRIBED BY PARTEC, GERMANY).

PROCEDURE

Twenty microliter (20 µl) of K_3ETA 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. 800 µl of CD4 buffer was gently added into the mixture and mixed gently. 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}.

DETERMINATION OF IMMUNOGLOBULIN G, A, AND M BY USING IMMUNOTURBIDIMETRIC METHODS (AS DESCRIBED BY LINEAR CHEMICALS, SPAIN).

PROCEDURE

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The immunoglobulin reagent and the Chemwell autoanalyser were pre-warmed to $37^{O}C$. Ten microlitre (10 µl) of reagent was used for IgM while 7 µl was used for IgG and IgA respectively. This was added 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.

DETERMINATION OF FULL BLOOD COUNT USING AUTOMATED ANALYSER (AS DESCRIBED BY SYSMEX, GERMANY).

PROCEDURE

The automatic voltage regulator, the uninterrupted power supply unit and the power button on the Sysmex KX-21N Haematology analyser were turned on. The "ready" for analysis was allowed to appear and there was a temporary wait for the analyser to come to "ready state". Then, the sample numbers of the subject entered into the device. Five millilitres (5 ml) of blood collected in K_3EDTA 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.

ERYTHROCYTE SEDIMENTATION RATE (AS DESCRIBED BY DACIE AND LEWIS, 2001) (14)

PROCEDURE

Trisodium citrate solution (0.4 ml) was dispensed into a unit with the help of a pipette. 1.6 ml of K_3EDTA 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.

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.

RESULTS

Table 1 shows that the mean ± 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 ± SD value of HCT (%) and LYM (%) were significantly higher in control subject

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when compared with DOTS administered MTB positives and MTB positives in each case ($p < 0.05$). Similarly, the mean ± SD value of MXD (%) and LYM (%) was significantly higher in MTB positives on DOTS compared with MTB positives ($p < 0.05$). Moreover, the mean ± 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 2, shows that 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 3 shows that the mean ± SD of IgA (mg/dl)and IgM of MTB positive subject was significantly higher when compared with MTB positive subjects on DOTS and control subjects ($p < 0.05$). Also, the mean ± 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 4 showed that mean ± SD value of parameter compared between male and female *Mycobacterium tuberculosis* positivesubjects were not statistically significant ($p > 0.05$).

Table 5 shows that mean ± SD value between male and female *Mycobacterium tuberculosis* positive subjects on DOTS of CD4 cells (cells/µl) was significantly higher in female than male ($p < 0.05$) while the other parameters showed no significant difference ($p > 0.05$).

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Table 6 shows that the mean ± SD value of ESR (mm/hr) and CD4 cells (cells/µl) for female in each case 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 subjects were found not to be significant ($p > 0.05$).

Table 1: Mean ± 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±12.60	12.33±2.07	39.02±5.65	44.45±12.04	13.73±6.73	41.82±12.29
(2) MTB positive on DOTS(n=50)	42.63±29.77	11.33±2.09	34.96±7.15	37.42±12.12	14.16±7.07	48.42±13.91
(3) MTB positive (n = 50)	42.13±22.27	10.21±1.63	34.28±4.17	16.25 ± 6.10	10.76±4.35	72.99± 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 ± 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 4 = mean ± SD of parameters between control and *Mycobacterium tuberculosis* positive subjects on DOTS compared (using t test).
p value of 1 vs 5 = mean ± SD of parameters between control and *Mycobacterium tuberculosis* positive subjects compared (using t test).
p value of 4 vs 5 = mean ± SD of parameters between *Mycobacterium tuberculosis* positive subjects on DOTS and *Mycobacterium tuberculosis* positive subjects compared (using t test).

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

PARAMETER	WBC ($x10^3$/µl)	CD4 (cells/µl)	LYM ($x10^3$/µl)	MXD ($x10^3$/µl)	NEUT ($x10^3$/µl)

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(1) Control subjects (n = 50)	5.72 ± 1.51	605.73± 406.13	2.49 ± 0.86	0.76 ± 0.36	2.60± 1.36
(2) MTB positive subjects on DOTS (n = 50)	6.19 ± 2.44	578.85± 295.79	2.23 ± 0.93	0.83 ± 0.41	3.13 ± 2.11
(3) MTB positive subjects (n = 50)	11.07 ± 4.18	526.80± 125.46	1.65 ± 0.40	1.07 ± 0.51	10.17± 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 ± 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 4 = mean ± SD of parameters between control and *Mycobacterium tuberculosis* positive subjects on DOTS compared (using t test).
p value of 1 vs 5 = mean ± SD of parameters between control and *Mycobacterium tuberculosis* positive subjects compared (using t test).
p value of 4 vs 5 = mean ± SD of parameters between *Mycobacterium tuberculosis* positive subjects on DOTS and *Mycobacterium tuberculosis* positive subjects compared (using t test).

Table 3: Mean ± 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 ± 107.45	1853.48 ± 666.10	196.80 ± 87.02
(2) MTB positive subjects on DOTS (n = 50)	346.13 ± 163.71	2559.50 ± 529.95	189.28 ± 98.43
(3) MTB positive subjects (n = 50)	498.40 ± 117.02	2687.70 ± 248.04	273.30 ± 112.59
F (p) value	17.37 (0.00)	3081 (0.00)	8.67 (0.00)

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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 ± 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 4 = mean ± SD of parameters between control and *Mycobacterium tuberculosis* positive subjects on DOTS compared (using t test).
p value of 1 vs 5 = mean ± SD of parameters between control and *Mycobacterium tuberculosis* positive subjects compared (using t test).
p value of 4 vs 5 = mean ± SD of parameters between *Mycobacterium tuberculosis* positive subjects on DOTS and *Mycobacterium tuberculosis* positive subjects compared (using t test).

Table 4: Mean ± SD of parameter compared between male and female *Mycobacterium tuberculosis* positivesubjects.

PARAMETER	MALE	FEMALE	p VALUE
ESR (mm/hr) n = 20	38.67 ± 22.89	40.80 ± 24.75	0.78
HGB (g/dl) n = 20	10.29 ± 1.62	10.76 ± 1.16	0.31
HCT (%) n = 20	33.79 ± 4.02	35.89 ± 3.31	0.11
LYM (%) n = 20	17.21 ±5.74	17.37 ± 5.77	0.94
MXD (%) n = 20	11.75 ± 4.49	11.55 ± 4.36	0.90
NEUT (%) n = 20	71.04 ± 8.85	71.08 ± 9.38	0.99
WBC ($x10^3$/µl) n = 20	10.63 ± 3.30	11.42 ± 4.66	0.52
CD4 (Cells/µl) n = 20	535.20 ± 142.20	513.07 ± 106.86	0.68
LYM ($x10^3$/µl) n = 20	1.49 ± 0.46	1.78 ± 0.37	0.94
MXD ($x10^3$/µl) n = 20	1.03 ± 0.66	1.13 ± 0.41	0.90
NEUT ($x10^3$/µl) n = 20	8.30 ± 4.25	10.24 ± 5.02	0.99
IgA (mg/dl) n = 20	507.00 ± 128.63	477.13 ± 108.94	0.58
IgG (mg/dl)	2617.73 ± 269.47	2731.87 ± 228.89	0.25

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n = 20			
IgM (mg/dl)	274.87 ± 107.21	268.60 ± 103.27	0.85
n = 20			

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

Table 5: Mean ± SD of parameter compared between male and female *Mycobacterium tuberculosis* positive subjects on DOTS.

PARAMETER	MALE	FEMALE	p VALUE
ESR (mm/hr)	37.95 ± 28.41	47.30 ± 31.09	0.33
n = 25			
HGB (g/dl)	11.98 ± 1.61	10.68 ± 2.33	0.05
n = 25			
HCT (%)	36.59 ± 6.84	33.34 ± 7.25	0.18
n = 25			
LYM (%)	35.69 ± 11.01	39.16 ± 13.18	0.3
n = 25			
MXD (%)	15.79 ± 7.74	12.51 ± 6.08	0.14
n = 25			
NEUT (%)	48.49 ± 13.60	48.35 ± 14.56	0.97
n = 25			
WBC ($x10^3$/µl)	6.15 ± 2.14	6.25 ± 2.76	0.86
n = 25			
CD4 (Cells/µl)	439.65 ± 214.57	718.05 ± 304.56	0.00
n = 25			
LYM ($x10^3$/µl)	2.12 ± 0.96	2.33 ± 0.91	0.45
n = 25			
MXD ($x10^3$/µl)	0.91 ± 0.36	0.76 ± 0.44	0.27
n = 25			
NEUT ($x10^3$/µl)	3.10 ± 1.66	3.16 ± 2.53	0.92
n = 25			
IgA (mg/dl)	363.20 ± 174.77	329.05 ± 154.10	0.59
n = 25			
IgG (mg/dl)	2554.30 ± 437.24	2564.70 ± 620.68	0.95
n = 25			
IgM (mg/dl)	210.80 ± 110.44	167.75 ± 81.93	0.18
n = 25			

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

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Table 6: Mean ± SD of parameters compared between male and female control subjects.

PARAMETER	MALE	FEMALE	p VALUE
ESR (mm/hr) n = 18	15.92 ± 7.15	29.62 ± 17.11	0.01
HGB (g/dl) n = 18	13.35 ± 2.28	11.78 ± 1.39	0.01
HCT (%) n = 18	41.71 ± 6.48	38.03 ± 3.79	0.04
LYM (%) n = 18	41.78 ± 15.13	47.19 ± 10.64	0.28
MXD (%) n = 18	11.50 ± 4.97	13.03 ± 5.60	0.57
NEUT (%) n = 18	46.72 ± 14.16	39.78 ± 12.97	0.22
WBC ($x10^3$/µl) n = 18	6.20 ± 1.77	5.91 ± 1.34	0.61
CD4 (Cells/µl) n = 18	502.31 ± 321.98	841.62 ± 518.09	0.04
LYM ($x10^3$/µl) n = 18	2.50 ± 0.97	2.78 ± 0.91	0.28
MXD ($x10^3$/µl) n = 18	0.71 ± 0.32	0.77 ± 0.39	0.57
NEUT ($x10^3$/µl) n = 18	3.08 ± 1.54	2.68 ± 1.45	0.22
IgA (mg/dl) n = 18	360.92 ± 91.62	376.70 ± 94.78	0.66
IgG (mg/dl) n = 18	1883.69 ± 575.76	2011.62 ± 776.64	0.71
IgM (mg/dl) n = 18	187.69 ± 95.63	218.23 ± 93.64	0.45

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

DISCUSSION

According to Al-Marri and Kirkpatrick (8), Sarkar *et al* (9), ESR is commonly done as an unspecific test during the initial diagnostic work-up for TB. In this study, the mean ± 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

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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 (7).

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. This finding is in agreement with the work of Akpan *et al* (15), which found that the mean ± SD value of HGB and HCT were significantly higher in control subject when compared with DOTS administered MTB positives in each case. 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 ± SD of haemoglobin concentration and packed cell volume (haematocrit) that was 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.

We also found that the mean ± 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 ± SD value of NEUT (%) on DOTS administered MTB positives and MTB positives in each case were significantly higher when compared with control subjects.

Accordingly, 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.

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This research also found that the mean ± SD value of WBC ($x10^3$/µl) 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 ± SD value of CD4 cells (cell/µl) were significantly higher in 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* (5) 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 Turneer *et al* (12) that 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* (17) 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* (18), 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* (12) that 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.

CONCLUSION

In this study, the following conclusions were reached:

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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.

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