

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).

41 *Mycobacterium tuberculosis* (MTB), one of the oldest known diseases and still a major cause
42 of mortality today, has many manifestations affecting the bone, the central nervous system
43 and many other organ systems. Tuberculosis (TB) is the leading cause of death in HIV
44 patients. The key components of the immune response in TB include T lymphocytes and
45 alveolar macrophages. The T helper type 1 subclass is the major effectors cell in cellular
46 immunity of TB or the policeman of TB control in the lung resulting in dissemination of
47 *Mycobacterium tuberculosis*. One study hypothesized that TB infection contributes to
48 additional reduction in CD4 cell count in HIV patients presenting with dual infection (4).

49 On the other hand, Lawn *et al* (5) showed that there is selective clonal depletion of
50 *Mycobacterium tuberculosis* specific CD4+ T lymphocytes. Additionally, Swaminathan and
51 Narendran (6) demonstrated the effect of tuberculosis (TB) on CD4 cells and they found
52 that TB develops at any level of CD4 count, though extra-pulmonary and disseminated
53 forms of the disease are more common as immunodeficiency increases. Ukpe and Southern
54 (7) studies in South Africa suggest that active TB is associated mostly with very high ESR
55 values (≥ 100 mm/h) irrespective of HIV status, and that concomitant HIV infection tends to
56 increase the proportion of TB cases with high erythrocyte sedimentation rate (ESR) values.
57 Likewise, Al-Marri and Kirkpatrick, (8), Sarkar *et al* (9) indicated that ESR is commonly
58 done as a non-specific test during the initial diagnostic work up for TB, which is a chronic
59 bacterial infection.

60 Immunoglobulin (Ig) is a large γ -shaped protein that is used by the immune system to
61 identify and neutralize foreign objects such as bacteria and viruses (10).

62 Notably, Kardjito *et al* (11) found that tuberculin-positive control subjects had higher levels
63 of antibody than tuberculin-negative controls in the IgG class only. Alongside, Turneer *et al*
64 (12) used p32 antigen of *Mycobacterium bovis* BCG to investigate the humoral immune
65 response in human tuberculosis. In their work, immunoglobulin G (IgG), immunoglobulin
66 A (IgA), and immunoglobulin M (IgM) were directed against p32 and measured by
67 enzyme-linked immunosorbent assay (ELISA). The mean IgG and IgA antibody levels
68 differed significantly ($p < 0.001$) between active tuberculosis patients and non-active
69 tuberculosis patients. According to them, mean IgG antibody levels, and mean IgA antibody
70 levels were higher ($p < 0.05$) in patients with positive microscopic examination for acid-fast
71 bacilli than in patients with negative microscopic examination. They found a positive
72 relation between mean levels and the extent of disease; but there was no difference in mean

73 IgM antibody levels between patients and controls. According to Turneer *et al* (12), only
74 active tuberculosis seemed to induce significant anti-p32 antibody levels and may be
75 associated with positivity. Due to findings from these independent researchers, the effect of
76 tuberculosis on immunoglobulin G, A, and M was explored in this work.

77 MATERIALS AND METHODS

78 Study Area

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

81 Study design

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

84 Study population

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

- 87 1. Fifty (50) Mycobacterium tuberculosis subjects on DOTS therapy. (They were either on
88 category I directly observed treatment short-course {rifampicin (150mg), isoniazid
89 (75mg), pyrazinamide (400mg) and ethambutol hydrochloride (275mg)} taken once
90 daily, category II directly observed treatment short-course {rifampicin (150mg),
91 isoniazid (75mg), pyrazinamide (400mg), ethambutol hydrochloride (275mg) and
92 streptomycin (1g)} taken once daily or category III {ethambutol hydrochloride (400mg)
93 and isoniazid (150mg)}). The amount (gram or milligram) of anti-tuberculosis drugs
94 administered to subjects were dependent on the weight (1).
95
- 96 2. Fifty (50) Mycobacterium tuberculosis subjects not on DOTS therapy.
- 97 3. Fifty apparently, healthy subjects from the staff and student population of same
98 hospital.
99

100 Ethical consideration and informed consent

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

104 Inclusion and Exclusion criteria

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

109

110 **Sample collection**

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

112 Three (3) ml of blood into tripotassium Ethylene diamine tetra-acetic acid (K₃EDTA)
113 vacutainer for CD4 count by flow cytometric method, and determination of haematocrit,
114 haemoglobin level, total white cell count and differential white cell count using an auto-
115 analyser and ESR using standard routine methods.

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

119

120 **LABORATORY METHODS**

121 **PROCEDURE FOR MYCOBACTERIUM TUBERCULOSIS DIAGNOSIS USING**
122 **ZIEHL NEELSEN STAINING METHOD**

123 **Sputum preparation:** The slide was labeled with the aid of Diamond pencil while wire loop
124 was used in making the sputum smear on a clean slide. The smear was passed over Bunsen
125 flame three times to inactivate the *Mycobacterium* and fix the smear on the slide. The fixed
126 smear thereafter was placed on staining rack.

127 **Sputum staining:** Strong carbol fuchsin was used in flooding the slide, and then Bunsen
128 flame was used in heating the smear-stain combination from the bottom of the slide for about
129 three minutes interruptedly. The slide was flooded with water and blotted afterward. 1 % acid
130 alcohol was applied to the slide to decolorize it for about 5 minutes. Methylene blue was used
131 in flooding the slide for about 30 seconds. Tap water was used in rinsing off the methylene
132 blue. The slide was allowed to air dry on the bench.

133 **Sputum microscopy:** The slide was viewed using the x100 objective and reported as sputum
134 smear positive (using appropriate grading) or sputum smear negative (1).

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

137 **PROCEDURE**

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 K₃ETA 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

176 The immunoglobulin reagent and the Chemwell autoanalyser were pre-warmed to 37°C. Ten
177 microlitre (10 µl) of reagent was used for IgM while 7 µl was used for IgG and IgA
178 respectively. This was added to 1 ml of sample.

179 The computer interphase attached to the Chemwell autoanalyser was programmed to suite our
180 analytical requirement and the process for analysis of IgA, IgG and IgM were undertaken
181 according to predefined program. The results obtained were recorded in mg/dl.

182 **DETERMINATION OF FULL BLOOD COUNT USING AUTOMATED ANALYSER** 183 **(AS DESCRIBED BY SYSMEX, GERMANY).**

184 **PROCEDURE**

185 The automatic voltage regulator, the uninterrupted power supply unit and the power button on
186 the Sysmex KX-21N Haematology analyser were turned on. The ready for analysis was
187 allowed to appear and there was a temporary wait for the analyser to come to ready state .
188 Then, the sample numbers of the subject entered into the device. Five millilitres (5 ml) of
189 blood collected in K₃EDTA vacutainer was mixed gently. The stopper of the vacuutainer was
190 opened to start analysis. The tube was set to the sample probe and the start switch was
191 pressed so that the device can aspirate the required volume. The tube was held against the
192 sample probe until the buzzer sounds twice. At the end of each analysis, the device displays
193 and prints a hard copy of the report for onward documentation. After the printing, it goes to
194 ready state so that it can analyze another sample.

195 **ERYTHROCYTE SEDIMENTATION RATE (AS DESCRIBED BY DACIE AND** 196 **LEWIS, 2001) (14)**

197 **PROCEDURE**

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

204 **STATISTICAL ANALYSIS**

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

209 **RESULTS**

210 Table 1 shows that the mean ± SD value of ESR (mm/hr) of MTB positive on DOTS and
211 MTB positive in each case were significantly higher than control subjects (p < 0.05). Also,
212 the mean ± 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 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 $\pm$ 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 $\pm$ 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 4 showed that mean $\pm$ SD value of parameter compared between male and female *Mycobacterium tuberculosis* positive subjects were not statistically significant ($p > 0.05$).

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

Table 6 shows that the mean $\pm$ SD value of ESR (mm/hr) and CD4 cells (cells/ μ l) 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 $\pm$ 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 4 = mean $\pm$ SD of parameters between control and *Mycobacterium tuberculosis* positive subjects on DOTS compared (using t test).

p value of 1 vs 5 = mean $\pm$ SD of parameters between control and *Mycobacterium tuberculosis* positive subjects compared (using t test).

p value of 4 vs 5 = 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 ($\times 10^3/\mu$ l)	CD4 (cells/ μ l)	LYM ($\times 10^3/\mu$ l)	MXD ($\times 10^3/\mu$ l)	NEUT ($\times 10^3/\mu$ l)
-----------	-------------------------------	-------------------------	-------------------------------	-------------------------------	--------------------------------

(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)

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 4 = mean $\pm$ SD of parameters between control and *Mycobacterium tuberculosis* positive subjects on DOTS compared (using t test).

p value of 1 vs 5 = mean $\pm$ SD of parameters between control and *Mycobacterium tuberculosis* positive subjects compared (using t test).

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

Table 4: Mean $\pm$ SD of parameter compared between male and female *Mycobacterium tuberculosis* positive subjects.

PARAMETER	MALE	FEMALE	p VALUE
ESR (mm/hr) n = 20	38.67 $\pm$ 22.89	40.80 $\pm$ 24.75	0.78
HGB (g/dl) n = 20	10.29 $\pm$ 1.62	10.76 $\pm$ 1.16	0.31
HCT (%) n = 20	33.79 $\pm$ 4.02	35.89 $\pm$ 3.31	0.11
LYM (%) n = 20	17.21 $\pm$ 5.74	17.37 $\pm$ 5.77	0.94
MXD (%) n = 20	11.75 $\pm$ 4.49	11.55 $\pm$ 4.36	0.90
NEUT (%) n = 20	71.04 $\pm$ 8.85	71.08 $\pm$ 9.38	0.99
WBC ($\times 10^3/\mu\text{l}$) n = 20	10.63 $\pm$ 3.30	11.42 $\pm$ 4.66	0.52
CD4 (Cells/ μl) n = 20	535.20 $\pm$ 142.20	513.07 $\pm$ 106.86	0.68
LYM ($\times 10^3/\mu\text{l}$) n = 20	1.49 $\pm$ 0.46	1.78 $\pm$ 0.37	0.94
MXD ($\times 10^3/\mu\text{l}$) n = 20	1.03 $\pm$ 0.66	1.13 $\pm$ 0.41	0.90
NEUT ($\times 10^3/\mu\text{l}$) n = 20	8.30 $\pm$ 4.25	10.24 $\pm$ 5.02	0.99
IgA (mg/dl) n = 20	507.00 $\pm$ 128.63	477.13 $\pm$ 108.94	0.58
IgG (mg/dl)	2617.73 $\pm$ 269.47	2731.87 $\pm$ 228.89	0.25

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.

Table 6: Mean $\pm$ SD of parameters compared between male and female control subjects.

PARAMETER	MALE	FEMALE	p VALUE
ESR (mm/hr) n = 18	15.92 $\pm$ 7.15	29.62 $\pm$ 17.11	0.01
HGB (g/dl) n = 18	13.35 $\pm$ 2.28	11.78 $\pm$ 1.39	0.01
HCT (%) n = 18	41.71 $\pm$ 6.48	38.03 $\pm$ 3.79	0.04
LYM (%) n = 18	41.78 $\pm$ 15.13	47.19 $\pm$ 10.64	0.28
MXD (%) n = 18	11.50 $\pm$ 4.97	13.03 $\pm$ 5.60	0.57
NEUT (%) n = 18	46.72 $\pm$ 14.16	39.78 $\pm$ 12.97	0.22
WBC ($\times 10^3/\mu\text{l}$) n = 18	6.20 $\pm$ 1.77	5.91 $\pm$ 1.34	0.61
CD4 (Cells/ μl) n = 18	502.31 $\pm$ 321.98	841.62 $\pm$ 518.09	0.04
LYM ($\times 10^3/\mu\text{l}$) n = 18	2.50 $\pm$ 0.97	2.78 $\pm$ 0.91	0.28
MXD ($\times 10^3/\mu\text{l}$) n = 18	0.71 $\pm$ 0.32	0.77 $\pm$ 0.39	0.57
NEUT ($\times 10^3/\mu\text{l}$) n = 18	3.08 $\pm$ 1.54	2.68 $\pm$ 1.45	0.22
IgA (mg/dl) n = 18	360.92 $\pm$ 91.62	376.70 $\pm$ 94.78	0.66
IgG (mg/dl) n = 18	1883.69 $\pm$ 575.76	2011.62 $\pm$ 776.64	0.71
IgM (mg/dl) n = 18	187.69 $\pm$ 95.63	218.23 $\pm$ 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 $\pm$ 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 (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 $\pm$ 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 $\pm$ 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 $\pm$ 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.

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.

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 ($\text{cell}/\mu\text{l}$) 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:

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.

REFERENCES

1. World Health Organization. Global tuberculosis control: WHO report. Geneva, World Health Organization 2011.
2. Konstatinos, A. (2010). Testing for tuberculosis. *Australian prescriber*. 2010; **33** (1): 12-18.
3. World Health Organization. Tuberculosis profile of Nigeria. Geneva, World Health Organization 2009.
4. Wanchu A., Kuttiatt, VS., Sharma A., Singh S. and Varma S. CD4 cell count recovery in HIV/TB co-infected patients versus TB uninfected HIV patients. *Indian Journal of Pathology and Microbiology*. 2010; **53**(4): 745-749.
5. Lawn SD., Butera SB. And Shinnick TM. Tuberculosis unleashed the impact of HIV infection on the host granulomatous response to *Mycobacterium tuberculosis*. *Microbes and Infection*. 2002; **4**: 635-646.
6. Swaminathan S. Narendran G. HIV and tuberculosis in India. *Journal of Bioscience*. 2008; **33**: 527-537.
7. Ukpe, I. S. and Southern L. Erythrocyte sedimentation rate values in active tuberculosis with and without HIV co-infection. *South African Medical Journal*. 2006; **96** (5): 427-428.
8. Al-Marri MR. and Kirkpatrick MB. Erythrocyte sedimentation rate in childhood tuberculosis: is it still worthwhile? *International Journal of Tuberculosis and Lung Disease*. 2000; **4**: 237-239.

9. Sarkar K., Baraily S, Dasgupta S, Bhattacharya SK. Erythrocyte sedimentation rate may be an indicator for screening of tuberculosis patients for underlying HIV infection, particularly in resource-poor settings: An experience from India. *Journal of Health, Population and Nutrition*. 2004; **22**: 220-221.

10 Litman GW, Rast JP, Shamblott MJ, Haire RN, Hulst M, Roess W, Litman RT, Hinds-Frey KR, Zilch A. Amemiya, CT. Phylogenetic diversification of immunoglobulin genes and the antibody repertoire. *Molecular Biology and Evolution*. 1993; **10**(1):60-72.

11 .Kardjito T, Handoyo I. Grange JM.. Diagnosis of active tuberculosis by immunological methods. 1: The effect of tuberculin reactivity and previous BCG vaccination on the antibody levels determined by ELISA. *Tubercle*. 1982; **63** (4): 269-274.

12. Turneer M, Vanvooren JP, De-Bruyn J, Serruys E, Dierck P Yernault, JC.. Humoral immune response in human tuberculosis: immunoglobulin G, A, and M directed against the purified P32 13 13. .protein antigen of *Mycobacterium bovis bacillus* Calmette-Guerin. *Journal of Clinical Microbiology*. 1988; **26**(9): 1714-1719.

14. Daniel NAT. and Evelyn A. Profiling haematological changes in HIV patients attending fevers clinic at the Central Regional Hospital in Cape Coast, Ghana: a case- control study. *Archives of Applied Science Research*. 2011; **3** (5):326-331.

15. Dacie. V. Lewis SM. Practical Haematology. 9th edition, Churchill Livingstone, Edinburgh. 2001; Pp. 19-51.

16. Akpan PA, Akpotuzor JO. Akwiwu EC. Some hematological parameters of tuberculosis (TB) infected Africans: the Nigerian perspective. *Journal of Natural Sciences Research*. 2012; **2** (1): 50-56.

17. Hamid E K, Seyedahmad S, Omid Z, Shadi H, Sara, J Duman S. Zahra A. Evaluation of serological tests using A60 antigen for diagnosis of tuberculosis *Acta Medica Iranica*. 2010; **48** (1): 21-26

18. Amilo GI, Meludu SC, Onyenekwe C, Ele PU, Ezechukwu C, Ekejindu GOC, Ifeanyichukwu MO. Nwobodo, E. Evaluation of CD4+ and CD8+T- lymphocyte counts and serum levels of immunoglobulins in pulmonary tuberculosis (PTB) patients with or without HIV co- infection in South Eastern Nigeria. *African Journal of Pharmacy and Pharmacology*. 2012; **6** (23): 1639-1643.