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Relationship of MTB Positive Cases with CD4+ Count and Qualitative Leucocytes and Values of Erythrocyte Sedimentation Rate in MTB Positive Cases in Gombe State

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Abstract: Tuberculosis caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) bacteria [link.springer.com] remains deadliest communicable disease. In this study the relationship of MTB positive cases with CD4+ count quantitative and qualitative leucocytes and values of erythrocyte sedimentation rate (ESR) in MTB positive cases of patients with suspected pulmonary tuberculosis in Gombe State, Nigeria was determined. Four hundred (400) sputum and blood samples were collected from tuberculosis suspects referred to the laboratory from TB clinics in three Senatorial Zones of Gombe State. The sputum samples were analyzed using GeneXpert MTB/RIF and culture on MGIT tubes and LJ slants methods. The CD4+count determination was carried out using BD FACSCOUNT flow cytometery automated machine (BD Biosciences, 2007 Germany). Complete blood count determination was carried out using 5 - Part SYSMEX XN-330 HAEMATOLOGY ANALYZER (SYSMEX CORPORATION 2017). Result from this study showed Males have higher percentage MTB positivity cases than the females. However, both males and female within the age groups 25 to 54 years showed high rate of MTB positive cases. Mean CD_{4+} count of MTB positive patients obtained in this study was significantly lower (337.0 ±1.4) than that of the apparently healthy control (753.6 ±1.28). In this study the mean Haemoglobin (Hb) values (9.8 ±3.12 g/dL) among TB patients were lower than those the control group (13.7±2.71 g/dL) for both males and females. Result from this study also showed that the mean neutrophil count value (55.1 ±13.8) of infected patients was higher than that of the healthy control group (46.3 ±22.19) and the mean lymphocytes count value (34.7 ±13.5) of infected patients was lower compared to healthy control group value (42.13 ±20.59). Report from this study showed high proportion of MTB positive patients in Gombe State have subnormal CD4+ count cells, lymphocytopenia, anaemia, neutropenia, thrombocytosis, leucocytosis and high erythrocyte sedimentation rate level before starting treatment.

Keywords: GeneXpert, *Mycobacterium tuberculoisis*, CD4+ Count, Haematology Parameters, Sputum, Culture

1. Introduction

Tuberculosis (TB) is a communicable disease that usually attack lungs. It can also spread to other parts of the body, like brain and spine (Global TB report, 2021). It is a major cause of ill health and one of the leading causes of death worldwide [1]. Until the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent, ranking above HIV/AIDS [1]. TB is caused by the bacillus *Mycobacterium tuberculosis* (MTB). About a quarter of the world's population is infected with MTB [1].

The effects of tuberculosis can be disastrous especially in those resource poor countries suffering from high burdens of both TB and HIV [1]. Every year, around 245,000 Nigerians die from tuberculosis (TB) and about 590,000 new cases occur (of these, around 140,000 are also HIV-positive). TB accounts for more than 10% of all deaths in Nigeria [2].

Early MTB detection is essential for early patient

management and successful outcomes [2].

Macrophages are important effector cells in the immune system and are being aimed by MTB, as its preferred habitat [3]. Resting macrophages may not kill MTB however; activated macrophages can control the growth of the microbe, although total eradication may not be achieved [3]. Different T-cell populations are required for the successful control of the pathogen. This dynamic interplay underlying protection is the reason for the long-term persistence of MTB [4]. CD4 lymphocytopenia is a well-defined risk factor for the development of active TB in patients infected with HIV [5]. TB may be also associated with CD4 and CD8 lymphopenia even in patients without HIV virus infection [5]. CD4 T cells play vital role in the function of the immune system. The cells are important mediators of immunologic memory, and when the cells are reduced in number or their functions are lost, the individual becomes susceptible to a wide range of infectious agents including MTB [6].

The protective and pathologic response to MTB infection is complex, involving many components of the immune system. It is therefore essential to determine CD4+ count as an immunological marker and haematological parameters at the baseline of anti-TB treatment for further consideration of supportive care and other treatment options that might be required for some patients to enhance the anti-TB treatment outcome in Gombe State..

2. Materials and Methods

2.1. Sample Size

The sample size was calculated using Yaro Yamane formula for a finite population (Alouysius, 2012)

$$n = \frac{\mathrm{N}}{1+\mathrm{N(e)2}}$$

Where n = sample size, N = finite population, e = level of significance (or limit of tolerable error), 1 = unity (a constant).

N = Gombe state population size 3,257,000 (Gombe population, 2022), e = 0.05.

Isolation and identification of Mycobacterium tuberculosis using GeneXpert and culture methods.

2.2. GeneXpert Method

Sputum samples of 400 patients with suspected pulmonary tuberculosis from eight Hospitals in three Senatorial Zones of Gombe State were examined for the presence or absence of MTB using GeneXpert MTB/RIF as described by Thomas Bodmer et al [9]. One milliliter of sputum was transferred into a falcon tube. Two milliliters GeneXpert MTB/RIF sample reagent was then added to make 2:1 v/v dilution ratio. The lid was replaced and the tube vortxed for 30 seconds then kept upright for 15 minutes at room temperature after vortexing. Specimens were inspected to make sure that the samples are liquefied. It was ensured that the label of GeneXpert MTB/RIF cartridge corresponds with the specimen identification. Two milliliter liquefied specimen added to the cartridge and cartridge lid closed. The GeneXpert MTB/RIF machine was turned on and the manufacturer's guide was followed in order to analyze the samples. After a full cycle (2 hours) of analysis electronic results were displayed and used for comparison.

2.3 Culture Using N-acetyl-L cysteine- sodium Hydroxide (NALC-NaOH) Method

All GeneXpert MTB negative samples were cultured. A maximum of 5 ml of sputum samples were transferred into a well labeled 50 ml sterile falcon tubes. Aerosol production was minimized by opening the specimen container slowly, avoiding vigorous shaking. Five milliliter of NALC-NaOH solution was added to specimen using a squeeze bottle**,** cap replaced and tightened and then vortexed for 20 seconds at 1000 x g. The same procedure was repeated for all the specimens. After vortexing, each tube was inverted 5 times to ensure that NALC-NaOH solution comes in contacts with the entire surface of the tube. The tubes were carefully inverted to avoid oxidation and inactivation of the NALC-NaOH solution; which were then allowed at room temperature (25°C) for 15 minutes for decontamination. Phosphate buffer (pH 6.8) then added to make up to a 45 ml mark of the tube to reduce the continued action of NaOH and lower the viscosity of the mixture using another squeeze bottle. The tubes were recapped tightly and inverted several times to mix the contents which was centrifuged again for 15 minutes at 3000 rpm. Supernatants were carefully dispensed into a discard container containing 5% phenol; the edges of the tubes were swabbed with disinfectant soaked gauze to ensure that the disinfectant did not enter the tube. The sediments were used for inoculation of Mycobacterium indicator tube (MGIT) [10].

2.4. CD4+ Count Determination

The CD4+count determination was carried out using BD FACSCOUNT flow cytometery automated machine (BD Biosciences, 2007 Germany). Following the manufacture`s instruction (BD Biosciences, 2007). BD FACSCount CD4+/CD3+ Reagent kits were labeled and vortexed before coring. Each reagent tube was then opened using a coring instrument. The patient`s whole blood was properly mixed using a blood mixer machine; fifty microliter (50 µl) of whole blood was dispensed into each tube then recapped and vortexed for 5 seconds, incubated in the dark for 60 minutes at room temperature (20°C). 50 µl of fixative solution was added and vortexed for 5 seconds then run on the BD FACS COUNT machine. The automated machine unit executes automatic analysis and the result displayed on machine`s screen after 5 minutes and printed out on a thermal paper.

2.5. Complete Blood Count Determination

The complete blood count determination was carried out using 5 - Part SYSMEX XN-330 HAEMATOLOGY ANALYZER, following the manufacture's instruction,

(SYSMEX CORPORATION 2017). Blood samples in EDTA tubes were properly mixed using a blood mixer machine. The machine was powered and displayed ready on its screen. The sample probe of the machine was placed into the mixed whole blood and the START button pressed. The tube was held to the sample probe until the buzzer sounded two times before removing the sample tube. The machine executed an automatic analysis of the sample and the result displayed on the machine screen and prints out the result after 3 minutes.

2.6. Determination of Erythrocyte Sedimentation Rate Determination (ESR) Using Westergren Method

This method was carried out as described by Saadeh C, 2018. Anticoagulated blood was sucke into glass Westergren pipettes and fixed in vertical position for one hour. The ESR was estimated by measuring the column of plasma at the top of pipette in millimeter per hour as the base unit [11]..

2.7. Statistical Analysis

The data was tabulated in Microsoft excel spreadsheet in a master chart and studied for correlation. Statistical analysis of the data was conducted with statistical package for the social science system version SPSS 22.0.

2.8. Ethical Approval

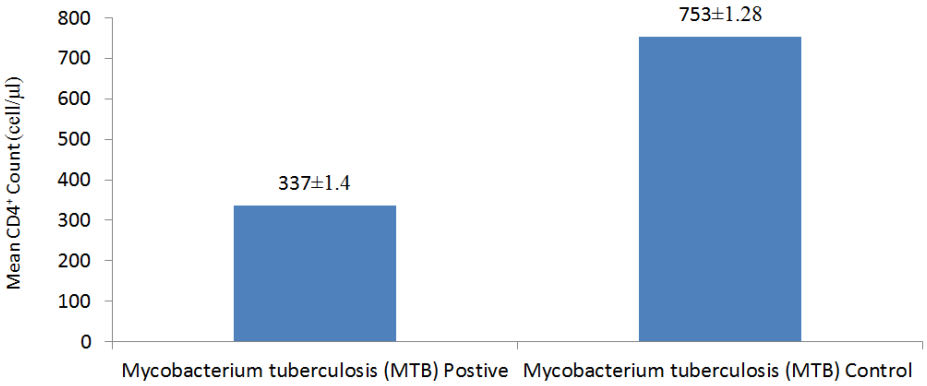
Ethical approval was obtained from Gombe State Ministry of Health through the ethics review committee. Support letter was obtained from the Gombe State TB and Leprosy control office. The purpose and benefit of the study was explained to each eligible study participant.

3. Results

Table 1 showed the occurrence of MTB positive cases among male and female in relation to age group. High occurrence of MTB positive cases was among the male than female, while high occurrence of MTB positive cases was among the age group 25- 54 years in Gombe State. Result from this study in figure 1 further showed low mean CD4+ count value among MTB positive patients compared to controls from Gombe State. Table 2 showed mean values of haematological parameters among MTB positive patients compared to MTB negative patients and controls.

Table 1. *Mycobacterium tuberculosis positive cases among male and female in relation to age group in Gombe State.*

Age (years)	Male	Female	Total
05 - 14	7	3	10
15 - 24	21	8	29
25 - 54	73	43	116
55 - 64	9	8	17
≥ 65	0	1	1
Total	110	63	173



The results are presented as mean ± SEM. Differences between means were assessed by t-test. Values of $p< 0.05$ were taken to imply statistical significance

Figure 1. *Relationship of CD4+ count with cases of pulmonary tuberculosis among patients from Gombe State.*

Table 2. *Mean values of the relationship of haematological parameters with MTB positive, MTB negative patients and controls.*

Haematological parameters	MTB positive mean ± SD	P-value	MTB negative mean ± SD	P-value	Control ± SD	P-value
WBC $X10^{9/L}$	16.2 ± 1.40	0.001	7.9 ±4.02	0.005	5.3 ±07.4	0.001
Hb (g/dL)	9.8 ±1.12	0.002	12.6 ±1.62	0.002	13.7 ±0.71	0.004
PCV (%)	31.9±1.56	0.001	37.6 ±05.31	0.001	40.2 ±1.41	0.002
Platelets $X10^{9/L}$	523.1±1.5	0.002	243.3 ±08.61	0.005	238 ±1.52	0.005
Neutrophils (%)	46.3 ±2.19	0.005	53.6 ±1.61	0.004	55.1 ±1.8	0.001
Lymphocytes (%)	42.13 ±2.59	0.001	36.9 ±1.09	0.001	34.7 ±1.05	0.004
Monocytes (%)	10.4 ±1.19	0.002	8.1 ±0.63	0.002	5.9 ±1.12	0.002
Eosinophils (%)	1.2 ±0.54	0.003	1.2 ±0.52	0.007	1.1 ±0.51	0.005
ESR (mm/hr)	100.8 ±08.31	0.001	8.9 ±1.06	0.002	6.5 ±1.3	0.001

The results are presented as mean ± SEM. Differences between means were assessed by one-way analysis of variance (ANOVA). Values of $p< 0.05$ were taken to imply statistical significance.

Key: WBC – White Blood cell Count, RBC – Red Blood Cell count, PCV – Packed Cell Volume, Hb – Haemoglobin, ESR – Erythrocyte sedimentation rate, mm – millimeter, hr – hour.

4. Discussion

Mycobacterium tuberculosis has different types of molecules on its surface which interact with the innate host response [12]. The interaction results in less optimal conditions for control of MTB growth [4]. A normal CD4+ count is from 500 to 1,500 cells per microlitre of blood [13]. Both CD_{4+} and CD_8 T-cells are important for successful immunity to TB [14]. In our finding, the mean CD_{4+} count of MTB positive patients was lower (337.0 ±1.4) than that of the apparently healthy control (753.6 ±1.28). The finding of this study is in agreement with a similar study reported by Uppal et al in Pune, India, which reported low CD_{4+} counts among MTB positive cases [13]. Low CD4+ cell levels in patients have been reported previously; in a study on hospitalized TB patients from Senegal, Kony et al found CD4+ cell counts below 300 cells/mm^3. [15].

The changes in the cytokine environment after CD4+ depletion may result in pulmonary trafficking of T effector cells [16]. Consistently, CD4+ T cell deficiency caused a shortage in total cell recovery from lungs in *M. tuberculosis* infection [17]. CD4+ T cells are needed to sustain the local pulmonary immune response. Decreases in pulmonary recruitment and accumulation of anti-TB T effector cells after CD4+ depletion and MTB infection suggest a role of CD4+ T cells and a potential mechanism for CD4+ T cell–induced immunity against TB. Pulmonary trafficking of IFN-g producing CD4+ and CD8+ effector cells is critical for protection against MTB infection [18].

This study further indicated that White Blood Cell (WBC) count in MTB positive subjects, was higher (16.2 ± 6.40) compared to the healthy control patients with a value of 5.3 ±27.4. WBCs increase among MTB positive cases might be due the increased polymorphonuclear leukocytes and macrophages as a part of the body's immune defense mechanism to combat the invading bacterial population. Infection can also lead to inflammation, which can in turn cause the number of white blood cells to increase.

Anemia can be defined as hemoglobin (Hb) levels below 12.5 g/dL for women and 13.5 g/dL for men [19]. Anemia is a common hematologic complication among TB patients and is a strong risk factor for mortality [20]. This study reported low haemoglobin (Hb) values (9.8 ±3.12 g/dL) among MTB positive cases than the corresponding control group (13.7±2.71 g/dL) for both males and females. Ciglenecki et al previously reported anemia as a common hematologic complication among TB patients and is a strong risk factor for mortality [20]. Result from this study is in agreement with a report by Lee et al who reported a prevalence of anemia among tuberculosis patients was reported to be 10.6 g/dL [22]. Previous studies in sub-Saharan Africa also found a high prevalence of anemia among TB patients in Malawi [26]. Several investigations have reported a prevalence of anemia at TB diagnosis ranging between 10.7 g/dL and 11.2 g/dL [19]. Despite its epidemiologic importance, careful investigation of causes of anemia in TB patients is not systematically performed in Gombe State. Low haemoglobin level among TB patients could be due to dampened hemoglobin synthesis as reported in anemia of chronic disease [21].

This study also reported high platelet count (523.1±123.5) among MTB positive patients than (238 ±124.52) for apparently healthy controls. This finding is supported by studies in Babylon province and Kirkuk city, Iraq [23], [24] Pakistan [26]. The difference might be due to reactive thrombocytosis which is found in pulmonary tuberculosis (PTB) due to increased thrombopoietic factors such as IL-6 which is released by inflamed cells as an inflammatory response [28]. The secretion of IL-6 in tuberculosis patients can stimulate the production of platelets [28]. A study conducted in India Institute of Medical Sciences, New Delhi, on TB patients indicated that thrombocytosis was more common in patients with PTB [27]. Similar study in Sao Paulo State University, Brazil, on PTB patients revealed that platelet count value was high among PTB patients [32]. This might be because at the beginning of the TB process, there was strong pro-inflammatory cytokine activity (IFN-γ & TNF-α) which stimulates expression of acute-phase proteins and thrombocytosis. Furthermore result from this study showed that the mean neutrophil count value (55.1 ±13.8) of infected patients was higher than that of the healthy control group (46.3 ±22.19) and the mean lymphocytes count value (34.7 ±13.5) of infected patients was lower compared to healthy control group value (42.13 ±20.59).

Immune responses of leukocytes to stressful events are shown by an increased neutrophil count and decreased lymphocyte count [29]. An increase in total WBC and neutrophils is an inflammatory reaction, particularly when caused by a bacterial infection [29]. Lymphocytopenia has also been described as a diagnostic marker of bacterial infection [21]. This study also indicated the mean monocytes count value (10.4 ±8.19) of MTB positive patients which was significantly higher ($p = 0.002$) than for apparently healthy control group (5.9 ±4.12). Interactions between MTB and host immune system resulted in the outcome of tuberculosis [13]. Monocytes/macrophages are derived from hematopoietic stem cells in the bone marrow and circulate in the bloodstream. These cells play a determinant role in innate immune response and act as a link to the adaptive immune response due to their antigen presenting function. In the progression of MTB infection, monocytes/macrophages can phagocytize and restrict mycobacterium and be recruited to form granuloma [31].

Increase of monocytes and decrease of lymphocytes may reflect the efficiency of immune response against infection [33]. Studies have shown that an elevated percentage of monocytes in peripheral blood are associated with the risk of tuberculosis [33]. The mean erythrocytes sedimentation rate (ESR) of infected patients was higher (100.8 ±28.31) compared to healthy control group (6.5 ±14.3). Erythrocytes sedimentation rate (ESR) is a non-specific measure of

inflammation. Non-inflammatory conditions that can cause raised ESR include anemia, kidney failure, obesity, ageing, and female sex. ESR is also higher in women during menstruation and pregnancy [30].

5. Conclusion

This study showed Males have higher percentage MTB positivity cases than the females. However, both males and female within the age groups 25 to 54 years showed high rate of MTB positive cases. Tuberculosis affecting the productive age group of the State will surely have a negative effect on economic productivity of the State. Report from this study showed high proportion of MTB positive patients in Gombe State have subnormal CD4+ count cells, lymphocytopenia, anaemia, neutropenia, thrombocytosis, leucocytosis and high erythrocyte sedimentation rate level before starting treatment. Findings from this study are indicative of reduced immune response against MTB infection. Determining CD4+ count values and haematological parameters as a base line investigation among MTB positive patients provides an important benefit of anti-tubercular treatment and case management.

Contribution to Field of Knowledge

First study that determined the relationship of MTB positive cases with CD4+ count quantitative and qualitative leucocytes and values of erythrocyte sedimentation rate (ESR) in Gombe Sate.

Knowledge of the base line immunohaematological value of MTB positive patient will maximize the potential benefit of anti-tubercular treatment and case management.

Challenges

a. Lack of financial resources posed a major challenge during this study.
b. Contamination of sputum sample was a big challenge during sputum culture method.
c. GeneXpert MTB/RIF was available only in few facilities in Gombe State.

Strengths of the Study

Our study has several strengths:

a. Controls were used in the three methods of MTB detection.
b. Large population size was used in the study.
c. Discipline and respect for right of patients was observed during sample collection.
d. Dedication, patience and determination was our watch words during the study.

Limitations of the Study

a. Demographic data may be incomplete and inaccurate.
b. Genexpert and Haematology analyzing methods were costly.
c. Difficulty in sputum collection, especially in the Children, or the chronicity condition of pulmonary TB or MDR-TB or HIV-TB.

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