Pattern of Hematological Indices in Tuberculosis (TB) Patients Attending St. Charles Borromeo Hospital, Anambra State, Nigeria

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ABSTRACT

Background: Tuberculosis is a highly prevalent chronic infectious disease caused by *Mycobacterium   tuberculosis bacilli.* Tuberculosis (TB) continues to be an important health and socio-economic issue, especially in developing countries.

Aim: The study was undertaken to determine the pattern of haemoglobin, red cell count, packed cell volume (PCV) and red cell indices in tuberculosis infected subjects (patients) visiting the TB clinic at St. Charles Borromeo hospital.

Materials and Method: This was a case controlled cross-sectional study conducted in Anambra State. Subjects was selected based on their relatively high stability and willingness to participate in the study. The data collection for the haematological investigation was carried out during morning hours (8:00 a.m.-10.00. a.m.). Samples of blood (5 ml) were obtained from each participant using a vacutainer and employing standard infection prevention procedures. The collected aliquots of blood samples were used to determine participants' PCV, haemoglobin concentration and RBC counts from which red cell indices were calculated.

Results and Discussion: The study consists of 20 (57.1%) TB positive subjects attending TB clinic at St. Charles Borromeo hospital, Anambra state and 15 (42.9%) control subjects with overall mean age of 31.29±9.64. The mean values were compared between cases and controls using *t*-test and One-way ANOVA (LSD Post Hoc Test) at 5% level of significance. Significant differences were recorded for RBC counts (TB Positive: 4.22±0.77, Control: 5.46±.84, *p*=0.000), Hb (TB Positive: 10.80±2.11, Control: 13.27±1.79, *p*=0.001), and MCHC (TB Positive: 31.51±1.74, Control: 33.36±.845, *p*=0.000) respectively while no significant differences were recorded for PCV (TB Positive: 34.05±7.15, Control: 38.27±6.70, *p*=0.085), MCV (TB Positive: 76.07±18.61, Control: 72.24±6.65, *p*=0.453) and MCH (TB Positive: 25.89±3.21, Control: 24.04± 2.51, *p*=0.075) respectively. Many haematological abnormalities have been demonstrated in tuberculosis patients in the present study.

Conclusion: The TB patients showed significantly lower Hb levels (mild anaemia), RBC count and PCV, while MCHC was the only significantly decreased RBC indices. While many of them are consistent with reported literature and reinforce the fact that they can become valuable tools in monitoring tuberculosis and also serve as indicators in assessment of response to therapy.

1. Introduction:

Tuberculosis is a highly prevalent chronic infectious disease caused by *Mycobacterium tuberculosis bacilli* (Parasappa *et al.,* 2013). Tuberculosis (TB) continues to be an important health and socio-economic issue, especially in developing countries (WHO, 2018). Among all infectious diseases that afflict humans, tuberculosis remains the deadliest (Rabita, 2012).

In 2017, 10 million people fell ill with TB, and 1.6 million died from the disease (including 0.3 million among people with HIV) (WHO, 2018)**.** Nigeria is among the 14 high burden countries for TB, TB/HIV and Multi Drug Resistant TB. The country is ranked seventh among the 30 high TB burden countries and second in Africa according to Global Health Education (GHE, 2017). The problem of TB in Nigeria has been made worse by the issues of drug resistant TB and the HIV/AIDS epidemic (GHE, 2017).

It is estimated that 407,000 people in Nigeria have TB each year (GHE, 2017). In addition, there are an estimated 63,000 HIV positive people that get TB each year. An estimated 115,000 HIV negative people die from TB in Nigeria each year and an estimated 39,000 HIV positive people also die (GHE, 2017).

Blood has two main components which are cells and plasma. Cells consists 40% to 45% of total amount of blood, and plasma consists 55% to 60% of total amount of blood; cells are the formed elements and are of three types, red cells (erythrocytes), white blood cells (leukocytes) and platelet (thrombocyte), and each has its own characteristic. Patients with sickle cell anaemia (SCA) have significantly impaired immunity and hence would be at increased risk of contracting TB (Salawu *et al.,* 2009). Nigeria is the most populous black nation that carries a heavy disease burden due to SCA, which affects about 2% of the general population (Salawu *et al.,* 2009).

Hence, from global perspective, Nigeria has more SCA patients than any other country in the world (Salawu *et al.,* 2009).

Tuberculosis generally affects the lung but can affect other parts of the body according to World Health Organization (WHO, 2015). Globally, *Mycobacterium tuberculosis* infection remains at an epidemic level affecting one third of world population (Parasappa *et al.,* 2013).

According to Centre of Disease Control (CDC, 2018), If not treated properly, tuberculosis disease can be fatal. People infected with TB bacteria who are not sick may still need treatment to prevent tuberculosis disease from developing in the future (CDC, 2018).

Reversible peripheral blood abnormalities are commonly associated with pulmonary tuberculosis. Insight into the relationship between haematological abnormalities and mycobacterial infection has come from an understanding of the immunology of mycobacterial infection.

The atypical and varied spectrum of clinical presentation of tuberculosis poses a diagnostic and therapeutic challenge to the physicians. As at the time of this research, there is little or non-existent literature on pattern of haematological indices, associated haematological abnormalities and the effect of anti-tuberculosis treatment on TB patients in the study area. Therefore, this study was undertaken to determine the pattern of haematological indices in TB infected individuals using patients visiting the TB clinic at St. Charles Borromeo hospital, Anambra State as cases studies.

2. Materials and Methods:

2.1. Study Population

The study population was made up of 20 selected TB positive subjects and 15 selected TB negative subjects between the ages of 20-60 years attending the TB clinic at St. Charles Borromeo hospital, Anambra state.

2.2. Study Design and Setting

This was a case controlled cross-sectional study conducted in Anambra State. Subjects was selected based on their relatively high stability and willingness to participate in the study during the year 2017-2018.

2.3. Selection Criteria

Inclusions: Individuals that had already been confirmed to positive for TB using sputum-based laboratory assays such as AFB and GeneXpert between the age bracket 20-60 years of age were recruited for the study.

Exclusions: Exclusion criteria for this study includes individuals not up to 20 years of age or above 60 years, individuals on diet restriction, exercise, stress, smokers, high altitudes, diuretics, chronic alcoholics, hyperlipidaemia, malaria patients, individuals with chronic diseases that can affect the red cell indices such as hypertension, diabetes mellitus and HIV.

2.4. Data Collection and Variable Specification

The data collection for the haematological investigation was carried out during morning hours (8:00 a.m.-10.00. a.m.). Samples of blood (5 ml) were obtained from each participant using a vacutainer and employing standard infection prevention procedures. The collected aliquots of blood samples were used to determine participants' PCV, haemoglobin concentration and RBC counts from which red cell indices were calculated.

2.5. Haematological Investigations

2.5.1. *Packed Cell Volume (PCV)*

The packed cell volume (PCV) was determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes after which the blood separates into distinct layers (Monica, 2006).

2.5.2. *Haemoglobin concentration (ctHb):* Haemi-*globincyanide - A Spectrophotometric Method Test principle*

Blood was diluted in a solution containing potassium ferricyanide and potassium cyanide.

Potassium ferricyanide oxidizes the iron in heme to the ferric state to form methaemoglobin, which is then converted to Haemiglobincyanide (HiCN) by potassium cyanide.

HiCN is a stable coloured product, which in solution has an absorbance maximum at 540 nm and strictly obeys Beer-Lambert's law.

Absorbance of the diluted sample at 540 nm was compared with absorbance at the same wavelength of a standard HiCN solution whose equivalent haemoglobin concentration is known.

2.5.3. *Reagent Diluent (modified Drabkin solution) (van Kampen and Zijlstra, 1961).*
Potassium ferricyanide (K3Fe (CN)6)   200 mg
Potassium cyanide (KCN)   50 mg
Dihydrogen potassium phosphate (KH2 PO4) 140 mg
Non-ionic detergent (e.g. Triton X-100)   1 mL
Above diluted to 1000 mL in distilled water

2.5.4. Manual Method

25 µl of blood was added to a 5.0 mL reagent, mixed and left to stand for 3 minutes.

Absorbance was read at 540 nm against a reagent blank and absorbance of HiCN standard was also measured in the same way.

2.5.5. Red Blood Cell count
2.5.5.1. *Principles*: In order to facilitate counting, whole blood was diluted with Gower's solution which haemolysed the white blood cell and prevented red cell lysis.

2.5.5.2. *Procedure*: a 1:200 dilution of blood was prepared by dispensing 20 µl of whole blood into a tube containing 3.98 mL (3980 µl) of Gower's solution.

The dilution was mixed continuously for 2-3 minutes with hand manually.

10 µl of the 1:200 dilution was dispensed into each side of the counting chamber.

Once the counting chamber was filled, it was left for approximately 3 minutes to allow the red blood cell to settle prior to counting.

2.5.5.3. *Count the red blood cell*: the counting chamber carefully placed on the microscope stage.

A 10x objective lens was used to scan for red cells and cells within each square was counted using 40x objective lens.

2.5.6. Calculation of RBC count

$$\text{Total count (Cells/L)} = \frac{\text{Cells counted} \times \text{dilution factor} \times 10^6}{\text{Volume}}$$

Example 10R: Total RBC count = 400 cells, dilution factor = 200

$0.2 \times 0.2 \times 0.1 \times 10$ cu.mm $\quad = 400$ cells

$$1 \text{ cu.mm} = \frac{400 \times 200}{0.2 \times 0.2 \times 0.1 \times 10}$$

$$= 2 \times 10^6 \text{ cells}$$

1 cu.mm = 1 µl, 10^6 µl = 1 L

Total RBC count/liter = 2×10^6 cells $\times 10^6$

$$= 2 \times 10^{12} \text{cells/L}$$

Normal range:
Male: 4.5-6.0 × 1012 cells/L
Female: 4.0-5.5 × 1012 cells/L

2.5.6. Statistical Analysis

Statistical Package for Social Sciences (SPSS, version 20.0) was used for all statistical analyses. Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were expressed as number (percentage, %). Using previously described methods, the mean values were compared between cases and controls using *t*-test and One-way ANOVA (LSD Post Hoc Test) at 5% level of significance. All reported *p*-values are two tailed, and statistical significances was set at 0.05 levels.

3. Results
3.1. Clinical Characteristics

The study consists of 20 (57.1%) TB positive subjects attending TB clinic at St. Charles Borromeo hospital, Anambra state and 15 (42.9%) control subjects with overall mean age of 31.29±9.64. The results obtained from this study are shown in Table 4.1.

Significant differences were recorded for RBC counts (TB Positive: 4.22±0.77, Control: 5.46±.84, *p*=0.000), Hb (TB Positive: 10.80±2.11, Control: 13.27±1.79, *p*=0.001), and MCHC (TB Positive: 31.51±1.74, Control: 33.36±.845, *p*=0.000) respectively while no significant differences were recorded for PCV (TB Positive: 34.05±7.15, Control: 38.27±6.70, *p*=0.085), MCV (TB Positive: 76.07±18.61, Control: 72.24±6.65, *p*=0.453) and MCH (TB Positive: 25.89±3.21, Control: 24.04± 2.51, *p*=0.075) respectively.

The mean values of RBC counts, Hb and MCHC were all significantly lower in TB positive subjects when compared to the control group. The mean values of MCV and MCH were insignificantly higher in TB positive subjects whereas their PCV was insignificantly lower when compared to the control respectively.

Table 4.1: RBCs counts, Hb, PCV and RBCs indices of TB patients and control:

Variables	TB Positive (n=20)	Control (n=15)	*p*-value[a]
RBC counts	4.22±0.77	5.46±.84	.000*
Hb	10.80±2.11	13.27±1.79	.001*
PCV	34.05±7.15	38.27±6.70	.085
MCV	76.07±18.61	72.24±6.65	.453
MCH	25.89±3.21	24.04±2.51	.075
MCHC	31.51±1.74	33.36±.84	.000*

Values are represented as mean ± SD, where SD= Standard deviation.

*Significant (p<0.05) a Differences determined by using 2-tailed t tests following Levene's test for equality of variances.

Table 4.2 shows the multiple comparison of RBC counts, Hb, PCV and Red indices by group and gender. The RBC counts and Hb of TBPM were significantly different from both TBPF and MC respectively and vice versa, RBC counts and Hb of FC was significantly different from both TBPF and MC, and the RBC counts and Hb of MC was significantly different from TBPM, TBPF and FC respectively. The PCV of MC were significantly different from the PCV of TBPM, TBPF and FC respectively. The PCV of MC were significantly different from both the PCV of TBPF and FC. MCHC of TBPM was significantly different from MCHC of both MC and FC, and MCHC of TBPF was significantly different from MCHC of both MC and FC.

From the population pyramid (Figure 4.1), it was illustrated that male TB positive subjects were distributed in all age groups whereas female TB positive subjects were found in only two of the age groups. Majority of the TB positive subjects were found in the youngest age group (50%).
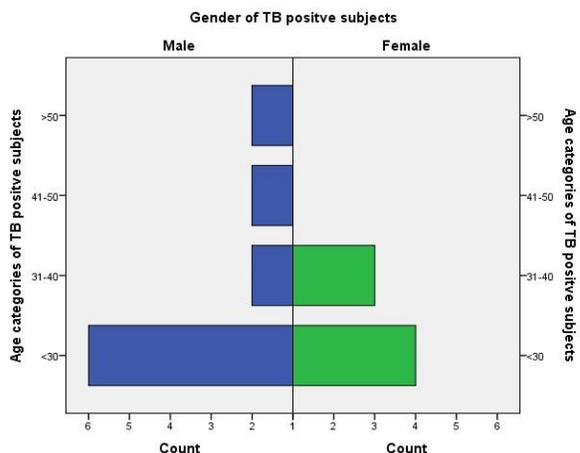
Figure 4.1. Distribution of TB patients' age according to gender.
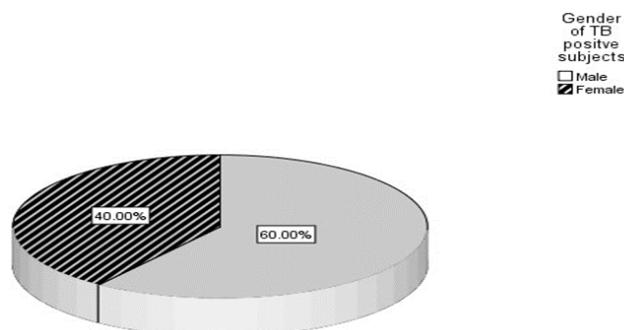
Figure 4.2. Gender distribution in tuberculosis patients

Figure 4.2 is a pie chart showing 60% (12) patients were male and 40% (8) patients were female which indicates men are more commonly affected by TB than women. This result may be due to small sample size which is not consistent with national representative sample.

Table 4.2: Multiple comparison of RBC counts, Hb, PCV and Red cell indices by group and gender

Variables	TBPM (n=12)	TBPF (n=8)	MC (n=8)	FC (n=7)
RBC Counts	4.52±0.77[ab]	3.77±0.56[acd]	6.02±0.57[bce]	4.82±0.61[de]
Hb	11.48±2.12[ab]	9.76±1.74[acd]	14.41±1.50[bce]	11.97±1.09[de]
PCV	36.23±7.52[a]	30.78±5.43[bc]	42.63±3.78[abcd]	32.29±5.82[d]
MCV	76.36±14.71	75.61±24.49	71.08±7.80	73.56±5.33
MCH	25.55±1.79	26.39±4.79	23.61±3.17	24.53±1.56
MCHC	31.43±1.98[ab]	31.64±1.44[cd]	33.23±1.03[ac]	33.51±0.62[bd]

Values are represented as mean ± SD

The mean difference is significant at the 0.05 level

Values with the same superscript in the same row are significantly different

Values with the varying superscript in the same row are not significantly different

Difference was determined using One-way ANOVA and LSD Post hoc test for multiple comparison TBPM: TB positive males, TBPF: TB positive females, MC: Male control, FC: female control, LSD= Least significant difference.

4. Discussion

Among all infectious diseases that afflict humans, tuberculosis remains the deadliest (Eyuel, 2016). In 2017, 10 million people fell ill with TB, and 1.6 million died from the disease (including 0.3 million among people with HIV) (WHO, 2018).

Nigeria is among the 14 high burden countries for TB, TB/HIV and Multi Drug Resistant TB. The country is ranked seventh among the 30 high TB burden countries and second in Africa according to Global Health Education (GHE, 2017).

The problem of TB in Nigeria has been made worse by the issues of drug resistant TB and the HIV/AIDS epidemic (GHE, 2017). It is estimated that 407,000 people in Nigeria have TB each year (GHE, 2017).

In addition, there are an estimated 63,000 HIV positive people that get TB each year. This was a case-controlled study conducted at the TB clinic of St. Charles Borromeo hospital, Anambra state to reveal changes in Haematological profile in TB patients who are clinically positive with acid fast bacilli (MTB) in sputum and under treatment.

The study consists of 20 (57.1%) TB positive subjects attending TB clinic at St. Charles Borromeo hospital, Anambra state and 15 (42.9%) control subjects with overall mean age of 31.29 ± 9.64. Of the TB positive subjects 60% and 40% were male and female respectively. Majority of the TB positive subjects were found in the youngest age group <30 (50%) and were mostly males (30%).

The results obtained from this study as shown in Table 4.1. demonstrated that significant differences were recorded for RBC counts (TB Positive: 4.22 ± 0.77, Control: $5.46\pm.84$, p=0.000), Hb (TB Positive: 10.80 ± 2.11, Control: 13.27 ± 1.79, p=0.001), and MCHC (TB Positive: 31.51 ± 1.74, Control: $33.36\pm.845$, p=0.000) respectively while no significant differences were recorded for PCV (TB Positive: 34.05 ± 7.15, Control: 38.27 ± 6.70, p=0.085), MCV (TB Positive: 76.07 ± 18.61, Control: 72.24 ± 6.65, p=0.453) and MCH (TB Positive: 25.89 ± 3.21, Control: 24.04 ± 2.51, p=0.075) respectively. The mean values of RBC counts, Hb and MCHC were all significantly lower in TB positive subjects when compared to the control group whereas the mean values.

The mean values of MCV and MCH were insignificantly higher in TB positive subjects whereas their PCV was insignificantly lower when compared to the control respectively. In order to investigate if there is significant difference in Hb levels between males and females among the patient group, the study of the Hb was carried out by the sex, and from the results in Table 4.2, it was shown that the RBC counts, Hb and PCV of both male and female TB positive subjects were significantly lower when compared to the control group respectively.

The variant values demonstrated that the distribution of Hb concentration and PCV were skewed toward the lower values. However, literature review showed that the mean RBC counts, Hb and PCV in Indian, Malaysian, Saudis and other parts of Nigeria TB patients were much higher than the values found in the current study (Akintunde et al., 1995; Singh et al., 2001; Araujo et al., 2003; Muzaffar et al., 2008; AL-Omar et al., 2009). A mild anaemia was observed in TB positive subjects (Hb, 10.80 ± 2.11) whereas study control subjects were not anemic (Hb, 13.27 ± 1.79). The presence of anaemia was defined using Lewis colour scale (Monica, 2006).

This observation is in accordance with the reports of several studies where mild anaemia was demonstrated to be a common feature among TB patients (Ebrahim et al., 1995; Das et al., 2003; Lee et al., 2006; Miah et al., 2007). All TB positive subjects presented significantly lower concentration of MCHC (male: 31.43 ± 1.98 and female: 31.64 ± 1.44) as compared to control group (male: 33.23 ± 1.03 male and female: 33.51 ± 0.62).

This finding is similar to study by Araujo et al. (2003). It has been reported that the lower MCHC values may be associated with a deficiency of iron which may be acquired through extrinsic factors. For example, malnutrition (Wessels et al., 1999; Araujo et al., 2003).

No significant difference of the mean MCV and MCH was found in both male and female patients as compared to the healthy controls.

Generally, literature review demonstrated that the anaemia in chronic diseases is of normocytic normochromic type, although in a few cases, microcytic hypochromic also occurred (Jenkins and Williams, 1994; Wickramashinghe, 2000; Iolascon et al., 2009). However, the findings of this study is supported by earlier and recent studies in which Haematological abnormalities was reported among TB patients (Morris et al.,1989; Dosumu.,2001; Shigh et al., 2001; Olaniyi and Akenova.,2003; Lee et al., 2006; Muzaffar et al.,2008).

5. Conclusion

Many haematological abnormalities have been demonstrated in tuberculosis patients in this study. The TB patients showed significantly lower Hb levels (mild anaemia), RBC count and PCV, while MCHC was the only significantly decreased RBC indices.

While many of them are consistent with reported literature and reinforce the fact that they can become valuable tools in monitoring tuberculosis and also serve as indicators in assessment of response to therapy. It is therefore recommended that iron profile should be done to confirm if there is iron deficiency anaemia.

Further studies should be done on this aspect. Also, the government should direct their efforts to establish health educational programmes about tuberculosis disease to minimize the distribution of the disease.

Conflict of Interest
The authors have no conflict of interest to declare.

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