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HAEMATOLOGICAL CHANGES IN PATIENTS WITH HUMAN TUBERCULOSIS.

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Abstract

Some haematological alterations due to active cases of tuberculosis caused by *Mycobacterium tuberculosis* were investigated in Enugu Urban of South East, Nigeria. The results revealed thrombocythaemia, leucocytosis and elevated Erythrocyte sedimentation rates ($p<0.05$). There was oligocythaemia, as well as reduced haematocrit and haemoglobin concentrations ($p<0.05$). The significant oligocythaemia, anaemia, reduced packed cell volume found in active TB positive persons are all attributed to invasion of haematopoietic organs by any of the *Mycobacteria tuberculosis* complex (MTBC) namely: *M tuberculosis, M. bovis, M africanum, M. canetti, M. microti,* and *M. leprae,* which reduced substantially the rate of erythropoiesis. The invasion of lymphoid organs such as lymph nodes, thymus and tonsils by MTBC stimulated the synthesis of leucocytes leading to leucocytosis ($p<0.05$). This could be an inflammatory response which prepared the victim to defend itself against any of the MTBC that invaded the lungs and might even invade other extrapulmonary organs. Thrombocythaemia in active TB is attributed to haemoptysis, since the latter occurs whenever there is a wound or a threat to tissue injury or damage. When these changes in haematological parameters are used in combination with other tests, microscopic and clinical methods, TB diagnosis and treatment could be well improved.

Key words: Tuberculosis, *Mycobacteria*, haematological parameters

INTRODUCTION

Tuberculosis (TB) is a common, and in many cases lethal, infectious disease caused by the bacterium, *Mycobacterium tuberculosis* (Kumar et al. 2007) This is a small, aerobic, non-motile bacillus (Dolin et al. 2010) *M. tuberculosis* complex (MTBC) includes four other TB causing *Mycobacteria* species; *M bovis, M. africanum, M. canetti,* and *M microti.* Other known pathogenic *Mycobacteria* species include *M. leprae, M. avium,* and *M. kansasii* with the latter two species being classified as "non–tuberculosis *Mycobacteria*" (NTM) (Panteix et al. 2010) NTM cause neither tuberculosis nor leprosy but can cause some lung diseases that resemble tuberculosis so closely (Thoen et al. 2006). The TB bacteria typically attack the lungs, but can also invade other extrapulmonary organs like kidney, liver, spleen, genital-urinary tracts, adrenal glands, bones, lymph nodes, testis, brain etc and in such cases are called extra pulmonary TB (Dauda et al. 2010).

The bacteria are spread through the air when someone who has active TB infection coughs, sneezes, speaks or in any way spreads respiratory fluid into the air (Kumar et al. 2007). Most infections are asymptomatic and latent, but about 5 to 10% of latent infections could eventually progress to acute disease, which, if not well treated, leads to 50% of deaths of those so affected (Kumar et al. 2007) About 20% of the world's population is infected with TB but only much less than 5 to 10% of the affected persons would develop active symptoms of TB. (WHO, 2011). People living with HIV, diabetics and alcoholics are at much greater risks than others (Mohan et al. 2000; Amdekar, 2009). A less common route of transmission is via the skin. However, laboratory technologists and pathologists who handle TB specimens could contract the infection via skin wounds (Nwachukwu, 2015) TB has also been reported in children who were circumcised with unsterilized instruments and in persons who received tattoos with unsterilized needles and blades (Escalante, 2009).

Symptoms of active TB include persistent cough, lasting three weeks or more, usually accompanied by one or more of the following signs:- chest pain, shortness of breath, night sweats, loss of appetite, weight loss, fever, chills and fatigue (Nwachukwu, 2015; Ulasi, 2016). Someone with an inactive infection, cannot transmit the disease. Primary TB does not produce noticeable symptoms in its early stages when it is not contagious (Kumar et al. 2007). Macrophages, immune cells that detect and destroy foreign matter could ingest the TB bacteria or transport them to the lymph nodes where they may be inhibited or destroyed (Guyton and Hall, 2011) However, if the immune cells fail to control the infection, the bacteria can then multiply and gradually develop into active TB infection (Houben et al. 2006). A chest X ray may show shadows in the lung or fluid collection between the lung and its lining (Thoen et al. 2006). If the immune system destroys the bacteria, the patient may experience no more than mild symptoms, such as occasional coughs (Panteix et al. 2010). If the bacteria are inhibited, rather than destroyed, the body's immune cells and the bacteria form a lump known as a granuloma, or tubercle (Pai et al. 2008). In effect, the immune cells form a wall around inactive bacteria. As long as the immune system remains strong, the TB bacteria remain walled off and inactive (Amicosante et al. 2010). The tubercles may appear as shadows in a chest X-ray. If the immune system later becomes weakened, the tubercle may open releasing the bacteria, and the infection may develop into secondary TB (Houben et al. 2006).

In secondary or post primary TB, the formerly dormant bacteria multiply and destroy the lung tissues and may also spread to other organs of the body via the bloodstream. Fluid or air may collect between the lungs and the lining of the lungs , while tubercles may continue to develop in the lungs , progressively destroying the lung tissue. Coughing of blood or phlegm may then occur (Lalloo et al. 2006).

A definite diagnosis of TB is made by identifying *M. tuberculosis* in a clinical sample (eg sputum, pus or a tissue biopsy). However, the difficult culture process for this slow-growing organism can take two to six weeks for blood or sputum culture. Thus, treatment is often begun before cultures are confirmed (Nwachukwu, 2015).

Nucleic acid amplification tests and adenosine deaminase testing may allow rapid diagnosis of TB (Bento et al. 2011). Although they are not routinely recommended, as they rarely alter how a person is treated. (Dwivedi et al. 1990). The mantoux tuberculin skin test is often used to screen people at high risk for TB. Those who have been previously immunized may give a false positive test result (Pai et al. 2008). The test may be falsely negative in those with sarcoidosis, Hodgkin's lymphoma, or are malnourished (Amicosante et al. 2010)

Since most of the above diagnostic methods for active TB do not investigate some haematological changes that accompany this disease, this study was therefore undertaken. The present study is aimed at investigating some haematological alterations that occur during active TB infection.

MATERIALS AND METHODS

Study Subjects

Sixty eight (68) patients who had a history of acute or chronic cough were referred to Akachukwu Diagnostic centre, Maryland-Enugu to screen for tuberculosis. The control subjects comprised of 47 apparently healthy subjects who had no complaints of cough or pulmonary problems, and whose sputum samples tested negative for the presence of mycobacteria.

Sample Collection and Processing

After ethical clearance from the relevant Hospital authorities, each patient was given a clean, sterilized bottle to put his/her early morning sputum. A monolayer smear of the sputum sample was made on a clean glass slide, allowed to air dry and stained using Ziehl-Neelsen (ZN) staining technique. (Steingart et al. 2009) It was examined under a light microscope for the detection of *Mycobacterium* species. A sample of the same sputum was confirmed for the detection of *Mycobacteria* using GeneXpert machine (WHO GeneXpert, 2012).

About 2.0ml of blood was then drawn from the vein of each patent found to be TB positive using a sterilized disposable syringe and transferred into EDTA (Ethylene Diamine Tetra acetic acid) tube to avoid clotting of blood. The tube was plugged with sterile cotton wool and kept in a refrigerator. The blood samples in test tubes, surrounded by ice chips were, within two hours brought into Physiology laboratory of the Department of Zoology and Environmental Biology, Abia State University Uturu-Nigeria for haematological analyses.

Haematological Analysis

The erythrocyte (EC), total leucocyte (TLC) and thrombocyte count (TC) were determined using an improved Neubauer haemocytometer following the method of Cheesbrough (2007). The haemoglobin content was determined by cyanmethaemoglobin method (Wharton and McCarty, 1972). Packed cell volume (PCV) was determined by the method of Cheesbrough (2007). The erythrocyte sedimentation rate (ESR) was determined by Westergren method as modified by Cheesbrough (2007). The above procedures were carried out on both the TB positive patients and on control subjects.

Statistical Analysis

The means, standard deviation and regression analysis were carried out as described by Zar (1984) Statistical comparisons between the experimental and control groups were made by the student's t-test and analysis of variance (ANOVA) Results were considered to be statistically significant if $p<0.05$.

RESULTS

Out of the 68 individuals who were tested for the detection of tuberculosis bacilli, namely:- *M. tuberculosis, M. bovis, M.africanum, M. canetti, M. microti,* and *M. leprae,* forty seven people (36 males and 11 females) were positive for TB. Comparison of the blood parameters of TB positive people with those of TB negative (control) revealed that TB positive persons had significant oligocythaemia, leucocytosis, thrombocythaemia, reduced Packed cell volume and haemoglobin concentrations respectively as well as elevated ESR ($p<0.05$). (Table 1).

Table 1.
The mean EC, TLC, TC, PCV and HC of TB positive patients when compared to TB negative (control) subjects.

Haematological parameter	TB negative (control)		TB positive	
	males	females	males	females
EC (million/mm^3)		4.8 ± 1.4	4.1 ± 1.5	4.0 ± 1.1
TLC (thousand/mm^3)	6.5 ± 1.7	6.1 ±1.9	33.3 ± 6.7	28.5 ± 5.5
TC(thousand / mm)	240 ± 58.7	225 ± 61.1	288 ± 69.8	263 ± 74.3
PCV (%)	45.4 ± 4.6	37.5 ± 5.1	31.3 ± 5.4	27.7 ± 8.0
HC(g/dl)	14.5 ± 1.3	13.4 ±1.5	10.3 ± 2.9	9.6 ± 3.3
ESR (mm/hr)	7.9 ± 2.6	11.2 ± 3.2	84.2 ± 16.6	80.5 ± 22.4

EC = Erythrocyte count.
TLC = Total leucocyte count.
TC = Thrombocyte count.
PCV = Packed cell volume.
HC = Haemoglobin content.
ESR = Erythrocyte Sedimentation Rate.

DISCUSSION

The significant oligocythaemia, reduced haematocrit and anaemia in TB positive persons could be attributed to invasion of haematopoietic organs like the spleen and bone marrow by any of the MTBC which reduced substantially the rate of haemopoiesis and especially erythropoiesis. The same invasion of some lymphoid organs such as lymph nodes, tonsils, thymus etc by *Mycobacteria* species stimulated the rate of leucopoiesis. This could be interpreted as an inflammatory response which prepared the victim to defend him/her self

against the bacteria that invaded the lungs and might even invade other body tissues, hence there was significant leucocytosis ($p<0.05$).

The elevation of E.S.R which was observed in the study is in accordance with the work of Nwachukwu (2015) who recorded a tremendous rise in ESR from <10.0 to 66.86 mm/hr in active TB patients. A very high level of ESR should thus be associated with TB and ought to be considered as a useful diagnostic tool.

In active pulmonary TB cases, *M tuberculosis* bacilli typically invade the lung tissues leading to haemoptysis. (blood in sputum). This explains the consequent thrombocythaemia. Thrombocythaemia occurs whenever there is a wound or serious threat to tissue damage or injury (Okafor and Chukwu, 2005).

Some other authors have, however, reported evidence of anaemia due to active pulmonary TB (Shafee et al. 2014). Also, sex seems to play a vital role in the prevalence of TB in this study; and the male/female ratio was 3:1. Globally, the male and female ratio for TB is 2:1 (WHO, 2011). TB also seems to be a poverty related disease which is associated with poor living conditions, lack of financial support, and less access to modern health care services (Ogboi et al. 2010) It has been severally reported that habitual smokers, diabetics, alcoholics as well as elderly people are at higher risks of acquiring active TB infection (Nwachukwu, 2015).

Tuberculosis continues to be an important communicable disease in the world and is a serious health problem in developing countries due to unhygienic environment and the unprotected state of those that are affected. The Governments of developing countries are hereby called upon to help in eradicating poverty since it is a major threat behind the spread of TB. Vitamin D and antibiotics should be recommended to TB patients which would lead to activation of macrophages and restrict intercellular growth of *Mycobacterium* species.
This paper concludes that patients infected with active Tuberculosis did exhibit certain changes in their blood parameters such as significant oligocythaemia, leucocytosis, reduced PCV, anaemia, thrombocythaemia and increased ESR, ($p<0.05$). When these changes in haematological parameters are used in combination with other tests, microscopic and clinical methods, TB diagnosis and treatment could be well improved.

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