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ALTERED PROTEIN AND IRON LEVELS OF PATIENTS WITH ACTIVE TUBERCULOSIS IN A NIGERIAN REFERENCE HEALTH FACILITY

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ABSTRACT
Backgound: Tuberculosis as a state of chronic inflammation impacts on haematologic functions of the body.

Objectives: This study aimed at assessing iron parameters and serum protein levels of ninety tuberculosis patients aged fifteen to sixty years, enrolled from Dr Lawrence Henshaw Memorial Hospital, Calabar, Nigeria. Ninety apparently healthy individuals age and gender-matched served as control subjects.

Methods: Packed cell volume, haemoglobin concentration, serum iron, total iron binding capacity, total protein, albumin and serum ferritin were determined using standard methods. Transferrin saturation, globulin concentration and albumin-globulin ratio were derived by calculation. Data were analysed using student T-test on SPSS version 20 software. Statistical significance was set at P-value less than 0.05.

Results: Packed cell volume, haemoglobin concentration, serum iron, total iron binding capacity, transferrin saturation, albumin levels and albumin-globulin ratio of tuberculosis patients were found to be significantly lower while serum ferritin and globulin were significantly increased ($p<0.05$) as compared with control values. Serum ferritin improved towards control values as anti-tuberculosis therapy progressed.

Conclusion: A reduction in haemoglobin, serum iron, total iron binding capacity and transferrin saturation and increase in serum ferritin as well as altered serum protein levels, occur in tuberculosis infection.

Key words: Tuberculosis, iron, serum protein

PROTÉINE ALTÉRÉE ET NIVEAUX DE FER CHEZ LES PATIENTS ATTEINTS DE TUBERCULOSE ACTIVE DANS UN ÉTABLISSEMENT DE SANTÉ DE RÉFÉRENCE DE L'

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RÉSUMÉ
Fond : la tuberculose comme un état d'inflammation chronique hématologique des impacts sur les fonctions de l'organisme.

Objectifs : Cette étude visait à évaluer les paramètres fer sérique et la teneur en protéines des quatre-vingt-dix patients âgés de quinze à soixante ans, inscrits par le Dr Lawrence Henshaw Memorial Hospital, Calabar Nigéria. Quatre-vingt-dix personnes apparemment en bonne santé appariés selon l'âge et le sexe ont servi de témoins.

Méthodes : l'hématocrite, du taux d'hémoglobine, le fer sérique, capacité totale de fixation du fer, protéines totales, albumine et la ferritine sérique ont été déterminées en utilisant les méthodes standard. La saturation de la transferrine, l'albumine et globuline globuline concentration ratio ont été obtenus par calcul. Les données ont été analysées à l'aide de student T-test sur le logiciel SPSS version 20. La signification statistique a été fixé à p est inférieur à 0,05.

Résultats : l'hématocrite, du taux d'hémoglobine, le fer sérique, capacité totale de fixation du fer, la saturation de la transferrine, l'albumine et l'albumine-globuline rapport entre les patients atteints de tuberculose ont été trouvés à être beaucoup plus faible alors que le taux de ferritine sérique et d'immunoglobuline humaine ont augmenté significativement ($p < 0,05$) par rapport aux valeurs des témoins. Le taux de ferritine sérique est améliorée vers les valeurs de contrôle comme thérapie anti-tuberculose progresse.

Conclusion : une réduction de l'hémoglobine, le fer sérique, capacité totale de fixation du fer et la saturation de la transferrine et la ferritine sérique en augmentation ainsi que le taux de protéines sériques modifié, se produisent dans l'infection par la tuberculose.

Mots clés : Tuberculose, fer à repasser, protéine sérique

INTRODUCTION

Tuberculosis (TB) is still a major concern of public health in Nigeria. Among the 22 high burden countries, Nigeria ranks eleventh [1]. Tuberculosis is primarily a pulmonary disease caused by *Mycobacterium tuberculosis* [2, 3]. It is an infectious disease which induces a state of chronic inflammation. The reference health facility for the diagnosis and treatment of tuberculosis in Cross River State, Southern Nigeria is Dr Lawrence Henshaw Memorial Hospital also called Infectious Disease Hospital located in the state capital, Calabar. This center is equipped to diagnose TB through microscopy, Gene Xpert, radiology and culture. The Directly Observed Therapy Short course (DOTS) program for the treatment of TB is fully implemented in this center. Iron is an essential micronutrient for all living organisms which plays a very crucial role in modulating the struggle for survival between mammals and pathogens. Its role is well known in metabolic processes such as cell respiration, growth and DNA synthesis [4]. It has been shown that iron overload promotes free radical induced tissue damage and organ failure, decreases immune protection and increases pathogen invasion. On the other hand, iron deficiency causes iron-deficiency anaemia with all its associated symptoms. An optimal level of iron is needed for natural immunity against the growth of pathogens [5]. Haematological abnormalities have been associated with tuberculosis infection. Decrease in packed cell volume and haemoglobin concentration and increase in erythrocyte sedimentation rate and relative plasma viscosity have been reported [6, 7]. It has also been reported that the majority of TB patients have significantly elevated level of antibodies against *Mycobacterium tuberculosis* in addition to acute phase response which involves release of plasma proteins as a consequence of inflammation [8, 9]. Hypoalbuminaemia, hyperproteinaemia and hyperglobulinaemia are findings associated with pulmonary tuberculosis [10]. There is paucity of information on iron parameters and serum protein levels in tuberculosis infection in our locality; hence this study was done to assess iron parameters and protein levels of patients with active tuberculosis in a reference health facility in Calabar, Nigeria.

METHODS

A total of one hundred and eighty subjects were recruited for this study. This consisted of ninety tuberculosis patients of both gender aged between fifteen and sixty years, enrolled from Dr Lawrence Henshaw Memorial Hospital in Calabar, Cross River State, Nigeria. TB patients included newly diagnosed cases and those on anti-tuberculosis therapy. Anti-tuberculosis therapy under the directly observed therapy short course (DOTS) lasts for a period of six (6) months. Those patients on anti-tuberculosis therapy from day one up to less than two (2) months are in the intensive phase while two to six months constitute the continuation phase of treatment. Ninety apparently healthy individuals age and gender-matched who were negative for tuberculin (mantoux) test, selected from residents of Calabar metropolis were the control subjects. Ethical permission was given by the Cross River State Ministry of Health and all participants gave their consent. Packed cell volume and haemoglobin concentration was by microhaematocrit and Cyanmethaemoglobin methods respectively [11]. Serum iron (SI), total iron binding capacity (TIBC), total protein (TP) and albumin (ALB) were measured by colorimetric method with test kits manufactured by GIESSE Diagnostics, Italy and RANDOX Laboratories, United Kingdom. Serum ferritin (SF) was determined by enzyme linked immunosorbent assay (ELISA) using CALBIOTECH USA produced kit. Transferrin saturation (TS), globulin concentration (GLOB) and albumin-globulin ratio (ALB-GLOB) were derived by calculation. Student's t-test was used to analyze data on SPSS version 20 software. Statistical significance was set at P-value less than 0.05.

Consent: Informed consent was obtained from all participants in this study.

Competing interest: No competing interest exists.

Authors' contributions: *This work is a result of collaboration between all authors. Akpan PA designed the study and wrote the first draft of the manuscript. Okafor IM performed the statistical analysis. Anakebe S managed the literature searches. All authors were involved in the laboratory analysis; the final manuscript was read and approved by all authors.*

RESULTS

Demographic data of TB patients and controls is presented in figure 1. Tuberculosis patients consisted of 63 males and 27 females while 60 males and 30 females served as control subjects. The TB patients as well as control were grouped into three based on age. Forty three (43) of the TB patients were aged 15-30 years with 36 and 11 aged 31-45 and 46-60 years respectively. Similarly, controls were 38, 40 and 12 in number for the 15-30, 31-45 and 46-60 years groups respectively.

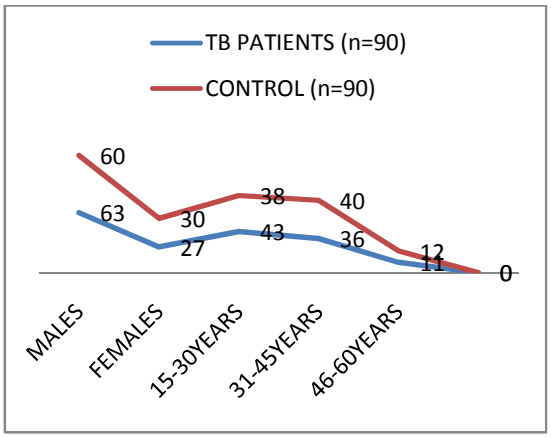

FIGURE 1: DEMOGRAPHIC DATA OF TB PATIENTS AND CONTROL

Table 1 shows iron parameters of TB patients and control subjects. The packed cell volume and haemoglobin of TB patients were observed to be significantly lower (p=0.004 and 0.001) than values obtained for control. Similarly, serum iron, total iron binding capacity and transferrin saturation were significantly lower (p=0.001) for TB patients when compared to control. Conversely, serum ferritin was significantly higher (p=0.001) for TB patients than their control.

TABLE 1: IRON PARAMETERS OF TUBERCULOSIS PATIENTS AND CONTROL SUBJECTS

Variable	TB patients (n=90)	Control (n=90)	P-value
Packed cell volume (L/L)	0.37±0.06*	0.41±0.04	0.004
Haemoglobin (g/L)	1.04±0.16*	1.33±0.13	0.001
Serum iron (µg/dl)	48.99±8.41*	106.00±24.30	0.001
Total iron binding capacity (µg/dl)	187.10±37.00*	257.95±47.00	0.001
Transferrin saturation (%)	26.28±4.32*	41.62±8.13	0.001
Serum ferritin (ng/ml)	361.25±267.00*	82.40±53.84	0.001

*: Significantly different from control subjects

In table 2, the serum protein levels of tuberculosis patients and control are presented. The TB patients and their control had similar values (p=0.226) for total protein. While the albumin concentration was significantly lower (P=0.001) for the TB patients, the globulin level was significantly higher (p=0.016) when compared to control values. The albumin-globulin ratio was significantly lower (p=0.001) for TB patients than their control.

TABLE 2: SERUM PROTEIN LEVELS OF TUBERCULOSIS PATIENTS AND CONTROL SUBJECTS

Variable	TB patients n=90	CONTROL n=90	P-VALUE
TOTAL PROTEIN (g/L)	94.46±21.33	88.95±22.71	0.226
ALBUMIN (g/L)	44.58±7.31*	50.18±7.10	0.001
GLOBULIN (g/L)	49.46±20.42*	38.78±21.20	0.016
ALB-GLOB RATIO	0.90±0.34*	1.32±0.44	0.001

*: Significantly different from control subjects

Figure 2a shows iron and protein levels of TB patients in different phases of treatment. Fifty two (52) of the ninety TB patients were in the intensive phase while the remaining thirty eight (38) were in the continuation phase of treatment. The serum iron, total iron binding capacity, transferrin saturation, total protein, ALB and GLOB did not change significantly between TB patients in both intensive and continuation treatment phases. However, the serum ferritin levels reduced significantly (p=0.001) from the intensive to the continuation treatment phase. In figure 2b, the PCV, HB and albumin-globulin ratio were observed to be comparable in both phases of treatment.

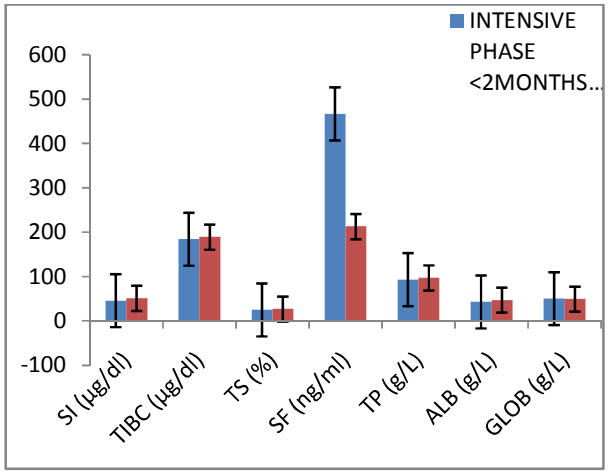

FIGURE 2A: IRON AND PROTEIN LEVELS OF TB PATIENTS IN INTENSIVE AND CONTINUATION PHASES OF TREATMENT

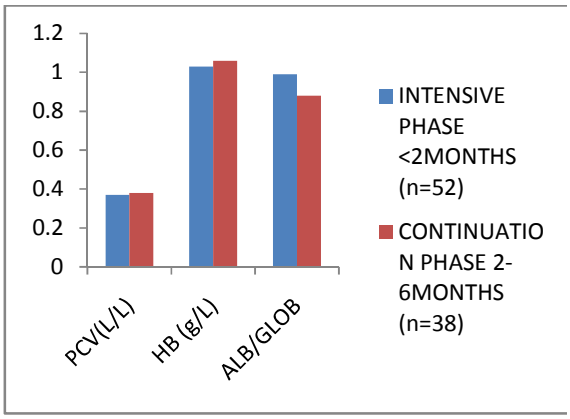

FIGURE 2B: PCV, HB AND ALBUMIN-GLOBULIN RATIO OF TB PATIENTS IN INTENSIVE AND CONTINUATION PHASES OF TREATMENT

DISCUSSION

This study has shown (table 1) that the packed cell volume and haemoglobin concentration of TB patients were significantly lower when compared to that of apparently healthy (control) subjects. Tuberculosis is a chronic ailment which could influence haemopoiesis leading to a reduction in erythropoiesis [12]. Reduced haemoglobin concentration below the reference range (11.0g/dl) which is termed anaemia, has been reported in tuberculosis infection [13, 7, 14]. This anaemia is classified as normocytic normochromic type showing features of anaemia of chronic disease and inflammation [15]. Other factors such as decrease in red cell survival and reduced erythroproietin response by the bone marrow erythroid cells are also known to cause anaemia in infections [12].

The serum iron (SI) level of TB patients was significantly lower than the value obtained for control subjects. According to Dacie and Lewis [11], SI level is low in anaemia of chronic disease and infection. Tuberculosis being a chronic inflammatory condition, it interferes with iron metabolism resulting in a low serum iron. Similarly, a lower total iron binding capacity (TIBC) was observed for TB patients when compared to control values. TIBC has been reported to be lower than reference values in infections and particularly in anaemia of chronic disease [16, 11]. Low serum iron as well as total iron binding capacity in infection has been attributed to increased hepcidin production. Hepcidin is an acute phase protein which regulates the absorption of iron in the small intestine and its release from macrophages. In an inflammatory state such as tuberculosis, hepcidin level increases but its normal function is impaired resulting in low SI and TIBC levels [17, 18]. Furthermore, hepcidin induces certain alterations in the metabolism of iron like less iron absorption from the gastrointestinal tract as well as trapping of iron in macrophages [19]. Transferrin saturation (TS) of the TB patients was significantly lower than values obtained for control subjects. Since the TS is calculated from SI and TIBC, a low TS follows the pattern of the low SI and TIBC. The low values of these iron parameters imply that erythropoiesis is ineffective and this accounts for the anaemia observed in chronic disease and inflammation.

The serum ferritin level of tuberculosis patients (361.25±267.00) was significantly higher ($p=0.001$) compared to control value (82.40±53.84). Serum ferritin is a valuable factor in the measurement of iron reserve of the body however it is a positive acute phase reactant protein which could be increased in tuberculosis infection due to inflammatory state [4]. As a reaction to injury, neutrophils and macrophages (local inflammatory cells) secrete several cytokines particularly interleukins (IL) 1, 6 and 8 as well as tissue necrotic factor alpha (TNFα) into the bloodstream. As a result, acute-phase reactants are produced by the liver in large amounts [20]. Also, the observed increase in ferritin level could be as a result of iron sequestration mechanism of the body during pathogen invasion. Iron supplementation which is the current practice in TB treatment, may be implicated in increased ferritin levels as the body withholds iron from the invading pathogen. It is therefore recommended that iron supplementation should be discouraged in the management of TB patients.

In this study, the total protein levels of TB patients were similar to control values and also comparable to previous finding [21]. TB patients had a significantly lower albumin and a significantly higher globulin level ($p<0.05$) in comparison to control (table 2). Reduction in albumin level correlates with some reports [22, 23] and disagrees with increase in albumin reported in another study [24]. Low albumin may be due to inadequate protein intake while increased globulin concentration might be due to immune response by the body against the invading TB bacilli. The albumin/globulin ratio of the tuberculosis patients was found to be lower than that of apparently healthy subjects (control).

It was observed that serum iron, TIBC, TS, albumin as well as globulin improved (though not significantly) in the continuation phase as compared to the intensive phase. On the other hand, serum ferritin reduced significantly ($p<0.05$) towards control values with progressive treatment. This could be attributed to adherence to anti-tuberculosis therapy under the DOTS program and the effectiveness of the anti-TB drugs in reversing the acute phase response. It also stands to reason that the body no longer withholds iron in the form of ferritin since the offending pathogen *Mycobacterium tuberculosis* is being cleared by the anti-TB drugs. Packed cell volume, haemoglobin and albumin-globulin ratio of TB patients were comparable for both phases of treatment.

Conclusion: This study has shown that reduction in packed cell volume, haemoglobin, serum iron, total iron binding capacity, transferrin saturation and albumin and increase in serum ferritin and globulin concentration occur in active tuberculosis; also, iron parameters and protein levels improved as anti-tuberculosis therapy progressed. It is suggested that early diagnosis and initiation of anti-tuberculosis therapy and strict adherence will alleviate the changes in iron stores.

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