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SERUM PROFILES OF INTERLEUKIN 6 CONCENTRATIONS AND WHITE BLOOD CELL COUNTS IN TUBERCULOSIS PATIENTS DURING THE COURSE OF SIX MONTHS TREATMENT IN ENUGU NIGERIA

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ABSTRACT
Aim: This study was carried out to determine the serum profiles of biomarkers of inflammation during the course of six months standard treatment of pulmonary tuberculosis in adults using the first line anti-tuberculosis drugs. Methods and Materials: The study was a prospective observational study. The protocol was approved by the Research Ethics Committee of University of Nigeria Teaching Hospital Enugu. The subjects gave informed consent. Treatment of the patients was undertaken by competent staff of our Teaching Hospital. Blood samples were taken before commencement of treatment, after 2 months, and then after 6 months treatment. Blood samples were also taken from healthy volunteers (controls). Serum concentration Interleukin 6 was determined using Human Elisa technique while white blood cell (leukocyte) count was done by routine manual microscopy. Results: Prior to treatment, the mean total white blood cell count of the patients was 4783.0±158.0/mm^3 and the mean serum concentration of Interleukin 6 was 26.9±2.2 pg/ml. After 6 months of treatment mean total white blood cell count was 4633.0±143.0/mm^3 whereas the mean serum Interleukin 6 concentration was 16.0±1.0pg/ml. For healthy controls, mean white blood cell count was 4817.0±131.0/mm^3 and mean serum Interleukin 6 was 15.3±2.3 pg/ml. There was statistically significant progressive decline in serum concentration of Interleukin 6 during the course of treatment ($p > 0.05$), but the differences in total white blood cell counts were not found significant. Conclusion: Serum Interleukin 6 is considered a potential biomarker for active tuberculosis.

KEYWORDS: Tuberculosis; cytokine; white blood cell count; leukocyte; interleukin 6.

INTRODUCTION
Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis.*[1] Despite much progress being made in the global efforts to eradicate tuberculosis, the disease has remained one of the greatest health challenges of mankind. In 2014 there were 9.6 million new cases of tuberculosis worldwide and 1.5 million people (1.1 million HIV-negative and 0.4 million HIV-positive) died of tuberculosis.[2] Some of the factors militating against disease elimination are the emergence of drug resistant strains of the pathogen, poor compliance with the treatment regimen that lasts for at least six months or more; and the concurrent epidemics of HIV/AIDS in some parts of the world.[3-5]

There are two arms to human immune response namely the innate immune response and the adaptive immune response.[6] Innate immunity which is responsible for the initial protective response to invading infective agents comprises of the skin, mucosal epithelium, and endogenously produced chemicals that possess anti-microbial action. It also includes the complement system, phagocytes, and natural killer cells. Adaptive immune response on the other hand, is made up of various types of lymphocytes, and antibodies produced by host cells. White blood cells or leukocytes (neutrophils, lymphocytes, basophils, eosinophils and monocytes) are vital cellular components of the human blood that work in concert with other components of the human immune system to rid the body of invading microbes and foreign antigens.[7]

The peripheral white blood cell (or leukocyte) count is known to be an important predictor of mortality or co-morbidity in tuberculosis.[8] White blood cells have roles to play in both the innate and the adaptive immune responses of the body[9-10] and their contribution in the natural immune response to microbial invasion of the body is thought to include sounding a systemic alarm through cytokine mediators.[6]

Cytokines are known to be active participants in inflammatory conditions including tuberculosis.[11] Several cytokines including Interleukin 6 that are elaborated during infection with tuberculosis serve to limit or exacerbate the disease process depending on their combination and balance.[12-14] Interleukin 6 is secreted by mononuclear phagocytes. It is also produced by fibroblasts, endothelial cells and activated T-cells. Its production is stimulated by microbial invasion of the body, as well as in response to the presence of tumor necrosis factor, interleukin 1 and some other cytokines.[15] Interleukin 6 has several biologic functions including the stimulation of the synthesis of acute phase proteins by the liver. It stimulates the bone marrow, along with colony-stimulating factors, to produce more neutrophils. It also serves as a stimulator of B-lymphocyte proliferation which enhances the production of immunoglobulin by differentiated B-lymphocytes. The receptor for interleukin 6 consists of a cytokine-binding protein and a 130-KD signal-transducing subunit.[16] Interleukin 6 thus plays a role in adaptive immune response.[16-17] Further understanding of human host immune responses to tuberculosis is expected to help point the way forward towards conquering this chronic debilitating disease. In a longitudinal study we determined the concentrations of serum Interleukin 6 as well as the total and differential peripheral blood white blood cell counts of adult tuberculosis patients at intervals during the course of their six months' treatment at Enugu in Eastern Nigeria.

MATERIALS AND METHODS

Subjects: We recruited successive drug-naïve tuberculosis patients who presented at the Chest Clinic of the University of Nigeria Teaching Hospital Enugu, Nigeria. They were adults of both gender and their diagnosis was based on positive clinical presentation, sputum microscopy and chest x-ray. They were HIV negative and had no other disease or inflammatory condition. Thirty one healthy adult human volunteers who had no evidence of disease and who were not taking any medicines were also included in the study as healthy controls after they gave informed consent.

Ethical consideration

The study protocol was approved by the Research Ethics Committee of the University of Nigeria Teaching Hospital Enugu. The study was carried out in line with the ethical principles of the Helsinki World Medical Assembly. Each study participant gave informed consent prior to enrolment.

Medications

As our study was not a drug trial, the patients had their normal consultation and treatment with the Chest Clinic physicians who prescribed standard anti-tuberculosis medicines for the patients. Appropriate doses of once daily oral isoniazid, rifampicin, ethambutol, and pyrazinamide were taken by the patients for the first 2 months, and thereafter isoniazid and rifampicin were taken once daily for the subsequent 4 months. The treatment for each patient lasted for at least 6 months. The study participants, like other tuberculosis patients of the chest clinic, were supplied their medicines by the clinic free of charge. The healthy volunteers were not offered any medications.

Laboratory tests

Blood sample was collected thrice during the course of the study: before treatment, after 2 months of treatment, and after 6 months of treatment from each enrolled patient but only once from the healthy volunteers. White blood cell count was carried out by manual microscopy on blood film slides prepared with Romanousky stain.[18-19] Serum was separated from clotted blood after centrifugation and frozen at -20 degrees centigrade until analysis. The concentration of Interleukin 6 in serum was measured by human ELISA (e-Bioscience Elisa Ready Set-Go, San Diego, CA) according to the manufacturer's instructions.

Data analysis

Data were analyzed using Graph Pad Prism version 6.0. Differences were considered statistically significant at p less than 0.05.

RESULTS

A total of 42 patients (24 males and 18 females) who had sputum smear-positive tuberculosis completed the study. Their mean age was 39 years. Four patients dropped out before completing the study. Those who completed the study responded very well to treatment. Prior to treatment, the mean total white blood cell count of the patients was 4783.0 ± 158.0/mm^3. After two months of treatment the mean white blood cell count was 4705.0 ± 106.0/mm^3, and after the completion of six months' treatment 4633.0 ± 143.0/mm^3. The mean total white blood cell count for healthy controls was 4717.0±131.0/mm^3. Figure 1 shows the slight decline of mean total white blood cell count during the course of treatment. The differences were however statistically insignificant. The mean serum IL-6 concentration was 26.9±2.2 pg/ml prior to commencement of anti-tuberculosis treatment. After 2 months, and after 6 months of treatment the mean Serum Interleukin-6 concentration were *19.6 ± 1.3* pg/ml and *16.0 ± 1.0* pg/ml respectively. *T*he mean serum IL-6 concentration for healthy controls was 15.3±2.3pg/ml. The difference between the pre-treatment value of IL-6 and the value obtained after the second month of treatment was statistically significant. Again the difference between the mean serum IL6 concentrations after 2 months and after 6 months of treatment was also statistically significant. The pre-treatment and the post-treatment serum concentrations of interleukin 6 were respectively significantly higher than those of the healthy controls (p less than 0.05). There was no significant difference between the post-treatment interleukin 6 serum concentration and that of healthy controls. Figure 2 shows the remarkable progressive decrease in mean IL-6

serum concentration of the tuberculosis patients during the course of treatment particularly after 2 months of treatment.

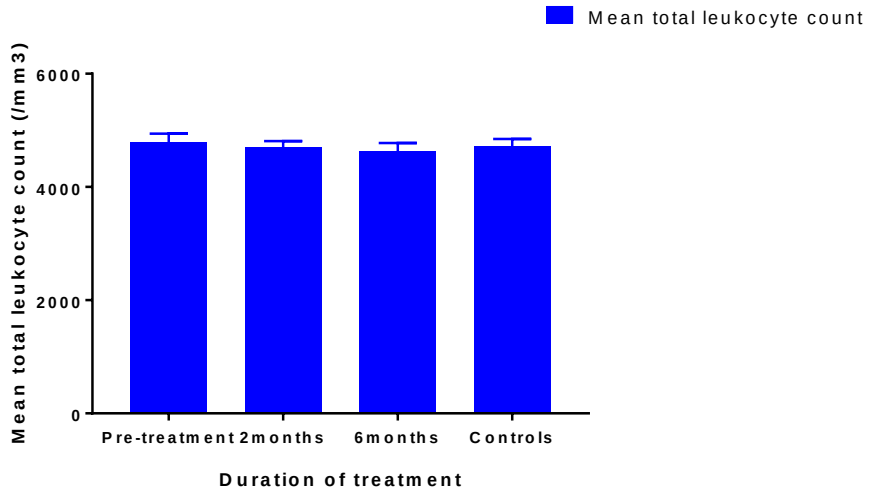

Figure 1: Showing mean total white blood cell or leukocyte counts during the course of anti-tuberculosis treatment (per mm^3).

Figure 2: showing the mean serum concentrations of interleukin 6 (pg/ml) during the course of anti-tuberculosis treatment.

DISCUSSION

The mean total white blood cell counts observed at the three various occasions for our patients were all within the normal range and showed no statistical difference when compared to healthy controls. Although a progressive decline was observed in the mean peripheral total white blood cell counts during the course of treatment of our cohort of tuberculosis patients, there were no statistically significant interval differences. This trend was in harmony with the report of Kassa and his co-researchers[20] but at variance with some other studies that reported leukocytosis in tuberculosis patients prior to treatment, particularly where there had been concurrent infections with some other bacteria.[21-23] The patients in our study were all HIV negative and had no obvious concurrent illnesses. A history of cigarette smoking had been considered to be a predictor of leukocytosis in active tuberculosis patients.[24] The mean concentration of IL 6 of the tuberculosis patients prior to commencement of anti-tuberculosis treatment was significantly higher than the mean serum concentration of IL 6 of the healthy controls. This indicated that IL 6 signaling was increased in active tuberculosis and agrees with the findings of Tang et al.[25] A murine study carried out in Mexico City[26] had also observed an increased production of interleukin 6 after the establishment of experimental tuberculosis in mice. That murine study also suggested that interleukin 6 was involved in the production of the histopathological changes observed in active tuberculosis particularly granuloma formation.[26] Unsal and his co-researchers[27] observed higher interleukin 6 serum concentrations in tuberculosis

patients than in healthy controls and additionally noted that interleukin 6 might have played a contributory role in the development of reactive thrombocytosis in the acute phase response in pulmonary tuberculosis. The remarkable decrease observed in the serum concentration of IL 6 of the patients in our study after 2 months of treatment (intensive phase of treatment) coincided with the period of sputum conversion for most of the patients. Thus treatment and commencement of resolution of active tuberculosis in our patients were accompanied with steep decline in serum concentration of IL 6. At the completion of six months short-course treatment the mean IL 6 serum concentration of patients approximated that of the healthy controls. We deduce that IL 6 is a key player in the cellular and molecular processes leading to the manifestation of clinical tuberculosis and the serum concentration of IL6 before and during treatment could serve as a useful biomarker for the diagnosis, treatment, and follow-up of patients with active tuberculosis. Larger prospective studies in both HIV sero-positive and sero-negative patients are hereby recommended.

CONFLICT OF INTEREST: None declared by the authors.

REFERENCES

1. Cambau E, Drancourt M. Steps towards the discovery of Mycobacterium tuberculosis by Robert Koch, 1882. Clin Microbiol Infect, 2014; 20(3): 196-201.
2. World Health Organization, Global Tuberculosis Report, 2015.
3. Maher D, Harries A, Getahun H. Tuberculosis and HIV interaction in sub-Saharan Africa: impact on patients and programs; implications for policies. Trop Med Int Health, 2005; 10(8): 734-742.
4. Babiarz KS, Suen S Goldhaber-Fiebert JD. Tuberculosis treatment discontinuation and symptom persistence: an observational study of Bihar, India's public care system covering >100,000,000 inhabitants. BMC Public Health, 2014; 14: 418.
5. Gabida M, Tshimanga M, Chemhuru M, Gombe N, Bangure D. Treatment Outcomes, New Sputum Smear Positive Patients in Kwekwe District, Zimbabwe, 2007-2011: A Cohort Analysis. Journal of Tuberculosis Research, 2015; 3: 126-135. http://dx.doi.org/10.4236/jtr.2015.34019
6. Hoebe K, Janssen E, Beutler B. The interface between innate and adaptive immunity. Nat Immunol, 2004; 5: 971-974.
7. Kobashi Y, Mouri K, Yagi S, Obase Y, Miyashita N, Oka M. Transitional changes in T-cell responses to Mycobacterium tuberculosis-specific antigens during treatment. J Infect, 2009; 58: 197-204.
8. Schwartz J, Weiss ST. Host and environmental factors influencing the peripheral blood white blood cell count. Am J Epidemiol, Dec, 15, 1991; 134(12): 1402-9.
9. Martineaus AR, Newton SM, Wilkinson KA. Neutrophil- mediated innate immune resistance to mycobacteria. J. Clin Invest, 2007; 117: 1988-94.
10. Persson YA, Blomgran-Julinder R, Rahman S, Zheng L, Stendahl O. Mycobacterium tuberculosis-induced apoptotic neutrophils trigger a pro-inflammatory response in Macrophages through release of heat shock protein 72, acting in synergy with the bacteria. Microbes infect, 2008; 10: 233-40.
11. Duque GA, Descoteaux A. Macrophage Cytokines: Involvement in Immunity and Infectious Diseases. Front Immunol, 2014; 5: 491.
12. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfefferk, Lowestein CJ, Schrelber R, Mak TW, Bloom Br. Tumor necrosis factor alpha is required in the protective immune response against mycobacterium tuberculosis in mice. Immunity, 1995; 2(6): 561-572.
13. Hanekom WA, Hughes J, Marin-kurve M. Novel application of a whole blood intracellular cytokine detection assay to quantitate specific T-cell frequency in field studies. J. Immunol Methods, 2004; 291: 185-95.
14. Walzi G, Ronacherk, Hanekom W, Scriba TJ, Zumla A. Immunological biomarkers of tuberculosis. Nat. Rev. Immunol, 11, 2011; 5: 343-54.
15. Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). The FASEB Journal, 1990; 4(11): 2860-2867.
16. Abbas AK, Lichtman AH. Cellular and molecular immunology. Saunders Publishers, 2005: Philadelphia, 114-121.
17. Schindler R, Mancilla J. Endres S, Ghorbani R, Clark SC, Dinarello CA. Correlations and Interactions in the Production of Interleukin-6 (IL-6), IL-1, and Tumor Necrosis Factor (TNF) in Human Blood Mononuclear Cells: IL-6 Suppresses IL-1 and TNF. Blood, 1990; 75(1): 40-47.
18. Ochei J, Kolhatker A. Medical Laboratory Science Theory and Practice. New Delhi: Tat-McGrawhill Publishers, 2000; 156-61.
19. Cheesborough M. District Laboratory Practice in Tropical Countries. Cambridge: Cambridge University Press, 2006; 112-27.
20. Kassa E, Enawgaw B, Gelaw A, Gelaw B. Effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients attending at University of Gondar Hospital, Northwest Ethiopia. BMC Hematology, 2016; 16: 1. DOI 10.1186/s12878-015-0037-1.
21. Sahin F, Yazar E, Yildiz P. Prominent features of platelet count, plateletcrit, mean platelet volume and platelet distribution width in pulmonary tuberculosis. Multi-disciplinary Respiratory Medicine, 2012; 7: 38.
22. Atomsa D, Abebe G, Sewunet T. Immunological Markers and Hematological Parameters among Newly Diagnosed Tuberculosis Patients at Jimma

University Specialized Hospital. Ethiop J Health Sci., Oct, 2014; 24(4): 311–318.
23. Abakay O, Abakay A, Sen HS, Tanrikulu AC. The Relationship Between Inflammatory Marker Levels and Pulmonary Tuberculosis Severity. Inflammation, 2015; 38(2): 691–696.
24. Schwartz J, Weiss ST. Cigarette smoking and peripheral blood white blood cell differentials. Ann Epidemiol, May, 1994; 4(3): 236-42.
25. Tang S, Cui H, Yao L, Hao X, Shen Y. Increased Cytokines Response in Patients with Tuberculosis Complicated with Chronic Obstructive Pulmonary Disease. PLoS ONE, 2013; 8(4): e62385. doi:10.1371/journal.pone.0062385.
26. Hernández-Pando R, Arriaga AK, Panduro CA, Orozco EH, Larriva-Sahd J, Madrid-Marina V. The response of hepatic acute phase proteins during experimental pulmonary tuberculosis. Exp Mol Pathol, 1998; 65(1): 25-36.
27. Unsal E, Aksaray S, Koksal D, Sipit T. Potential role of interleukin 6 in reactive thrombocytosis and acute phase response in pulmonary tuberculosis. Postgrad Med J., 2005; 81: 604–607. doi: 10.1136/pgmj.2004.030544.