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Full Length Research Paper

Plasma levels of C-Reactive Protein and Fibrinogen in Pulmonary Tuberculosis Patients in Ibadan, Southwest, Nigeria

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ABSTRACT

Tuberculosis (TB) is an infectious disease which remains a global health problem till date. A number of cytokine and chemokine have been reported to be associated with MTB clearance, reactivation or cure but there are no consensus biomarkers to differentiate the severity of TB. In this study, we determined the changes in plasma C- reactive protein (C-RP) and Fibrinogen levels in Drug sensitive Tuberculosis (DSTB) patients at diagnosis, Multi drug resistant tuberculosis (MDRTB) patients at diagnosis and during chemotherapy. Twenty-four (24) patients MDRTB patients and 24 newly diagnosed DSTB patients from University College Hospital (UCH) Ibadan, Nigeria were recruited for the study by a Consultant Chest physician using clinical history, Chest X-ray and GENE Xpert test. Five (5) milliliters of blood was drawn from the anti-cubital fossa vein into lithium heparin tubes before the commencement of chemotherapy, 2 months, 4 months and 6 months of anti-TB therapy. Plasma obtained was analyzed for C-RP and fibrinogen using ELISA. Data was presented as mean ± SD. Student t-test was used for mean comparison. Statistical significance was set at $p \leq 0.05$. Mean CRP level in multi-drug resistant tuberculosis patients was significantly higher at diagnosis compared with controls. Mean fibrinogen levels in the drug sensitive and multi drug resistant tuberculosis were significantly lower at diagnosis compared to controls. The mean CRP and fibrinogen levels in multi-drug resistant tuberculosis were significantly reduced during chemotherapy compared with before commencement of chemotherapy. Plasma CRP and fibrinogen are suggested as possible markers for monitoring treatment response in MDRTB patients.

Keywords; Tuberculosis, Treatment monitoring, Acute phase proteins, drug resistant.

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INTRODUCTION

Tuberculosis (TB) is a lethal infectious disease caused by various strains of mycobacteria, especially Mycobaterium tuberculosis (Shaikh *et al* 2012). In Nigeria, the number of new TB cases rose from 9 million in 2013 to 9.6 million in 2014 with the number of TB associated deaths in 2013 and 2014 remaining constant at 1.5 million (WHO 2014).

Despite the widespread prevalence of TB and the popularity of Directly Observed Short course (DOTS) chemotherapy against TB, there exists no efficient method to successfully monitor the efficacy of anti-tubercular treatment. A total of 36 million TB patients were successfully treated in DOTS programs, and up to 6 million deaths were averted. The treatment success rate (~86%) achieved in DOTS cohorts worldwide exceeded the global target of 85% in 2007 (WHO, 2014). But the progress in TB control and eradication has been threatened by the emergence of drug resistant strains of Mycobacterium tuberculosis.

Multidrug-resistant TB (MDRTB) strains of MRB are resistant to the two most potent anti-TB drugs, viz., Isoniazid and Rifampicin. Factors related to the development of drug resistance TB include the following: inadequate or inefficient administration of effective treatment; use of substandard drugs; inadequate or irregular drug supply; ignorance of health care workers in the treatment and control of TB; interruption of chemotherapy due to side effects; non-adherence of patients to the prescribed regimens; availability of anti-TB drugs without prescription; illiteracy; low socioeconomic status of patients; massive bacillary load; laboratory delays in identification and

susceptibility-testing of M. tuberculosis isolates; and the lack of the use of uniform laboratory methodology and quality control measures.(Paramasivan, 2005).

Diagnosis of pulmonary tuberculosis rely on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture of bodily fluids. Lack of reliable biomarkers to indicate or predict the different clinical outcomes of M. tuberculosis infection has been given as a key reason for the failure of developing new diagnostic and prognostic tools, drugs and vaccines against tuberculosis. Presently, only sputum culture is widely used to monitor response to the anti-tubercular treatment (WHO, 2003).

Cell-mediated immunity (Th1 immunity) is the major component of host defence against TB. This type of response involves the participation of resident alveolar macrophages, dendritic cells, T lymphocytes (TCD4+,TCD8+, Tγδ), and release of pro-inflammatory cytokines, interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-12, IL-18,tumour necrosis factor-α (TNF-α) and chemokines [(IL-8, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1α)]. These lead to the recruitment of additional cells to the infection site for the formation of granuloma that contains and kills tuberculosis bacilli, but also provide long-time niche needed for M. tuberculosis (Co *et al*., 2004; Day *et al*., 2010). This is an indication that inflammation is a common feature in tuberculosis patients.

C-reactive protein (CRP) is an acute-phase protein that serves as an early marker of inflammation or infection. During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours (Young *et al*., 1991). Fibrinogen is an acute-phase reactant that becomes elevated with tissue inflammation or tissue destruction, and it is also a vital part of the coagulation process (Holley *et al*, 1999). Since pulmonary tuberculosis is an inflammatory disease, the levels of fibrinogen and CRP may therefore be useful in determining the progress of TB and the efficacy of the anti-tubercular treatment.

In previous studies, significantly increased CRP levels were reported in pulmonary tuberculosis patients (Murphy *et al*, 2005; Akpotuzor *et al*, 2008; Van der Broek *et al*, 2008; Ojo *et al*, 2011). These finding are in contrary to the finding of Jeremiah *et al* (2013) who reported a low CRP value in pulmonary tuberculosis patients at diagnosis. Declining level (Awodu *et al*, 2007) and non-significantly different level (Aduku *et al*, 2014) of fibrinogen were found in pulmonary tuberculosis undergoing treatment. Based on these contrasting reports and to provide the prognostic values of CRP and fibrinogen in TB patients, the study determined the levels of CRP and fibrinogen in different severities (drug sensitive and drug resistant) of TB patients and in multi-drug resistant TB patients undergoing treatment. This is to find out the usefulness of these acute phase proteins in the differentiating the two groups of TB patients and to monitor treatment response in MDR-TB.

The objective of this study is to determine the plasma levels of C-RP and fibrinogen in patients with drug sensitive pulmonary tuberculosis (DSTB), multi-drug resistant pulmonary tuberculosis (MDRTB) at diagnosis and during 6months of chemotherapy compared with controls. The aim is to find out if plasma CRP and fibrinogen have diagnostic and prognostic values in TB patients.

MATERIALS AND METHODS

This is a longitudinal study carried out at the MDRTB unit and Medical Out Patient Clinic of the University College Hospital, Ibadan. Ethical approval was obtained from Joint Ethics Committee of University of Ibadan/University College Hospital, Ibadan, Nigerian. Patients were selected by Consultant Chest physician based on diagnosed as being infected with M. tuberculosis using clinical history, chest X-ray and GENE Xpert test. HIV positive patients, those with other severe illness and Patients with Extra pulmonary tuberculosis were excluded. 5ml of blood obtained from all participants was spun in lithium heparin to obtain plasma to analyse for C-RP and fibrinogen using AssayMax Human Enzyme-Linked Immunosorbent Assay kit. All the reagents were brought to room temperature before use. Fifty (50) µl of human CRP standard or sample was added per well which was covered with a sealing tape and incubated for 2 hours. The preparations were washed five times with 200 µl of wash buffer manually. The plate was inverted to decant the content. Fifty (50 µl) of biotinylated human antibody or 50 µl of streptavidin-peroxidase conjugate was seperately added to each well and incubated for 30 minutes each time. Chromogen substrate was added per well and incubated for 15 minutes before the reaction was stopped with stop solution. The absorbance was read on a microplate reader at a wavelength of 450 nm immediately. The concentration of CRP or fibrinogen was read from standard curves. The result was presented in mean and standard deviation (SD). Student t-test was used to compare the mean values. The p-value ≤ 0.05 was considered as statistically significant.

RESULTS

A total of 48 PTB patients (24 MDR-TB patients and 24 DSTB patients) and 24 healthy controls were recruited for this study. The mean age of the healthy control subjects 31.21 ± 4.34 years was not significantly different from that of DSTB patients (37.25 ± 16.12 years) and MDRTB (31.75 ± 8.55 years). Table 1 shows the means of plasma CRP and fibrinogen of the controls, DSTB, MDRTB patients at diagnosis and MDRTB at various periods of treatment.

CRP (C-reactive protein) : There was no difference in plasma CRP level of DSTB patients before commencement of chemotherapy (2.05±0.22 mg/dl) compared with controls (2.00±0.67mg/dl). Mean CRP level of MDRTB before commencement of chemotherapy (2.28±0.38mg/dl) was significantly increased compared with DSTB before commencement of chemotherapy (2.05±0.22mg/dl) and controls (2.00±0.67mg/dl). There were significant decreases in CRP levels of MDRTB patients at 2months of treatment (1.72±0.34mg/dl), 4months of treatment (1.53±0.21mg/dl) and 6months of treatment (1.46±0.21mg/dl) compared with level before commencement of chemotherapy (2.28±0.38mg/dl).

Table 1:
The Levels of Plasma CRP and Fibrinogen in Tuberculosis Patients Before Commencement of Chemotherapy and During Chemotherapy Compared with Control.

	Control	$DSTB^0$	MDR^0	MDR 2months	MDR 4months	MDR 6months
CRP (mg/dl)	**2.00±0.67**	**2.05±0.22**	**2.28±0.38**	**1.72±0.34**	**1.53±0.21**	**1.46±0.20**
p'		0.762	0.010*	0.074	0.002*	0.000*
p''			0.080			
P'''				0.000*	0.000*	0.000*
Fibrinogen (mg/dl)	**374.31±50.0**	**312.3±56.78**	**366.30±38.84**	**308.74±60.19**	**274.57±38.19**	**261.05±36.10**
P'		0.000*	0.538	0.000*	0.000*	0.000*
p''			0.000*			
P'''				0.000*	0.000*	0.000*

*Significantly different from control.
$DSTB^0$: Drug sensitive TB before commencement of chemotherapy.
MDR^0: Multi-drug resistant TB before commencement of chemotherapy.
P': Compared with control.; P'': Compared with $DSTB^0$. P''': Compared with MDR^0

The levels of CRP of MDRTB patients at 4 months of treatment (1.53±0.21mg/dl) and 6months of treatment (1.46±0.21mg/dl) were also significantly decreased compared with levels in the controls (2.00±0.67mg/dl).

Fibrinogen: Mean plasma fibrinogen level in DSTB before commencement of chemotherapy (312.3±56.78mg/dl) was significantly reduced when compared with control (374.31±50.0mg/dl). Fibrinogen levels of MDRTB at 2months of treatment (308.74±60.19mg/dl), 4months of treatment (274.57±38.19mg/dl) and 6 months of treatment (261.05±36.10mg/dl) were significantly reduced when compared with levels in MDRTB patients before commencement of chemotherapy (366.30±38.84mg/dl) or controls (374.31±50.0mg/dl).

DISCUSSION

C-reactive protein (CRP) is an established marker of acute inflammation and therefore, its blood level is assessed to determine the state of inflammation (Shaikh *et al*., 2012). Fibrinogen takes part in acute phase response after tissue damage and inflammatory reactions thus contributes to the rise in erythrocyte sedimentation rate (Holley *et al*, 1999). The levels of fibrinogen and CRP are therefore, expected to be change in pulmonary tuberculosis.

In this present study, the mean plasma CRP level in multi-drug resistant tuberculosis was significantly increased before commencement of chemotherapy compared with the level in DSTB patients before commencement of chemotherapy and control. This finding is similar to a previous study by Shaikh *et al* (2012). Breen *et al* (2008) found that an elevated CRP in 85% of proven tuberculosis cases in London while Chalmers *et al* (2008*)* also showed elevated CRP in patients with TB as high as 44 mg/L. It is therefore postulated that the magnitude of the increase of plasma CRP concentration reflects the extent of the tissue injury and may determine the course of the illness. The level of C-reactive protein is an indication of pathology and its decrease may be concomitant with effectiveness of drugs used in treatment. This explains significant decreases in CRP levels of MDRTB patients at 2months of treatment, 4months of treatment and 6months of treatment compared with level before commencement of chemotherapy. It has been earlier observed that plasma CRP concentration is a useful marker of treatment and pointer of potential defaulters in drug treatment (Pepys & Hirschfield, 2003).

The mean fibrinogen levels in this present study were found to be significantly lower in DSTB patients as well the multi-drug resistant tuberculosis patients before commencement of chemotherapy compared to control. The result of the study was at variance with a Ghanaian study in which there was no statistically significant difference in mean fibrinogen concentration of newly diagnosed treatment naïve pulmonary tuberculosis patients compared to that of patients who had become acid fast bacilli negative after anti-tuberculosis therapy (Aduku *et al*, 2014).

Fibrinogen levels of MDRTB patients at 2months of treatment, 4months of treatment and 6 months of treatment were significantly reduced when compared with levels in MDRTB patients before commencement of chemotherapy or controls. Our finding agrees with an earlier report from Benin City, Nigeria among pulmonary tuberculosis patients undergoing therapy which revealed a decline in fibrinogen levels with therapy (Awodu *et al*, 2007). Observation of high fibrinogen levels in TB patients is also in agreement with findings of a previous studies (Mosesson, 2005).

Fibrinogen is a blood-clotting factor therefore elevated levels of fibrinogen would be associated with an increased risk of heart attack. CRP has also been linked with the risk of coronary disease. Thus reductions of CRP and fibrinogen in MDRTB patients on treatment are indications that heart diseases might not be ongoing in these patients.

In conclusion, increased plasma CRP concentrations in MDR-TB patients before commencement of chemotherapy compared with DSTB patients before commencement of chemotherapy or control is indicative of the usefulness of CRP in assessing severity of pulmonary tuberculosis. Furthermore, changes in plasma CRP and fibrinogen in MDR-TB patients on anti –tuberculosis chemotherapy depicts a possible prognostic value of CRP and fibrinogen in monitoring response to chemotherapy of MDR-TB patients.

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