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Plasma Adenosine Deaminase Enzyme Reduces with Treatment of Pulmonary Tuberculosis in Nigerian Patients: Indication for Diagnosis and Treatment Monitoring

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Summary: Tuberculosis(TB)-specific host biomarkers for diagnosis and monitoring of treatment response have been identified as priorities for TB research. Macrophage and T cell lymphocytes play vital roles in *Mycobacterium tuberculosis* immune response and their associated biomarkers could form good candidates for diagnosis and treatment monitoring. The enzyme adenosine deaminase (ADA) is produced mainly by monocytes and macrophages and increase in biological fluids in the course of infection with microorganisms infecting macrophages. This study comprised sixty-eight (68) participants; twenty-four (24) multi-drug-resistant TB(MDR-TB) patients, twenty-four (24) drug-sensitive TB patients(DS-TB) and twenty (20) non-TB apparently healthy individuals. Five (5) milliliters of blood was drawn before commencement of chemotherapy and 6 anti-TB therapy. In DSTB and MDR-TB patients before commencement of chemotherapy and 6 months of anti-TB treatment, the mean plasma levels of ADA were significantly increased compared with control. At 6 months of anti-TB chemotherapy of DSTB or MDR TB patients, ADA level was significantly decreased compared with before chemotherapy. Plasma ADA in DSTB patients before and 6 months of chemotherapy were not significantly different compared MDR TB patients. Plasma ADA level is a promising biomarker for the screening and treatment monitoring of pulmonary tuberculosis but not to differentiate MDR TB from DSTB patients.

Keywords*:* Tuberculosis Patients, Biomarkers, Chemotherapy, Lymphocytes.

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INTRODUCTION

Tuberculosis (TB) is a major global health challenge. It causes ill-health among millions of people each year and ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide (WHO, 2015). Recent estimates indicate an incidence of 9.6 million new TB cases with 1.5 million deaths annually (WHO, 2015). In addition, the global emergence of multidrug-resistant TB, extensively drug-resistant TB, and more recently, totally drug-resistant TB present a formidable challenge to TB control especially in sub-Saharan Africa, Asia and Eastern Europe (Alexander and De, 2007).

Timely diagnosis and proper treatment of TB have been identified as essential factors for successful TB control. It is estimated that availability of a widely used rapid diagnostic test for TB could avert 625,000 TB deaths annually (Keeler et al, 2006). Also, studies have demonstrated delays in TB diagnosis due to drawbacks of the presently available diagnostic tools (Storla et al, 2008; WHO, 2006). Mycobacterium culture that is the gold standard for TB diagnosis takes eight weeks before result is available. Sputum smear microscopy, a quick screening method is not a sensitive method while polymerase chain reaction (PCR) test is expensive, requires sophisticated equipment and cannot be used for monitoring treatment response (Adekambi et al, 2015). Hence, there is need for more biomarkers to monitor treatment and diagnosis of TB.

Adenosine deaminase (ADA) is an enzyme of the purine metabolic pathway (Shore, 1981). Itcatalyses the irreversible conversion of adenosine and 2' deoxyadenosine to inosine and 2' deoxyinosine respectively (Piras et al, 1978). ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and is essential in the maturation of monocytes to macrophages. High concentration of adenosine or deoxyadenosine as a result of non-conversion to inosine or deoxyinosine is toxic to lymphocytes and macrophages (Zavialov et al, 2010). Also, ADA deficiency has a direct effect on the lungs, aslung damage and inflammation have been associated with elevated adenosine and deoxyadenosine in lungs of ADA-Severe Combined Immuno-Deficient patients (Blackburn et al, 1998). Both adenosine and 2' deoxy

adenosine have potent physiological effects on cells. Adenosine elicits its actions on cells by engaging G proteins coupled with receptors on the cell surface (Olah and Stiles, 1995) while 2' deoxyadenosine has been associated with disruption of cell growth and development and influence apoptosis (Liu et al, 1996). These pathways have been identified to play important roles in many aspects of lung inflammation and damage (Jacobson and Bai, 1997).

Given the roles T cells and macrophages play in protection against *Mycobacteriam tuberculosis* (Mtb) infection, ADA levels may be reflective of shifts in protection against Mtb infection and treatment response.Data regarding ADA levels in PTB concentrated in pleural, peritoneal and pericardial fluids has been documented (Cimen et al, 2008; Greco et al, 2003). Moreover, few reports regarding blood levels of ADA in PTB are inconsistent, was not determined in treatment follow-up and did not classify TB into DSTB and MDR TB (Boonyagars and Kiertiburanakul 2010; Afrasiabian et al, 2013)..This study determined plasma ADA levels in DSTB and MDR TB pulmonary TB patients before and at 6 months of anti-TB chemotherapy compared with non-TB controls.

MATERIALS AND METHODS

Study participants

Sixty eight (68) participants were recruited for this study which comprised of twenty four (24) MDR-TB patients, twenty four (24) drug-sensitive TB patients and twenty (20) non-TB apparently healthy individuals after obtaining written informed consent. MDR-TB patients had been previously diagnosed as being infected with isoniazid and rifampicin resistant strains of Mtb using clinical history, chest Xray and GENE Xpert test and were admitted into the MDR TB centre, University College Hospital (UCH) Ibadan, Nigeria for treatment. DS-TB patients were recruited from the Medical Out-patient clinic, University College Hospital, Ibadan, Nigeria by a consultant Chest Physician after Zeihl Neelsen staining technique, Sputum culture, chest X-ray and clinical history. The study protocol was reviewed and approved by the University of Ibadan/University College Hospital Institutional Research Ethics Committee.

Five (5) milliliters of blood was drawn from the anti-cubital fossa vein into lithium heparin tubes before commencement of chemotherapy and after 6 months of anti-TB therapy. Blood samples were centrifuged and plasma obtained were analyzed.

PTB Treatment protocol

All bacteriologically confirmed MDR-TB patients received intensive phase for 6-8 months in the hospital followed by 12 months of continuation phase in the community based on World Health Organization (WHO) updated guidelines in 2011 (WHO, 2011). Standardized treatment regimen was used including five drugs: kanamycin/Amikacin, Levofloxacin, Prothionamide, Cycloserine, Pyrazinamide (with Pyridoxine). This present study was conducted during the intensive phase of treatment.

Sputum smear positive DSTB patients received DOTS intensive phase for 2 months and 4 months continuation in the hospital based on WHO updated guidelines in 2011 (WHO, 2011). Standardized treatment regimen with fixed drugs containing; Rifampicin, Isoniazid, Pyrazinamide and Ethambuthol during intensive phase, and Rifampicin and Isoniazid in continuation phase, were used.

Biochemical analysis

Enzyme-linked immunosorbent assay (ELISA) was used for the measurement of adenosine deaminase (Human ADA; Lot: AK0016MAR21058, Elabscience, China). Assay protocol was as specified by the manufacturer and the absorbance was measured at 450nm with an ELISA reader (SpectraMax Plus 384, Molecular Devices LLC, USA).

Statistical analysis

Data obtained were analyzed using statistical package for social sciences (SPSS) version 17.0. Independent Student t-test was used to compare the mean values of PTB patients and controls while paired t-test was used to compare the mean values of PTB patients before commencement of chemotherapy and 6 months of anti-TB chemotherapy. Values were considered significant at $p<0.05$.

RESULTS

In DSTB and MDR TB patients before and at 6 months of anti-TB treatment, the mean plasma levels of ADA were significantly increased when compared with control. (Table 1)

At 6 months of anti-TB chemotherapy of DSTB or MDR TB patients, mean ADA level was significantly decreased when compared to the mean value before

Table 1. Mean comparison of ADA levels in PTB patients with controls

Group	ADA (ng/ml)	t	p
Control	28.09±6.15		
DSTB 0	79.38±10.98	16.508	0.000*
DSTB 6 months	37.56± 6.84	4.222	0.000*
MDR 0	72.04 ± 17.64	9.453	0.000*
MDR 6 months	38.23±6.47	4.666	0.000*

*Significant at $p<0.05$ compared with control

DSTB 0 = Before commencement of chemotherapy in drug sensitive TB, DSTB 6 months= 6 months of chemotherapy in drug sensitive TB, MDR 0 = Before commencement of chemotherapy in multidrug resistant TB, MDR 6 = 6 months of chemotherapy in multidrug resistant TB

Table 2. Mean comparison of ADA level in DSTB and MDR TB patients before and at 6 months of anti-TB chemotherapy

Group	DSTB	MDR	t	p
0 months	79.38±10.98	72.04 ± 17.64	1.498	0.143
6 months	37.56± 6.84	38.23±6.47	-0.300	0.766
t'	14.080	8.543		
p'	0.000*	0.000*		

t,p – DSTB compared with MDR-TB, t',p' – 0 months compared with 6 months

chemotherapy in DSTB or MDR TB patients respectively. But the level of ADA in DSTB patients at 6 months of chemotherapy was not significantly different compared with ADA level of MDR TB patients at 6 months of chemotherapy. (Table 2)

DISCUSSION

TB-specific host biomarkers for diagnosis of active TB and monitoring of treatment response have been identified as priorities for TB research (Wallis et al, 2009). These biomarkers are being explored to reduce disease misdiagnosis, ensure proper prognostication, monitor treatment response, and provide markers for evaluating efficacy of newly developed therapeutic drugs and vaccines (Rozot et al, 2015; Bloom et al, 2013). Macrophages and T cells play important roles in the formation of lung granulomas and eventual cavity formation, which are key features of TB immunopathology in active lesion (Ernst, 2012). ADA is produced mainly by monocytes and macrophages and is increased in biological fluids in the course of infection with microorganisms infecting macrophages (Boonyagars and Kiertiburanakul, 2010).

This present study found increased plasma ADA in both DSTB and MDR TB patients when compared with controls. This shows that ADA level is useful in differentiating Mtb infected patients from uninfected controls. Our finding is supported by previous studies that reported increased ADA in serum of pulmonary TBpatients (Afrasiabian et al, 2013; Srinivasa Rao et al, 2010; Cimen et al, 2008). Our previous study shows slight increases in total white blood cells count, percent leucocyte migration and percent nitroblue tetrazolium index in TB patients at diagnosis compared with controls (Edem and Arinola, 2015). Increased plasma ADA in PTB patients compared to controls in this present study might be due to activation, proliferation and differentiation of monocytes to macrophages which presents Mtb antigen toCD4+ T cells. Full functionality of Cell Mediated Immunity has been associated with normal lymphocyte metabolism regulated partially by the purine salvage enzyme such as ADA (Giblette et al, 1972). Studies have also shown that monocytes undergoing differentiation and macrophages continuously secrete ADA which induces proliferation of CD 4+ T cells (Zavialov et al, 2010). This is therefore indicative of continuous activation of CMI in TB patients, thus supporting clinical usefulness of plasma ADA as a biomarker for PTB diagnosis. Other studies have demonstrated increased ADA in effusion fluids in extra-pulmonary tuberculosis (Mathur et al, 2006; Zaric et al, 2007 and Gupta et al, 2010;).

At 6 months of anti-TB chemotherapy, plasma ADA level decreased significantly in TB patients compared with ADA levels before commencement of chemotherapy. At 6 months of chemotherapy when plasma ADA levels were reduced compared with before chemotherapy, sputum smear microscopy showed that the patients were sputum smear negative for Mtb.This finding is similar to the report of Swami (2016) who reported decrease in pleural fluid ADA following anti-TB treatment. Our previous report showed slight decreases in white blood cell counts, percent leucocyte migration and percent nitroblue tetrazolium index in TB patients at 6 months of anti-TB (Edem and Arinola, 2015). Thus, plasma ADA level may be a useful biomarker to monitor treatment in either DSTB or MDR TB patients. Sputum smear microscopy is currently used to monitor treatment response in TB patients on anti-TB chemotherapy. However, there is need for blood biomarkers of treatment response since sputum production becomes increasingly difficult to obtain during treatment in TB. Reduced ADA level at 6 months of chemotherapy might be explained by reduced Mtb specific CMI (reduced Mtb specific monocyte and T cell activation) as a result of reduced Mtb antigen or other regulatory immune factors. A previous study reported a shift in CMI response during anti-TB chemotherapy (Cardoso et al, 2002) which was attributed to sequestration of Mtb-specific T cells at the site of disease leading to reduced frequency in peripheral blood, the release of anti-inflammatory cytokines by PBMCs and depression of T-cell responsiveness (Wilkinson et al, 1988).

This present study also observed similar levels of plasma ADA in multi-drug resistant TB patients when compared with drug-sensitive TB patients at diagnosis and at 6 months of chemotherapy. This implies that plasma ADA level may not be useful in distinguishing drug sensitive from drug resistant TB patients and indicating that there may be no difference in the nature of CMI response in DSTB and MDR TB patients. Basile et al (2011) however reported that MDR-Mtb strains induce stronger IL-17 than drug susceptible strains in vitro and MDR TB patients showed high IL-17 expression. This might be explained by the fact that ADA is not involved in all aspects of lymphocyte activities. Plasma ADA after 6 months of treatment of both DSTB patients and MDR TB patients were significantly higher than control values. This indicates

that at 6 months of anti-TB chemotherapy all physiological effects of Mtb infection are not completely repressed. Thus studies with longer follow-up period using larger sample size are required to suggest which duration post TB-chemotherapy reflects complete reversal of physiological effects of Mtb infection.

In conclusion, plasma ADA level is a promising biomarker for the screening and treatment monitoring of pulmonary TB but not to differentiate MDR TB from DSTB patients.

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