Research Article

Total Antioxidant Capacity (TAC) in Patients with Haematological Malignancies in Niger Delta-region of Nigeria

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Abstract

Introduction: Haematological malignancies (HM) are malignant disorders involving the haemopoietic system. Oxidative stress has been implicated in the development and evolution of these disorders. To measure total antioxidant capacity (TAC) in patients with HM and to determine if any, the relationship between TAC and total lipid in these patients.

Presentation of the case: This is a multicentre cross sectional study. Patients were sampled with self administered questionnaire that documented their biodata and the various types of haematological malignancies they had. Blood samples from 31 patients with HM and 11 controls were assessed for TAC and total lipids using RelAssay and TECO diagnostic kits respectively. Difference between means was compared using student T-test. The relationship between TAC and TL was measured using Pearson's correlation coefficient.

Total antioxidant capacity was significantly lower in patients with HM. The mean total lipid concentrations of the cancer patients were higher than in the controls but this was not statistically significant ($p > 0.05$). A negative correlation was found between TAC and total lipids in patients with HM.

Conclusion: This study has further affirmed that patients with HM have a significantly lower antioxidant activity. However, further investigations are required to fully elucidate the mechanism and clinical implications of reduced TAC in these patients.

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Introduction

Haematological malignancies (HM) are cancers of the blood, bone marrow and lymphatic tissues. They include leukaemias (lymphoid and myeloid), lymphomas (non Hodgkins and Hodgkin's) and plasma cell neoplasm.

Oxidative stress is an imbalance in the redox potential of the body in favour of oxidizing reactions over antioxidant activities as a result of increased production of reactive oxidizing species (ROS) and depletion of neutralizing antioxidant. When the body is unable to control this stress, disorders develop.[1] Oxidative stress has been implicated in the development and evolution of HM.[2,3] The ROS may act as initiators and or promoters of carcinogenesis through their mutagenic effect on the DNA.[4-6] Furthermore, cancer chemotherapeutic agents used in the management of HM also generate ROS some of which may in addition to destroying the cancer cells have lethal effects of normal host cells.[1] The body antioxidant mechanisms are responsible for the neutralization of ROS. It comprise of both enzymatic and non enzymatic components. The non enzymatic components include exogenous vitamins such as vitamins A, C, E; reduced glutathione (GSH) and other endogenous proteins. Enzymatic component include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).

Several studies on oxidative stress in patients with haematological malignancies have focused on specific components of the antioxidant system and their findings have not been consistent.[7-10] A study on the total antioxidant capacity (TAC) which is a measure of the sum of endogenous and food derived antioxidant capacity measurable with various methods[11,12] may be a better measure of oxidative stress in haematological malignancies. There is paucity of studies on total antioxidant capacity in patients with HM especially in Nigeria. We hypothesized that patients with HM have reduced TAC. The objective of this study therefore is to measure the TAC in patients with HM and to determine if there is any the relationship between TAC and plasma total lipids (TL).

Patients and Methods

Study Design: This is a multicentre cross sectional studies conducted in two areas with a tertiary health facility namely University of Benin Teaching Hospital (UBTH), Benin City with a 700 bed-space capacity and Delta state University Teaching Hospital (DELSUTH), Oghara in Nigeria. The total catchment's population of the hospital used is 15.3 million and are major referral centre serving the South South geopolitical zone of Nigeria. Controls recruited from the study areas comprise non-cancer healthy volunteers who were seronegative for HIV 1 and 2, hepatitis B surface antigen and hepatitis C virus. Patients were sampled with self administered questionnaire that documented their age, gender and the````````` various types of haematological malignancies they had. The cytomorphological diagnostic techniques recommended by the various Cancer Institutes' including blood smears, bone marrow studies, lymph node histology and immunohistochemistry were applied in the diagnosis of HM patients in this study. The patients were placed on the appropriate anti-neoplastic agents for the various HM. All the participants gave informed consent after the study was approved by the hospital ethical committee.

Laboratory Assay Methods

Sample Collection: Ten milliliters of venous blood was aseptically obtained from each participant after 12-14 hours of fasting. In this

way, the possible influence of dietary factors on the level of free radicals was avoided. The samples were dispensed into specimen bottles without anticoagulants and were allowed to clot and retract. The blood was thereafter centrifuged for 10-15 minutes at 3000rpm and the clear sera harvested with clean Pasteur pipettes, put in sera bottles and stored at -20^{0}C until analyzed.

TAC Assay Method: TAC was measured with TAC assay kit manufactured by RelAssay Diagnostics (Turkey) Lot number RL020. The antioxidants in the sample reduce dark blue green coloured radicals to colourless forms. The change in absorbance at 660nm is related to the TAC of the sample in Trolox Equivalent/L.

Total Lipids Assay: Total lipids were assayed using a laboratory total lipid kit manufactured by TECO Diagnostics (Anaheim, USA). The sulfo-phospho-vanillin (SPV) colorimetric method for the determination of total lipids described by Charbol et al[13] and modified by various investigators[14,15] was used. The assay was quality controlled using controls supplied alongside.

Statistical Analysis: The means and standard errors of mean (SEM) of TAC and total lipids for both patients and controls were computed using the statistical Package for Social Sciences (SPSS) version 16. T- test was used to calculate the difference in means of both groups. The one way analysis of variance (ANOVA) test was used to compare the difference in means between the various HM with more than one participant. The relationship between TAC and TL was determined using Pearson's correlation test. P-values < 0.05 was considered significant.

Results

A total of 31 patients with hematological cancers were seen during the study period (July to August, 2012). This comprised of 11 males (35.5%) and 20 females (64.5%) with a male-to-female ratio of 0.6:1. These were compared with 10 non-cancer apparently healthy subjects (8 males and 2 females) who were prescreened and found without any history of clinical disorder. The mean age of the patients group was 57.1±12.8 years with a median age of 59 years (range, 30-80years) while the mean age for the control was 31.8±4.3 years with a median age of 32.5years (range, 26-39 years)

The study population of 31 patients with haematological malignancies comprised of 3 (9.7%) acute leukaemias (2 acute lymphoblastic and 1 acute myeloblastic leukaemias), 9 (29.0%) chronic lymphocytic leukaemias, 8 (25.8%) lymphomas (7 non Hodgkin's lymphoma and 1 Hodgkin lymphoma), 7 (22.6%) multiple myeloma and 4 (12.9%) myeloproliferative diseases (2 chronic myeloid leukaemias, 1 Polycythaemia Rubra Vera and 1 essential thrombocythaemia). Chronic leukaemias and Lymphomas were the common haematological cancers seen during the study period.

The mean and standard error of mean of total antioxidant capacity for the merged cancer patients and the controls were 3.8±0.3 and 5.2±0.3 Trolox Equivalent/L respectively as revealed in table 1. This shows that the mean TAC of the patients were significantly lower than the mean level observed in controls (p<0.05). The mean total lipid concentrations of the cancer patients were higher than in the controls but this was not statistically significant (p>0.05). The analysis of variation (ANOVA) test did not show any significant difference in the means between the various haematological malignancies. Table 2, shows the descriptive statistics of patients with various haematological malignancies. A negative correlation however exist between TAC and total lipids in the patients group (r=0.48 and p<0.01) as shown in table 3.

Table 1 the mean standard error of mean of total antioxidant capacity and total lipids in both patients and control

Parameters	Patients	Control	P Value
TAC	3.8 ±0.3	5.2 ±0.3	0.005
TLC	584.6±29.0	564.4±52.0	ns

TAC-total antioxidant capacity; TLC-total lipids concentration; ns-not significant
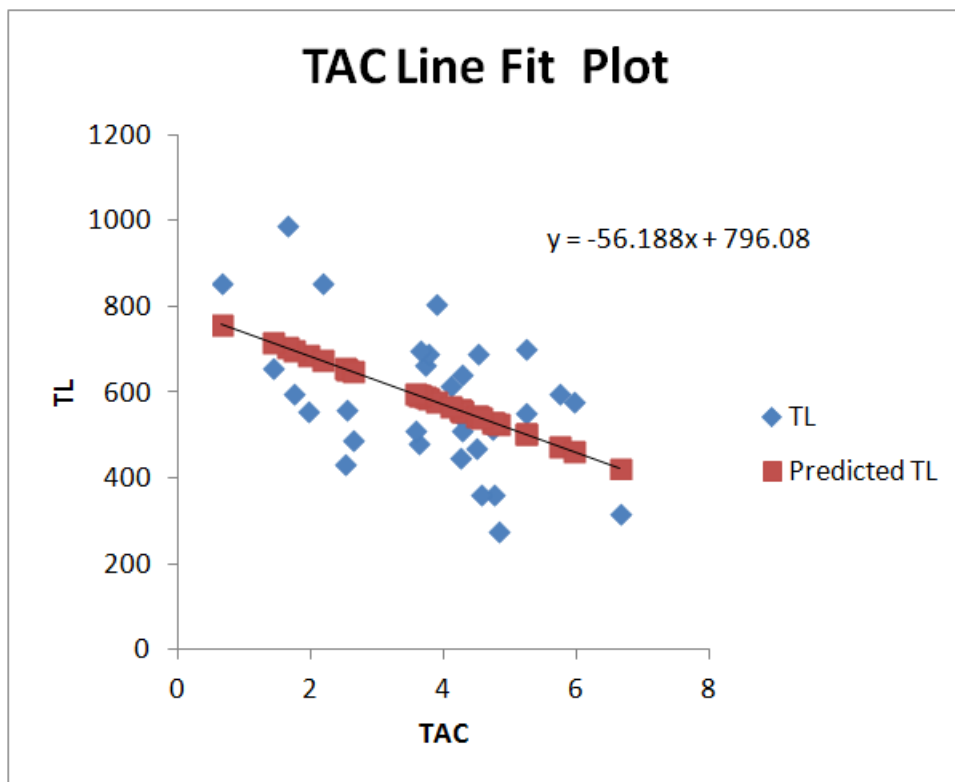
Figure 1 Correlation between TAC and total lipids in patients with hematological malignancies

Table 2 Descriptive Statistics of the various hematological malignancies

HAEMATOLOGICAL MALIGNANCIES		TAC	TL
	N	Mean ±SEM	Mean ±SEM
ACUTE LEUKAEMIA	3	3.8 ±1.1	616.9 ±40.1
CLL	9	3.7 ±0.6	622.5 ±70.3
NHL	7	3.7 ±0.5	600.6 ±75.8
MYELOMA	7	3.8 ±0.5	545.3 ±27.8
MPD	4	3.7 ±0.8	570.4 ±74.3
HD	1	4.7 ±0.0	365.1 ±0.0
TOTAL	31	3.8 ±0.3	584.6 ±29.0

Table 3 the correlation between TAC and total lipids in patients group

		TAC	TOTAL LIPIDS
TAC	Pearson Correlation (r)	1	-.496**
	Sig. (2-tailed)		.005
	N	31	31
TOTAL LIPIDS	Pearson Correlation (r)	-.496**	1
	Sig. (2-tailed)	.005	
	N	31	31

Discussion

There are limited studies on total antioxidant capacity (TAC) in patients with haematological malignancies (HM) in our environment. Several studies have evaluated enzymatic antioxidant activities in patients with HM and their findings are not consistent. Devi et al [7]reported increased superoxide dismutase (SOD) and GPX in patients with HM but Sonali et al[8] reported the reverse.

Oltra et al[3] reported a lower SOD and catalase (CAT) in patients with CLL. Similarly reduced SOD and CAT have been documented in patients with ALL.[9] These inconsistent findings may suggest that TAC may be a better assay in measuring antioxidant status. Variation in enzymatic activities may be attributable to the redox buffering potential of non enzymatic antioxidant component such as GSH, responsible for the maintenance of the overall redox balance in cells.[16]

Papageorgiou et al[17] in a study on TAC in children with HM reported no significant difference in the TAC in patients and control groups prior to commencement of chemotherapy. However, on commencement of chemotherapy, there was a progressive decline in TAC in patient group. The decrease in TAC was attributed to the oxidant stress of chemotherapy. Kuku et al[18] reported a significant decline in enzyme antioxidant capacity of patients with multiple myeloma undergoing chemotherapy.

In this study, we observed a significantly lower TAC in our patients' compared to controls. Though there is a disparity in age between our patient and control groups, it has been shown that TAC does not differ significantly with age.[19, 20] Chrzczanowicz et al[19] in a study of 422 males' with an age range of 19.2 – 89 years demonstrate that TAC does not differ significantly with age. The significant difference in the TAC of both study groups may be attributable to the oxidative stress of chemotherapy and low intake of exogenous antioxidants. The tumoricidal action of anti-cancer drugs has been suggested to be mediated through a free-radical dependent mechanism. Kelkel et al[21] in a review on antioxidants documented that some dietary antioxidants have the potential of preventing and treating haematological malignancies. However, more randomized studies may be required to validate this report.

Haematological malignancies as well as other cancers have been associated with abnormal lipid metabolism.[22-24] Several of these indicate that patients' with malignancies tend to have hypocholesterolaemia and hypertriglyceridaemia. There are dearth of studies on the correlation between TAC and lipids. Though in our study there is no significant difference in the total lipids between the patient and control groups, we found a negative correlation between TAC and total lipids. However there may be need for further studies to establish the relationship between the various lipid fractions and TAC.

In conclusion, patients with HM have significantly low TAC than controls and there is a negative correlation between TAC and total lipids in these patients. Further investigation is required in order to fully determine the correlation between TAC and the various lipid fractions.

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