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Total Antioxidant Capacity (Tac) in Hypertensive Patients

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Abstract: Hypertension has been an age long ailment that has affected the entire population of the world with its many complications. This study investigated the relationship between hypertension and TAC in different age groups. Samples were collected from Bomadi general hospital in Bomadi Local Government Area of Delta State, Nigeria and the project was also conducted there. A total of 40 consenting subject between the age of 45-65 years were randomly selected for the study of which 20 were control subjects (non-hypertensive individuals). The age groups 40-49 years in hypertensive patients and 60-69 years in normotensive individuals indicate statistical significance when compared to their corresponding age group (0.9±0.25mMol/L and 1.68±0.32Mmol/L, respectively). In hypertensive subject, male shows a statistically insignificant ($p \leq 0.05$) mean higher value than female (1.14±0.18Mmol/L) and 1.01±0.21Mmol/l, respectively) while in normotensive subjects, female shows a statistically significant ($p \leq 0.05$) mean lower value than male (1.66±0.35mMol and 1.77±0.06, respectively). Total antioxidant capacity is lower in hypertensive patients and as a result these individuals are predisposed to ills associated with reduced TAC. This includes cell damage by free radicals.

Keywords: Antioxidant capacity, hypertensive patients, normotensive

INTRODUCTION

The term antioxidant originally was used to refer specially of oxygen, extensive study was devoted to the uses of antioxidant in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber and the polymerization of fuel in fueling of internal combustion engines (Kelly, 1998).

However, it was the identification of vitamins A, C and E as antioxidant that revolutionalized the field and lead to the realization of the importance of antioxidant in the biochemistry of living organisms.

Although oxidation reactions are crucial for life, they can be damaging, hence plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, vitamin E, as well as enzymes such as catalase and SuperOxide Dismutase (SOD) (Burton and Ingold, 1981).

Antioxidadants may be synthesized in the body or obtained from the diets. The different antioxidants are present at a wide range of concentration in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, which others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and those compounds can be important in pathogens and can be virulent factors (Rice-Evans and Miller, 1994). The action of one antioxidant may therefore, depend on the proper function of other members of the antioxidant system.

Hypertension or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. It is the opposite of hypotension. Hypertension is classified as either primary (essential) or secondary. About 90-95% of cases are termed “primary hypertension” for no medical cause which refers to high blood pressure can be found.

The remaining 5-10% of cases (secondary hypertension) is caused by other conditions that affect the kidneys, arteries, heart, or endocrine system. Persistent hypertension is one of the risk factors for stroke, myocardial infarction, heart failure and arterial aneurysm and is leading cause of chronic kidney failure, moderate elevation of arterial blood pressure leads to shortened life expectancy. Dietary and lifestyle changes can improve blood pressure control and decrease the risk of associated health complications, although drug treatment may prove necessary in patients for whom lifestyles changes prove ineffective or insufficient (Carretero and Oparil, 2000).

Antioxidants protect the body against the destructive effect of free radicals. Antioxidant neutralizes free radicals by donating one of their own electrons, thereby rending the electron stealing reactions, known as chain reaction. Antioxidant

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themselves do not become free radicals by donating an electron because they are stable in either form.

Antioxidants help to maintain the concentration of free radicals at an optimum level, thereby preventing oxidative stress. In addition, antioxidants play a key role in these defense mechanisms (Sies, 1993).

MATERIALS AND METHODOLOOGY

Equipments:
Centrifuge (C56C clinical,Vulcan technology USA)
Digital photo-calorimeter (SNO 2040 3219, surgefriend medicals,England)
Refrigerator (EHT-173K, whirl pool,USA)
Incubator (DNP-9022)
Calculator (YH-2000, PorPo, china)
Bio-tek plate reader (ELx800, cayman chemicals,USA)plate.
Bio-tek Reagents and Chemicals
Trolox equivalent total antioxidant asay kit supplied by layman chemicals USA
Antioxidant assay buffer = 5um
Antioxidant assay h_20_2= 4um
Antioxidant assay trolox = 0.5mm
Antioxidant assay chromogen
Antioxidant assay metmyoglobin

Specimen: Blood sample

Sample collection and handling: Forty consenting apparently healthy individuals 20 hypertensive and 20 non-hypertensive) between the ages of 40-65 years were selected from individuals residing within Bomadi and its environs.

Blood sample Collections: 2 mL of blood sample was collected from each consenting subject into an EDTA anticoagulant container using the vein puncture technique .The whole blood was centrifuged at 1200 rpm for 5-min at room temperature at (29°C-31°C) to separate the plasma which was decanted into the bijou bottle for analysis. The total antioxidant capacity was estimated using the trolox equipment total antioxidant assay supplied by cayman chemicals USA.

Statistics: The unpaired student "t-test" was used to analyze the data obtained and the level of significance was set at p = 5 %($\propto$ = 0.5).

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY (CAYMAN METHOD)

Principle of reaction: The caymanantioxidant assay is used to measure the total antioxidant capacity of the serum. The oxidation process of 2, 2-Azino-di-3ethylbenzthiazoline sulphonate to $ABTS^+$ by metmyoglobin was inhibited by the presence of antioxidants in the serum. The amount oxidized $ABTS^+$ is measured at 750 nm and compared to trolox: A hydrophilic tocopherol analogue is proportional to the concentration of the total antioxidant (mm) present in the Rice-Evans and Miller (1994).

Table 1: Results of Trolox standard prepared in seven test tubes A-G

Test-tube	Trolox (uL)	Assay Buffer (uL)	Final Concentratin (mm trolox)
A	-	1000	0
B	30	870	0.045
C	60	940	0.090
D	90	910	1.135
E	120	880	0.180
F	150	850	0.225
G	220	780	0.330

Procedure: Trolox standard was prepared in seven test tubes A-G as shown by the Table 1, Samples serum was diluted with assay buffer at 1:20.10uL of final concentration of trolox standard A-G was pipette into labeled A-G well-plates, respectively. Another 10uL of serum sample was also pipette into well-plates labeled S_1-$S_{40,}$ respectively. Again 10ul of metmyoglobin, 150uL of chromogen and 40uL hydrogen peroxide was also pipette to each well-plates A-G and S_1-S_{40}, respectively. Then coversheets and mixed thoroughly by shaking for 5 min.

Removed the cover sheets and read the absorbance of each mixture (A-G and S_1-S_{40}) at 750 nm using a plate reader and was recorded for calculation.

Calculation: A graph was plotted using the absorbance of trolox standards labeled A-G against final concentration A-G, respectively from the table above. The y-intercept and the slop of the graph was determined therefore, the y-intercept and slop derived from the graph was used to calculate the concentration of samples labeled S1-S_{40}

$$\text{Conc.ofTAC(mmol/L)} = \text{Absorbance of samples (S1-S40) - y- I ntercep /slope}$$

RESULTS

The total antioxidant capacity of the subjects was measured and the results obtained are presented in Table 2 to 3. Values are expressed as mean ±SD for "n"subject.

Normal range: TAC.0.5-2mmol/L As age group increase, TAC. Increase from 40-49 years to 50-59 years and decrease from 50-59 years to 60-69 years in both hypertensive subjects (hypertensive: 0.01±0.25, 1.75±0.24, 11.1±0.15 and normoten sive: 1.75±0.06, 1.87±0.08, 1.68±0.32 for 40-49, 50-59 and 60-69 years, respectively) with only 40-49 years showing statistical significance when compared with 60-69 years in normotensive subjects. Values are expressed as mean ±SD for "n" subjects.

Table 2: Age influence on the TAC of subjects

Age group (years)	Total antioxidant (mmol/L) Capacity			
	Hypertensive	n	Normotensive	n
40-49	0.91±0.25	6	1.75±0.06	10
50-59	1.75±0.24	10	1.87±0.08	8
60-69	1.11±0.15	4	1.68±0.32	2

Table 3: The effect of gender on TAC of hypertensive and normotensive subjects

Gender	TAC (mmol/L) Hypertensive	n	Normotensive	n
Male	1.14±0.18	12	1.77±0.08	11
Female	1.01±0.21	8	1.66±0.35	9
Total	1.09±0.19	20	1.81±0.24	20

DISCUSSION

Hypertensive has been recently seen as a major health problem affecting both the developed, developing and most under-developed countries (Krouf *et al*., 2003). It was hypothesized that high blood pressure which is the clinical manifestation of hypertensive, is associated with loss of balance between per oxidation and various antioxidant factors which are reactive oxygen species (Krouf *et al*., 2003). It was hypothesized that high blood pressure which is the clinical manifestation of hypertension, is associated with loss of balance between per oxidation and various antioxidant factors which are reactive oxygen and species (Krouf *et al.*, 2003).

In this present study, age was shown to influence the Total Antioxidant Capacity (TAC) as TAC increased also age increases but at a much older age, TAC decreased (Table 2) although hypertensive subjects shown mean values lower.

TAC when compared with the normotensive subject ($p<0.05$) which is statically significant.

In 40-49 years, hypertensive subject showed a lower mean TAC than normotensive (0.91±0.25 and 1.75±0.06) but this mean difference is stastistically insignificant. In 50-59 years, normotensive subjects have mean valueshigher than hypertensive (1.87±0.08 and 1.75±0.06) but this mean difference is statistically significant ($p\leq0.05$). in 60-69 years, hypertensive subject showed values when compared to normotensive (1.11±o.15 and 1.68±0.32, respectively).

The result also showed that male gender have mean higher TAC with only normotensive male subjects being significant when compared to female subject. In overall, male and female hypertensive subject showed a statistically significant mean lower TAC when compared to normotensive subject as seen in Table 3.

A brief explanation for the obtained result may be due to reduction of overall body function and hormonal organ depletion as human grows older (Guyton and Hall, 2006).

This may account for the lower TAC in age 60-69 years in both normotensive and hypertensive patient. The increase in TAC in 50-59 years may not be far from the increase in thought and other emotional demand as one grow older leading to increases impulse and generation of free radicals (reactive oxygen species) leading to hypertension (Evans, 2008; Knight, 1998). It has been established that male leads activate lives than female and this may also lead to increase muscular activities and lipid peroxidation which will in turn lead to increase in TAC production (Lenaz, 2001) from the fore going, it has been established that free radicals production thus stimulate thus stimulate the production of antioxidants (Nelson and Cox, 2005).

Hypertension results from excessive generation of free radicals (Subash *et al*., 2010) and this account for the presence of adequate antioxidants (Alsaif, 2009). This therefore may accounts for statistically significant lower mean TAC observed in hypertensive subject.

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