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Full Length Research Paper

Low blood selenium: A probable factor in essential hypertension

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The possible association between selenium and essential hypertension was investigated in this study. Blood selenium (BSe) and plasma glutathione peroxidase (pIGSH-Px) activity were measured as biochemical markers of selenium status of 103 hypertensive patients (44 males and 59 females) and 88 apparently healthy subjects (40 males and 48 females). The hypertensive patients were classified into three groups based on the severity of the disease namely: mild (Group 1), moderate (Group 2) and severe (Group 3). The healthy and the hypertensive subjects were recruited from Abeokuta and Ibadan (South-Western Nigeria). The mean age of the hypertensive patients was 41.9 ± 10.3 (range 21 – 68) years, while the mean age of the healthy subjects was 37.8 ± 8.6 (range 18 – 52) years. The weight, height, blood pressure and pulse rates of all subjects were measured and their body mass indices (BMI) computed. BSe was determined by atomic absorption spectrophotometry (AAS) while pIGSH-Px activity was measured by spectrophotometric method. The mean BSe concentration was significantly lower in the hypertensive patients (0.136 ± 0.028 mg/L) than in the healthy group (0.188 ± 0.026 mg/L) ($P < 0.001$). However with respect to pIGSH-Px activity, there was no statistically significant difference between the hypertensive patients (0.126 ± 0.019 U/mL) and the healthy group (0.127 ± 0.022 U/mL). Blood Selenium concentration was found to decrease with the severity of the disease. The difference in BSe concentration of Group 1 and Group 2 patients was not significant. However, there were significant differences in the BSe levels of Group 2 and Group 3 patients ($P < 0.05$) and Group 1 and Group 3 patients ($P < 0.05$). The observed low BSe in hypertensive subjects implies that low BSe is probably a predisposing factor to essential hypertension or a consequence of the disease. The severity of the disease was also observed to be inversely related to the level of BSe, suggesting that BSe level may have a role in the prognosis of the disease. Alteration in BSe status appears to confirm the elemental basis of the aetiopathogenesis of certain diseases. Despite the reduction in BSe level in the hypertensive patients, it was still adequate to maintain pIGSH-Px activity at a level comparable to those of the healthy group. This suggests that BSe may exist in another functional form, which plays a role in the pathogenesis or prognosis of the disease.

Key words: Selenium, essential hypertension, biochemical markers, disease, patients, aetiopathogenesis.

INTRODUCTION

The role of selenium in heart disease has been intensely debated since an inverse correlation was reported between mortality from arteriosclerosis and hypertensive heart disease and the selenium content of crops and water in countries such as Finland and New Zealand (Samberger et al., 1978). In the USA, an inverse correla-tion was reported between the plasma selenium level and the severity of coronary arteriosclerosis (Moore et al., 1984). Earlier, Westermark (1977) found that blood selenium level in patients with acute myocardial infarction were lower than those of healthy adults but there were no differences in the heart and liver selenium level of patients who died from myocardial infarction and those who died from other diseases.

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Table 1. Classification of the hypertensive patients.

*Group	No. of Subjects	Average of Blood Pressure(mmHg)
Group 1 (Mild Hypertension)	35	DBP 90-99 or SBP 140-159
Group 2 (Moderate Hypertension)	42	DBP 100-109 or SBP 160-179
Group 3 (Severe Hypertension)	26	DBP ≥ 110 or SBP ≥ 180

*After WHO-ISH, 1999. DBP = Diastolic blood pressure; and SBP = Systolic blood pressure.

Attempts were also made by a number of investigators to control for potential confounding factors by using controls match for five major coronary heart disease risk factors namely age, sex, serum cholesterol, smoking history and history of angina pectoris and in women of menopausal status. Also, attempts to correlate blood selenium and GSH-Px activity with accepted risk factors for cardiovascular disease, has shown diverse results. (Lockitch, 1989).

About 40 years after the discovery of the importance of selenium, there is still controversy over the cause and effect of selenium deficiency in a variety of clinical states. This scientific work therefore focuses on the possible involvement of selenium in one human disease of multiple aetiopathogenesis, hypertension. There is increased incidence of this disease in this environment. Babalola et al. (2003) had earlier determined the selenium levels in blood of apparent healthy adults in South Western Nigeria and observed that there is no selenium deficiency in our environment. The present study is designed to compare the levels of blood selenium and plasma glutathione peroxidase of the hypertensive with those of the apparent healthy subjects. This is expected to advance our understanding of the pathogenesis of the disease, and probably, come up with new approaches to its diagnosis, management and treatment.

MATERIALS AND METHODS

Selection of hypertensive patients

One hundred and three adult Nigerians of both sexes aged between 21 and 68 years with essential hypertension were selected for the study. All patients were examined by the consultant physiccians and their complete medical history and physical examination were documented. The diagnosis of essential hypertension was based on at least two separate blood pressure readings within a minimum of two weeks intervals and laboratory examinations. The patients were considered to be hypertensive as defined by an average of two or more diastolic blood pressure in the range of 90 - 120 mmHg and/or the average of multiple systolic blood pressure in the range of 140 – 200 mmHg in the sitting position, on two of more subsequent visits to the clinic. The patients were divided into three groups based on the severity of the disease namely: mild (Group 1), moderate (Group 2) and severe (Group 3) hypertension. Table I shows the three groups and the criteria for the classification. They were recruited from Abi Memorial Hospital, Abeokuta, Federal Medical Center (F. M. C), Abeokuta, and University College Hospital (U. C. H.), Ibadan. These comprised of newly diagnosed patients and those that were previously on antihypertensive agents such as Propanolol and Moduretic. The blood pressure (in mmHg) and pulse rate (per min) were again taken at the time of sample collection using Omron Automatic Digital Blood Pressure Monitor HEM - 713C (Omron Healthcare Inc. Vernon Hill Illinois 60061). The mean of three reading was calculated for each patient.

The following exclusion criteria were employed in this study:

(i) All cases of hypertension secondary to other diseases were excluded from the study.
(ii) Patients with history of alcoholism, cigarette smoking, drug abuse or any form of mental disorder.
(iii) Patients with angina pectoris.
(iv) Female patients were neither pregnant nor lactating mothers nor on any oral contraceptives at the time of the study.
(v) All obese patients (BMI of patients not ≥ 27 Kg/m^2)
(vi) Patients with diabetes mellitus.
(vii) Patients with evidence of renal disease.

The following Inclusion Criteria was also adopted: Participation in the study was purely voluntary. Informed consents were obtained from patients after they were properly educated about the benefit of the study.

Selection of healthy subjects group (Controls)

These consist of eighty-eight apparently healthy subjects of both sexes, aged between 18 and 52 years. The subjects were all apparently healthy with their blood pressure in the normal range. They were all non-smokers and non-alcoholics and were mainly students, office workers and traders. They were not taking any medication during the period of the study. The control group was selected as much as possible to match the hypertensive group in age, sex and BMI. Their blood pressure and pulse rates were similarly measured at the time of sample collection. Also, none of the apparently healthy subjects was obese. They were also classified into male and female subgroup. Inform consents were obtained from all participants after being educated on the benefit of the study.

Anthropometric indices

The current ages of the subject were noted. Also the current body weight was measured with minimal clothing using a balance beam scale. Heights were also measured barefooted, using a meter rule. Height (m) and Weight (Kg) were used to calculate the body mass index (BMI) (kg/m^2).

Collection of blood

About 10 ml of venous blood was obtained from the antecubital fossa vein using disposable pyrogen-free needle and syringes about 3 ml of blood was dispensed into heparinized tubes containing lithium heparin this was frozen as heparinized whole blood sam-

Table 2. Age, weight, height and body mass index (BMI) of healthy and hypertensive subjects.

Parameter	Healthy Subjects (n = 88)	Hypertensive Subjects (n = 103)	t	P
Age (years)	37.8 ± 8.6 (18 – 52)	41.9 ± 10.3 (21 – 68)	2.99	P<0.01
Weight (kg)	62.2 ±9.3 (42 – 78)	64.3 ± 10.7 (56 – 78)	1.45	NS
Height (metres)	1.66 ± 0.09 (1.45 – 1.80)	1.65 ± 0.07 (1.45 – 1.75)	0.85	NS
Body Mass Index (BMI; kg/m^2)	21.06 ± 2.74 (15.57 – 26.70)	21.57 ± 3.84 (18.83 – 26.92)	1.07	NS

Values are in mean ± SD; n = No. of subjects; BMI = Body Mass Index; SD = Standard Deviation; and NS = no significant difference.

Table 3. Age, weight, height and BMI in the three groups of hypertensive patients.

Subject/Group	Age (years)	Weight (Kg)	Height (m)	BMI (Kg/m^2)
Group 1 (n = 35)	41.7 ± 10.9	69.9 ± 6.4	1.68 ± 0.05	23.37 ± 2.03
Group 2 (n = 42)	41.2 ± 10.4	64.7 ± 5.1	1.63 ± 0.06	20.92 ± 1.77
Group 3 (n = 26)	42.0 ± 8.2	65.9 ± 5.5	1.65 ± 0.07	21.42 ± 1.48
t	0.20 a,NS	3.89 a,**	3.99 a,**	5.58 a,**
	0.35 b,NS	0.89 b,NS	1.21 b,NS	1.25 b,NS
	0.12 c,NS	2.62 c,*	1.65 c,NS	4.35 c, **

Values are in mean ± SD; Group 1 = Mild Hypertension; Group 2 = Moderate Hypertension; Group 3 = Severe Hypertension; BMI = Body Mass Index; n = No. of subjects; SD = Standard deviation; a = Comparison of Group 1 and Group 2; b = Comparison of Group 2 and Group 3; c = Comparison of Group 1 and Group 3; NS = Not significant; * = P < 0.01; and ** = P < 0.001.

Table 4. Blood pressure and pulse rates of healthy and hypertensive subjects.

Parameter	Healthy Subjects (n = 88)	Hypertensive Subjects (n = 103)	t	P
Diastolic BP (mmHg)	81.6 ± 6.8	104.9 ± 10.16	18.86	<0.001
Systolic BP (mmHg)	107.0 ± 14.05	173.2 ± 22.32	24.87	<0.001
Pulse rate (/min)	73.1 ± 1.80	74.6 ± 7.22	2.03	<0.05

Values are in mean ± SD; BP = Blood pressure; and n = No. of Subjects.

sample; the remaining blood sample was dispensed into plain vacutainer tube containing EDTA. The sample was centrifuged at 450 g in IEC centrifuge for about 5 min to obtain plasma, which was transferred with a pipette into plain vacutainer tubes and then frozen. Samples were kept frozen at -70^{o}C until analysed.

Determination of plasma glutathione peroxidase (EC 1.1.11.9)

GSH-Px activity was assayed by a modification of the couple method of Paglia and Valentine (1967) using hydrogen peroxide as substrate and monitoring the oxidation of NADPH at 340 nm. One unit of enzyme activity is defined as 1 µmole NADPH oxidized/min. and the result is expressed as unit/ml of plasma.

Determination of blood selenium

Selenium in blood was determined with atomic absorption spectrophotometer (AAS) by the method of Pleban et al. (1982) Atomic absorption spectrophotometric measurements of blood selenium concentration were performed on a Perkin Elmer model 703 (Perkin – Elmer Oak Brown, Illinois, USA).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Student t-test was used to determine significance between means. The 5% ($p < 0.05$) level of significance using the two-tailed 't' table was used to compare the calculated and critical 't' value from the table and thus statistical significance.

RESULTS AND DISCUSSION

Tables 2 and 3 show the results of the anthropometrics indices of the healthy and the hypertensive subjects. Tables 4 and 5, the results of the blood pressure and pulse rate of the healthy and the hypertensive subjects while Tables 6 and 7 show the results of their BSe and plGSH-Px activity.

In the present study we assessed the selenium status of hypertensive patients (specifically patients with essential hypertension) classified into three major groups based on the severity of the disease. Earlier, we had establi-

Table 5. Blood pressure and pulse rates of the three groups of hypertensive patients.

Parameter	Group 1 (n = 35)	Group 2 (n = 42)	Group 3 (n = 26)
Diastolic BP (mmHg)	95.5 ± 2.5	103.7 ± 2.6	119.4 ± 7.5
Systolic BP (mmHg)	150.5 ± 12.8	174.9 ± 17.2	186.8 ± 20.4
Pulse rate (/mins)	74.2 ± 6.3	73.8 ± 6.4	76.1 ± 4.7

Values are in mean ± SD; BP = Blood pressure; and n = No. of Subjects.

Table 6. BSe and plGSH-Px activity in healthy and hypertensive subjects.

Parameter	Healthy subjects (n = 88)	Hypertensive subjects (n = 103)	t	P
Bse (mg/L)	0.188 ± 0.026	0.136 ± 0.028	15.71	<0.001
PlGSH-Px (U/ml)	0.127 ± 0.022	0.126 ± 0.019	0.34	NS

Values are in mean ± SD; BSe = Blood selenium; plGSH-Px = plasma glutathione activity; NS = No significant difference; and n = No of subjects.

Table 7. BSe and plGSH-Px in the 3 groups of hypertensive patients.

Parameter	BSe (mg/L)	PlGSH-Px U/mL)
Group 1	0.137 ± 0.019	0.125 ± 0.028
Vs		
Group 2	0.135 ± 0.014	0.126 ± 0.027
t	0.52	0.16
P value	NS	NS
Group 2	0.135 ± 0.014	0.126 ± 0.027
Vs		
Group 3	0.129 ± 0.013	0.124 ± 0.027
t	1.80	2.98
P value	P<0.05	p<0.05
Group 1	0.137 ± 0.019	0.125 ± 0.028
Vs		
Group3	0.129 ± 0.013	0.124 ± 0.027
t	1.95	1.39
P value	P<0.05	NS

Values are in mean ± SD; Group 1 = Mild hypertension; Group 2 = Moderate hypertension; Group 3 = Severe hypertension; BSe = Blood Selenium; plGSH-Px = Plasma glutathione peroxidase; and NS = Not significant.

shed that the values of blood selenium and plasma glutathione peroxidase activities for healthy adults in this environment is comparable with those from other areas of the world where selenium concentration in soil and crops are said to be adequate (Babalola et al., 2003).

Our interest is based on the fact that selenium is an integral component of the ubiquitous antioxidant enzyme, GSH-Px (Zagrodzki et al., 2000) and tissues that are highly vulnerable to oxidant damage are those with sustained metabolic activities, such as the heart (Diplock, 1974) and the blood vessels. Indeed other workers had observed that increased oxidative damage in patients with essential hypertension, might caused a decrease in the activity of glutathione peroxidase in response to increased production of reactive oxygen species in hypertensive subjects (Lockitch, 1989). Furthermore, the fact that epidemiological studies have related low dietary selenium with increased incidence of cardiovascular mortality in low selenium areas of the world (Shamberger et al., 1975) suggests that selenium probably have a role in the pathogenesis of cardiovascular disease.

For the study, the selenium status of hypertensive patients was compared with those of healthy adults, so as to establish any possible association between selen-ium and the disease. The cases and the controls were comparable for most of the confounding factors. The differentces in their mean weights, heights and BMIs were not significant, there was a slight different in their mean ages (P<0.01), these however fall into the middle age bracket. From our results, there was significantly lower BSe in hypertensive patients compared with the healthy subjects (P < 0.001). Though the mean plGSH-Px activity was slightly reduced in hypertensive patients compared with the healthy group; the difference was not statistically significant. Also there was a negative correlation between BSe and blood pressure and between plasma GSH-Px and blood pressure in hypertensive patients (r = -0.275, P<0.005 and r = -0.185, P<0.05, respectively). No such correlations in these parameters were found in healthy group.

This alteration in BSe level appears to confirm the elemental basis of the aetiopathogenesis of certain disease. The observed low BSe in hypertensive patients also implies that low BSe is probably a factor in essential hypertension, either a predisposing factor or a consequence of the disease. This also appears to justify the hypothesis that selenium deficiency may render the heart susceptible to injury (Shamberger, 1978), if central role of selenium in GSH-Px antioxidant activity is considered.

However, despite the significant reduction of BSe levels, plGSH-Px activities were not significantly different in healthy subjects and hypertensive patients. There are many possible explanations for these discrepancies. Since the recognition of the essentiality of selenium, decreese dietary supplies have been associated with a large number of clinical conditions. Also since the discovery of GSH-Px and the recognition of the importance of selenium in the enzyme, attempts were made to explain the role of selenium in prevention of many diseases as a consequence of its antioxidant effect through GSH-Px.

With the discovery and characterization of several other selenoproteins, it is probable that the mechanism by which selenium exerts its role on disease may not only be through its antioxidant action. Secondly from earlier studies, it was observed that there was an excellent correlation between human whole blood selenium concentration and GSH-Px activity only at concentration below 0.1 mg/L (Thomson et al., 1977). However above this concentration, the activity of the enzyme is not noticeably increased, which suggests either that this concentration is optimal and that an intake that maintained this concentration is adequate for function as measured by GSH-Px activity, or, that above this concentration, other factors may play a greater role in influencing GSH-Px activity. It is probable that since the whole blood selenium concentration in hypentensive subjects was above 0.1 mg/L, the blood selenium level was still adequate to maintain the activity of the GSH-Px in the blood of hypertensive subjects at level comparable to those of the healthy control group. Thirdly, only about 10% of the total selenium in human blood is associated with glutathione peroxidase (Behne and Wolters, 1979). This means that the “non glutathione peroxidase” selenium accounts for about 90% of the total selenium in human blood. Thus, the role of selenium in glutathione peroxidase may not be the only function of the element. It is probable that the whole blood selenium fully represents other functional forms of the element, which may be associated with the disease.

In addition, most selenium compounds were known to be very efficiently absorbed from the duodenum (Levander, 1984), absorbed selenium is readily taken up by the liver, erythrocyte (Griffiths et al., 1976) and many other tissues. The distribution of selenium in human organs varies from one organ to another (Schroeder et al., 1970). GSH-Px represents the only “functional” selenium in tissue, not selenium that has been non-specifically incorporated into protein or those that have formed biologically inactive complexes with heavy metals (Hoekstra, 1975). Thompson et al. (1976) suggested that the reason for high blood selenium levels but low blood GSH-Px activities in some species could perhaps be related to an increased level of these ‘non functional’ selenium in the blood such as selenomethionine that has been non specifically incorporated into protein as a substitute for methionine. Thus it is probable that selenium intake must be markedly low before GSH-Px activity can be altered significantly. Consequently it is probable that there may be other functional forms of selenium, associated with this disease, since the pathophysiological consequence of low BSe was not inextricably linked to change in GSH-Px activity. This observation suggests that there were "non-glutathione peroxidase" function of selenium.

A number of physiological mechanisms are involved in the maintenance of normal blood pressure and their derangement may play a part in the development of essential hypertension. It is probable that there are very many interrelated factors that contribute to a raised blood pressure in hypertensive patients and their relative roles may differ between individuals. Among the factors that have been intensively studied are salt intake, obesity and insulin resistance, the renin-angiotensin aldosterone system and the sympathetic nervous system. The pathophysiologic factors that have been implicated in the genesis of essential hypertension include increase sympathetic nervous system activity, overproduction of an unidentified sodium-retaining hormone, chronic high salt intake, inadequate dietary intakes of potassium, calcium, and magnesium, increase or "inappropriate" renin secretion, deficiencies of various vasodialatory substances such as prostaglandins and congenital abnormalities of the resistance vessels. It is probable from the results obtained from this study that low BSe is a factor that contribute to raised blood pressure in hypertensive patients.

There are numerous pathophysiological features of essential hypertension, many of which undoubtedly represent compensatory mechanism that offset primary abnormality. Unlike animal model however where experimental deficiencies of a single nutrient can be produced, low blood selenium in man is only one factor in a complex set of other variables that may predispose to or protect against disease. Moreover essential hypertension is said to be a genetically based disease with underlined inherited biochemical abnormalities, consequently the possible involvement of selenium in this disorder still need to be fully clarified.

The hypertensive patients were also classified into three groups based on the severity of the disease in accordance with WHO-ISH 1999 guidelines for classifycation of hypertensive patients; this is to ascertain the relationship between selenium and the severity of the disease. The mean ages of the three groups were similar. BSe concentrations were however found to decrease with the severity of the disease suggesting that selenium probably have a central role in the pathogenesis of the disease. The mean BSe concentration of Group 1 patients was higher than that of Group 2 patients, the difference was however not statistically significant. The mean BSe concentration in Group 2 patients was significantly higher than that of Group 3 patients, also BSe level in Group 1 patients was significantly higher than that of Group 3 patients. A negative correlation was also obser-

ved between BSe concentration and blood pressure in hypertensive patients, this again appears to corrobo-rate the view that selenium has a pivoted role in the patho-genesis of hypertension. Unlike BSe, however, the plGSH-Px activities in the three groups of hyper-tensive patients were similar.

These various observations appear to confirm that the severity of hypertension is inversely related to the level of BSe, suggesting that apart from the possible role of sele-nium in the pathogenesis of the disease, BSe may also have a role in the prognosis of the disease.

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