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ANTIOXIDANTS STATUS OF ACUTE ISCHAEMIC STROKE SUBJECTS IN SOKOTO, NIGERIA.

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ABSTRACT

Acute ischaemic stroke (AIS) is characterized by elevated levels of oxidative stress indices and declined antioxidant defences. Increased oxidative stress is thought to play a critical role in the development of AIS and its associated complications. We investigated malondialdehyde (MDA), BP, FBS, SOD, GPx, CAT, vitamins (A,C and E), Zn, Cr, Cu, Mn and Se of 79 AIS subjects admitted in Neuro Medical Ward of Usmanu Danfodiyo University Teaching Hospital (UDUTH),Sokoto,Nigeria and the results compared with 20 non-acute ischaemic stroke (NAIS) subjects of comparable age and social status. The results suggested significant increase *(p<0.05)* levels of MDA, BP, FBS and significant decrease *(p<0.05)* levels of vitamins A, C and E, SOD, GPx, CAT, Zn, Cr, Mn and Se and non significant decrease *(P>0.05)* level of Cu in AIS subjects compared with NAIS subjects. It is concluded that acute ischaemic strokes in the study area have depletion of antioxidant defense mechanism, an indication that the AIS subjects are predisposed to increased oxidative onslaught.

KEYWORDS: Antioxidants, acute ischaemic stroke, oxidative stress.

INTRODUCTION

Acute ischaemic stroke (AIS) is characterized by the sudden loss of circulation to an area of the brain, resulting in a corresponding loss of neurologic function.[1] AIS is a syndrome caused by disruption of the blood flow to part of the brain due to occlusion of a blood vessels as a result of either embolism or thrombosis, resulting in injury to cells and causing sudden loss of focal brain functions.[2] The prevalence of stroke varies greatly between communities. It accounted for over 56,000 deaths in England and Wales in 1999, which represent 11% of all deaths.[3] In USA and England approximately 795,000 and 900,000 respectively.[4] In Africa is about 19% with Ibadan, Nigeria having 17% (Valery *et al*., 2009).

Stroke has been classified into ischaemic and haemorrhagic types. Acute ischaemic stroke, intracerebral haemorrhage and subarachnoid haemorrhage accounts for 87%, 10% and 3% respectively.[5] Oxidative stress is increasingly being recognized as central to the underlying pathophysiology of acute ischaemic stroke. The deleterious effect of the excessive production of free radicals or reactive oxygen species can be prevented by body's antioxidant defense mechanism which may include antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and antioxidant vitamins (A,C and E) as well as minerals (Zn, Cr, Cu, Mn and Se).[6] Antioxidants defense mechanism help in neutralizing the effect of free radicals by mopping them off which further limit ischaemic stroke disease and its associated complications.[6]

In this study, BP, FBS, MDA,SOD, GPx, CAT, vitamins A, C and E, Zn, Cr, Cu, Mn and Se were determined in acute ischaemic stroke subjects presented within 72 hour of symptoms onset and the results compared with non-acute ischaemic stroke subjects of comparable socio-economic status. It is expected that this study will stimulate interests, discussion and further studies on lipid peroxidation and antioxidant micronutrients vis-à-vis complications of acute ischaemic stroke.

MATERIALS AND METHOD

Participants: The subjects employed for this study were 79 AIS subjects presented within 72 hour of symptoms onset of both sexes admitted at Neuro Medical Ward of UDUTH, Sokoto and 20 NAIS subjects of comparable socio-economic status. The consents of all the participants were sought for and obtained. Ethical Committee approval was also obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Sample Collection: Blood samples of 72 hour onset of symptoms of AIS confirmed by CT scan were collected by venipuncture and delivered into clean dry tubes and allowed to clot at room temperature. The samples were centrifuged at 3000 rpm for 5 minutes using bench top centrifuge and the serum separated and kept in labeled sample bottles at (-20^{0}C) until required.

Chemical and Reagents: All chemicals and reagents were of analytical grade. MDA, GPx, SOD and CAT assay kits were purchased from Enzo's Life Science United Kingdom. Glucose assay kit, chemicals for vitamins and trace elements were obtained from Randox Laboratory Limited, Switzerland.

Biochemical Analysis: MDA was determined by method of[7], Glucose was determined by method of[8], SOD was determined by method of[9], GPx was determined by method of[10] and CAT determined by method of.[11] Vitamin A was determined by method of[12], vitamin C was determined by method of[13] and vitamin E by method of.[14] Trace elements (Zn, Cr, Cu, Mn and Se) were determined by method of.[15]

Data Analysis: All data were presented as mean±SD. Levels of significance was assessed using Student t-test. Turkey-Kramer multiple comparison test (In stat 3 Software San Diego, USA). Significant difference was taken at 5% *(p<0.05)*.

RESULTS

The result of the serum levels of MDA and activity of SOD, GPx and CAT of AIS and NAIS subjects were presented in Table 1. The result show a significantly *(P<0.05)* higher level of MDA in AIS subjects compared to NAIS. The activity of antioxidant enzymes (SOD, GPx, CAT) were significantly *(P<0.05)* lower in AIS subjects compared to NAIS subjects.

Table 2 shows serum antioxidant vitamins levels in AIS subjects.The results indicate significant decrease levels of vitamins in AIS than control subjects.

The result of antioxidant minerals of AIS subjects is presented in Table 3. It revealed significant decrease levels of all studied minerals except copper in AIS subjects when compared to NAIS subjects.

Correlation coefficient between mean arterial blood pressure (MABP) and malondialdehyde (MDA) and antioxidant enzymes is presented in Table 4. The results indicated strong positive correlation between MABP and MDA and strong negative correlation between MABP and antioxidant enzymes of AIS subjects.

Table 5 shows correlation coefficient of MABP and antioxidant micronutrients of AIS subjects. The results revealed strong negative correlation between micronutrients and MABP.

Table 6 and 7 show correlation coefficient of FBS against MDA and enzymes and FBS against antioxidant micronutrients of AIS subjects respectively.

Table 1: Serum Malondialdehyde and Antioxidant enzymes of Acute Ischaemic Stroke Subjects.

Parameter	Acute Ischaemic Stroke (n=79)	Non-Acute Ischaemic Stroke (n=20)
MDA (nmol/ml)	297.06± 70.17^a	110.69± 22.73^b
SOD (µ/ml)	4.31± 0.30^a	8.05±0.63^b
GPx (nmol/min/ml)	22.30± 1.51^a	57.71± 2.96^b
CAT (nmol/min/ml)	41.93± 2.25^a	73.97± 3.27^b

Values are mean±SD. Values bearing different superscripts on a row differ significantly *(p<0.05)*. n=number of participants; MDA=malondialdehyde;SOD=superoxidedismutase; GPx=glutathione peroxidase; CAT=catalase.

Table 2: Serum Antioxidant Vitamins of Acute Ischaemic Stroke Subjects

Parameter	Acute Ischaemic Stroke (n=79)	Non-Acute Ischaemic Stroke (n=20)
Vitamin A (µg/dL)	13.39± 1.37^a	31.58± 2.65^b
Vitamin C (mg/dL)	0.47± 0.07^a	1.46±0.23^b
Vitamin E (mg/dL)	0.57± 0.07^a	1.55± 0.20^b

Values are mean±SD. Values bearing different superscripts on a row differ significantly *(p<0.05)*. n= number of participants.

Table 3: Serum Antioxidant Minerals of Acute Ischaemic Stroke Subjects.

Parameter	Acute Ischaemic Stroke (n=79)	Non-Acute Ischaemic Stroke (n=20)
Zinc (mg/L)	0.08±0.06^a	0.42±0.03^b
Chromium(mg/L)	0.54±0.06^a	0.85±0.04^b
Copper (mg/L)	0.54±0.05^a	0.42±0.03^a
Manganese (mg/L)	0.45±0.03^a	0.81±0.03^b
Selenium (mg/L)	10.50±0.51^a	19.62±0.51^b

Values are mean±SD. Values bearing different superscripts on a row differ significantly *(p<0.05)* and the same superscripts show no significant difference *(p>0.05)*. n= number of participants.

Table 4: Correlation Coefficient between Mean Arterial Blood Pressure (MABP) and MDA and Antioxidant Enzyme.

Parameter	Correlation Coefficient between MABP
MDA	0.17
SOD	-0.23
GPx	-0.09
CAT	-0.11

There is strong positive correlation between MABP and MDA. Strong negative correlation between MABP and antioxidant enzymes.

Table 5: Correlation Coefficient (r)between MABP and Antioxidant Micronutrients of AIS subjects.

Parameter	Correlation Coefficient between MABP
Vitamin A	-0.31
Vitamin C	-0.11
Vitamin E	-0.24
Zinc	-0.14
Copper	-0.24
Chromium	-0.15
Manganese	-0.07
Selenium	-0.14

There is strong negative correlation between MABP and antioxidant micronutrients.

Table 6: Correlation Coefficient (r) between FBS and MDA, SOD, GPx and CAT of AIS subjects.

Parameter	Correlation coefficient between FBS
MDA	0.51
SOD	-0.45
GPx	-0.43
CAT	-0.24

There is strong positive correlation between FBS and MDA. Strong negative correlation between FBS and enzymes of AIS subjects.

Table 7: Correlation Coefficient (r) between FBS and Antioxidant Micronutrients of AIS subjects.

Parameter	Correlation coefficient between FBS
Vitamin A	-0.42
Vitamin C	-0.27
Vitamin E	-0.25
Zinc	-0.20
Copper	-0.25
Chromium	-0.32
Manganese	-0.27
Selenium	-0.51

There is significant *(p<0.05)* negative correlation between antioxidant micronutrients and fasting blood sugar of AIS subjects.

DISCUSSION

Oxidative stress and thrombosis are suggested to be potential contributor to the development of AIS and the associated complications.[16] This may be connected to the fact that the antioxidant status may be inadequate in AIS subjects. The metabolic significance of the evaluation of oxidative stress and antioxidants status in AIS is therefore of paramount importance.

During ischaemic stroke, superoxide anion is primarily generated radical through several ways, including mitochondrial electron transport process[17], xanthine oxidase system which is thought to be a major source for the generation of oxygen free radicals in ischaemia and reperfusion[18,19] and metabolism of arachidonic acid through the cyclooxygenase pathways. H_2O_2 is formed from superoxide anion and it is the source of OH. NO is generated from L-arginine by nitric oxide synthases (NOS) which are Ca^{2+} -dependent. NO can react with superoxide anion to produce peroxynitrite ($ONOO^-$), another highly toxic oxygen species.[20] On the other hand, antioxidants like SOD, GPx and CAT degrade superoxide anion into H_2O. Despite these defenses, the brain is vunerable to oxidative stress resulting from ischaemia and reperfusion.

The results of the current study indicated significant *(p<0.05)* increase level of MDA and significant (p<0.05) decrease levels of all studied antioxidants enzymes and micronutrients except copper, which is not significantly decreased *(p>0.05)* in AIS than the values obtained from NAIS subjects.

The increased MDA in AIS is due to an altered intracellular ratio between free radicals and antioxidant capacity which may leads to oxidative stress.[21] This finding is consistence with previous work of.[17,18,19] who reported increased level of MDA in AIS subjects.

Several studies reported decrease antioxidant enzymes and micronutrients in AIS subjects.[18,22] Our findings are in line with this. The reduction could be due to their exhaustion during the challenge of free radical stress in AIS subjects. The reduction of antioxidant vitamins and minerals could also be due to decreased availability of cofactors/co-enzyme of antioxidant enzymes during oxidative stress challenge.

The negative correlation established between serum levels of antioxidant vitamins, minerals and enzymes and mean arterial blood pressure and fasting blood sugar indicates that, the increase in the levels of MABP or FBS is accompanied by a decrease in serum antioxidant vitamins, minerals and enzymes of AIS patients. The positive correlation established between malondialdehyde and blood pressure and fasting blood sugar indicates that, the increase in the levels of either or

both is accompanied by an increase level of malondialdehyde, a marker of lipid peroxidation. This illustrated an evidence that mean arterial blood pressure and fasting blood sugar are risk factors of acute ischaemic stroke.

CONCLUSION

In conclusion, the present study indicate that severe depletion in antioxidant system is unable to combat oxidative stress, this antioxidant system could be an important protective system against oxidative damage but tends to be severely impaired in AIS leading to its associated complications.

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