ejpmr, 2018,5(6), 130-133

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIOXIDANT MICRONUTRIENTS AND HEMATOLOGICAL INDICES OF DIABETIC ISCHEMIC STROKE SUBJECTS

Wali U.[1], Yusuf S.[2], Suleiman A. I.[2], Balarabe S. A.[3*], Bilbis L. S.[2] and Sahabi D. M.[2]

[1]Department of Chemical Pathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]Department of Medicine, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

***Corresponding Author: Dr. Balarabe S. A.**
Department of Medicine, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Article Received on 31/03/2018 **Article Revised on 21/04/2018** **Article Accepted on 11/05/2018**

ABSTRACT

Diabetic Ischemic stroke (DIS) is characterized by elevated level of oxidative stress indices, declined antioxidant defenses and hematological impairment. Increased oxidative stress is thought to play a role in the development of DIS and its attendant complications. We investigated serum fasting blood sugar (FBS), malondialdehyde (MDA), Special enzymes such as superoxide dismutase (SOD), glutatuione peroxidase (GPx), Catalase (CAT), Vitamins (A, C, and E), Cu, Cr, Zn, Mn and Se, Rbc counts, Hb, Wbc counts and platelet counts and BP of 37 diabetic Ischemic strokes (DIS) attending Neurology clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria and the results compared with 42 non-diabetic ischemic stroke (NDIS) subjects. The results suggested significant increased ($P<0.05$) levels of FBS, MDA and non significant increase ($P>0.05$) of BP, Wbc counts, Rbc counts and platelet counts and decreased levels of antioxidant enzymes (SOD, GPx and CAT), Vitamins (A, C, and E), minerals (Cu, Cr, Zn, Mn and Se),RBC, counts and Hb in diabetic Ischemic strokes subjects compared with non-diabetic Ischemic stroke subjects. It is concluded that diabetic Ischemic strokes in the study area contribute to depletion of antioxidant defense mechanism and impaired hematological Indices, an indication that the diabetic Ischemic stroke patients are predisposed to increased oxidative onslaught.

KEYWORDS: Antioxidants, Diabetic Ischaemic stroke, oxidative stress micronutrients hematological Indices

INTRODUCTION

Stroke is a vascular disease with cerebral consequences, disorders like diabetes mellitus is a key player. It is defined as 'a clinical syndrome typified by rapidly developing signs of focal or global disturbance of cerebral functions, lasting more than 24 h or leading to death, with no apparent causes other than of vascular origin' (Mackay J, Mensah GA, Mendis S, Greenlund K.2004). Stroke is characterized by the sudden loss of blood circulation to an area of the brain, resulting in a corresponding loss of neurologic function (Wu *et al*., 2013). Ischaemic stroke (DIS) 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 (Enrigue *et al.*, 2008). 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 (Mant *et al.*, 2004). In USA and England approximately 795,000 and 900.000 respectively (Roger *et al.*, 2012). In Africa is about 19% with Ibadan, Nigeria having 17% (Valery *et al*., 2009). Conventional stroke risk factors are divided into those which cannot be influenced such as aging, male gender, family history and race; and those which can potentially influenced such as hypertension, diabetes mellitus, atrial fibrillation, hypercholesterolaemia, obesity, physical inactivity, smoking, excessive alcohol intake and prothrombotic factors (Morren and Salgado, 2012; Warlow *et al.*, 2001).

Most strokes (85%) are ischaemic and 15–20% are haemorrhagic. Oxidative stress is increasingly being recognized as central to the underlying pathophysiology of diabetic acute Ischaemic stroke. In humans there is a complex endogenous defense system designed to protect tissues from ROS/RNS induced cell injury. Special enzymes such as superoxide dismutase (SOD), Catalase (CAT), and glutatuione peroxidase (GPx) (including their co-factors selenium, zinc, manganese and iron), sulfhydryl group donors glutathione and vitamins (A, C and β-carotene) form a network of functionally over injury defense mechanisms.

In diabetic acute Ischaemic stroke patients there are reduced stores of antioxidants, reduced plasma or intracellular concentrations of free electron scravengers or cofactors and decreased activities of enzymatic systems involved in the detoxification of ROS in acute Ischaemic subjects. In this study, blood pressure, serum fasting blood sugar malondialdehyde, antioxidant enzymes (SOD, GPx and CAT) Vitamins (A, C, and E), minerals (Cu, Cr, Zn Mn and Se) as well as haematological Indices (RBC-counts, Haemoglobin, WBC-count and platelet counts) were determined in diabetic Ischaemic strokes and the results compared with non-diabetic Ischaemic strokes of comparable socio-economic status. It is expected that this study will stimulate interests discussion and further studies on the role of antioxidants and haematological indices via-a-vis complications of diabetic Ischaemic strokes.

MATERIALS AND METHODS

Participants: The subjects employed for this study were 37 diabetic Ischaemic strokes and 42 non-diabetic Ischaemic stroke patients of both sexes who were attending the Neuro clinic of the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. The consents of all the participants were sought for and obtained. Ethical committee approval was also obtained for the study.

Blood samples: Fasting blood samples of 72 hours onset of acute Ischaemic strokes 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 minute using benchtop centrifuge and the serum separated and kept in labeled sample bottles at (-200 C) until required. Glucose was estimated immediately.

Reagents: All chemicals and reagents used were of analytical grade. MDA, GPx, CAT and SOD assay kits were purchased from Enzo's life science, United Kingdom. Glucose kit and Chemicals for Vitamins and trace elements were obtained from Randox laboratory Limited, Switzerland.

Analytical methods: Serum glucose level was estimated by method of Trinder, (1969), MDA was estimated by method of Chandra et al., (1994), BP was taken by method pickering et al., (2008), SOD was estimated by method marklund, (1980), GPx was estimated by method marklund, (1980), GPx was estimated by method of paglla and valentine, (1969), and CAT estimated by method of Johansson and Borg, (1988). Vitamin A was estimated by method of Bassey *et al.*, (1946) ,Vitamin C was estimated by method Roe and Kuether, (1954), and Vitamin E by method of Hashim and Schuttringer (1966). Trace elements (Cu, Cr, Zn, Mn and Se) were estimated by method of Bhatti et al., (2006). Full blood counts estimated using fully antomated analyser by method of Buker and Silmverton's (1998).

Data AnalysisL: 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% (5<0.05).

RESULTS

Table 1: Serum FBS, MDA and BP of Diabetic Ischaemic Stroke and Non-Diabetic Ischaemic Stroke Subjects.

Parameter	Diabetic (n=37)	Non-Diabetic (n=42)
FBS (mmol/l)	9.96 ± 1.50a	5.45 ± 0.71b
MDA (nmol/ml)	314.70 ± 29.52a	276.87 ± 45.57b
Systolic BP (mm Hg)	160.33 ± 13.62a	157.52 ± 22.16a
Diastolic BP (mm Hg)	89.65 ± 8.74a	96.57 ± 45.57a

Values are mean ± SD. Values bearing different superscripts on horizontal column differ significantly (*P<0.05*),the same superscripts show no significant difference (*P>0.05*). n =number of subjects.

Table 2. Antioxidant Enzymes and Vitamins of Diabetic Ischaemic Stroke and Non-Diabetic Ischaemic Stroke subjects.

Parameter	Diabetic (n=37)	Non-Diabetic (n=42)
SOD (µ/ml)	4.26 ± 0.14a	4.40 ± 0.20a
GPx (nmol/min/ml)	22.03 ± 0.70a	22.59 ± 0.98a
CAT (nmol/min/ml)	41.27 ± 1.03a	41.99 ± 0.96a
Vit. A (µg/dl)	12.77 ± 0.37a	13.86 ± 0.95a
Vit. C (mg/dl)	0.44 ± 0.04a	0.50 ± 0.05a
Vit. E (mg/dl)	0.56 ± 0.05a	0.50 ± 0.04a

Values are mean ± SD. There is no significant different (*P>0.05*) between diabetic Ischaemic stroke and non-diabetic Ischaemic stroke subjects.

Table 3: Antioxidant Minerals of Diabetic Ischaemic Stroke and Non-Diabetic Ischaemic Stroke Subjects.

Parameter	Diabetic (n=37)	Non-Diabetic (n=42)
Zn(ppm)	0.06 ± 0.01a	0.09 ± 0.03a
Cu (ppm)	0.12 ± 0.02a	0.14 ± 0.04a
Cr (ppm)	0.54 ± 0.03a	0.55 ± 0.04a
Mn (ppm)	0.44 ± 0.02a	0.46 ± 0.02a
Se (ppm)	10.39 ± 0.28a	10.68 ± 0.37a

Values are mean ± SD. There is no significant different (*P>0.05*) between diabetic Ischaemic strokes and non-diabetic Ischaemic stroke subjects. n=number of subjects

Table 4. Haematological Indices of Diabetic Ischaemic Stroke and Non-Diabetic IschaemicStroke Subjects

Parameter	Diabetic (n=37) (n=42)	Non-Diabetic
RBC Count x 106/µl	2.73 ± 0.60a	3.14 ± 1.08a
Hb(g/dl)	11.26 ± 1.56a	0.14 ± 2.25a
WBC count x 106/ µl	10.76 ± 0.93a	9.85 ± 1.58a
Platelet count x 106/ µl	267.94 ± 45.63a	239.96 ± 59.89a

Values are mean ± SD. There is no significant different (*P>0.05*) between diabetic Ischaemic strokes and non-diabetic Ischaemic subjects..n= number of subjects.

DISCUSSION

It was observed that diabetic Ischaemic stroke had higher levels of malondialdehyde (MDA), fasting blood sugar, white blood cell counts, platelet counts and blood pressure (BP) and decreased levels of antioxidant enzymes (SOD, GPx and CAT), vitamins (A, C and E) and minerals (Cu, Cr, Mn, Zn and Se), red blood cell counts and Haemoglobin (*P>0.05*) compared with non-diabetic Ischaemic stroke subjects. The finding of significant increase (*P<0.05*) in serum fasting blood sugar (FBS) and blood pressure (BP) in DIS is in agreement with previous studies (Durgesh *et al.,* 2012; Aygul *et al.*, 2006; Bhatia *et al*., 2004) who reported significant elevations of FBS and BP in AIS subjects compared with controls. Oxidative stress has been suggested to contribute to insulin resistance and play a critical role in the pathogenesis of endothelial dysfunction (Esper *et al*., 2006). Elevated blood glucose has been implicated as a poor prognostic factor for cerebral ischaemia (Nedergusril, 1984). Animal studies have demonstrated the aggrevation of ischaemic injury by hyperglycaemia (Alex and Baron, 1982). Diabetic ischaemic strokes are often associated with large infart size and poor outcome due to increased autoregulation and changes in blood coagulability (Caraleting *et al.,* 1986).

There is strong evidence pointing out that the production of free radicals during the Ischaemic and reperfusion is one of the important mechanisms causing brain damage. Due to certain reasons, the brain tissue is especially prone to the deleteriors effects of the free radicals (Chen *et al.,* 2011; Zcelik *et al.,* 2012). Several studies indicated decrease antioxidant vitamins and minerals in AIS subjects (Aygul *et al.,* 2006; Demirkaya *et al.,* 2001). Our findings are in line with this. The reduction could be due to their exhaustion diring the challenge of free radical stress in AIS subjects.

Several published studies observed an increased leucocytes count, raised ESR and decreased haemoglobin (Hb) in acute Ischaemic stroke (Nayak *et al.*, 2011; Bhatia *et al*., 2004). Our study is in line to these findings. Possibly, changes in haematological parameters at the onset of stroke play an important role in altering the cerebral blood flow (Chamorro *et al.,* 1995). Leukocytosis influences the prognosis, several mechanisms by which leukocytes may be implicated in parenchymal brain injury include vessel pluggeding, release of hydrolytic enzymes, oxygen free radicals or initiation of thrombosis (wilnelson *et al.,* 1984).

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 diabetic Ischaemic stroke leading to the derangement in haemotological Indices.

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