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Research Article
Association of a Product of Free Radical Injury with Parameters of Lipid Profile in Patients with Stroke

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Abstract

Background and Objectives: Dyslipidemia and free radical injury are important risk factors in the pathogenesis of stroke. The observations of various researchers on the association between the parameters of lipid profile and malondialdehyde (product of free radical injury) level in patients with stroke are still inconclusive. This study estimated the plasma concentration of Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein-Cholesterol (LDL-Chol), High Density Lipoprotein-Cholesterol (HDL-Chol) and serum level of malondialdehyde (MDA) in patients with stroke. **Materials and Methods:** A total of 60 patients with stroke, 40 age and sex-matched volunteers were recruited after obtaining their informed consent. Plasma and serum were extracted from whole blood collected from each subject and control. The serum MDA as well as plasma TC, TG, LDL-Chol and HDL-Chol were determined by various methods in the laboratory. **Results:** The mean values of MDA (11.02 vs. 7.82 nmol mL^{-1}), TG (1.61 vs. 0.84 mmol L^{-1}) and LDL-Chol (2.74 vs. 2.06 mmol L^{-1}) were significantly higher in patients with stroke than the controls ($p<0.001$). The mean value of HDL-Chol was significantly lower in stroke patients (1.10 mmol L^{-1}) than the controls (1.60 mmol L^{-1}) ($p<0.001$). However, no significant difference was observed between the mean TC in stroke patients and controls (4.37 vs. 4.16 mmol L^{-1}, $p = 0.215$). Total cholesterol has a significant positive correlation with LDL-Chol ($r = 0.757$, $p<0.01$) and MDA ($r = 0.258$, $p = 0.047$) whereas, it has significant negative correlation with HDL-Chol ($r = -0.316$, $p = 0.014$). **Conclusion:** Stroke is shown to be associated with free radical injury as a result of increased serum MDA. Significant positive correlation between MDA and Total Cholesterol (TC) further explains the link between free radical injury and dyslipidaemia in stroke patients.

Key words: Stroke, malondialdehyde, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Globally, the incidence of stroke reportedly[1] varies between 100/100,000-330/100,000. In Nigeria, the report of a study in Lagos gave an overall crude prevalence rate[2] of 1.14/1000. Thus, stroke accounts for 9-10% of all deaths in the world[3], making it the second leading cause of death[4].

Stroke is a syndrome comprising sudden focal (non-convulsive) or global neurological deficits. These deficits persist for more than 24 h or leading to death with no obvious cause other than pathology of blood vessel[5]. A Transient Ischemic Attack (TIA) comes with stroke like symptoms but lasts less than 24 h. Transient ischemic attack generally does not cause permanent brain damage but it is a serious warning sign for impending stroke[6]. Stroke is characterized by a reduction in blood flow to the brain caused by blockage in a cerebral artery by a clot or embolus (ischemic stroke) or rupture of blood vessel (hemorrhagic stroke). This process can lead to free radical formation worsening the situation for the patient. Increased plasma lipids may also worsen the patient's clinical conditions.

Raised levels of certain lipids, particularly cholesterol are risk factors for atherosclerosis, coronary artery and other cardiovascular diseases[6]. Lipids especially polyunsaturated fatty acid located in cellular membrane can be a target of free radical attack in a process called lipid peroxidation[7]. This may further complicate the clinical conditions of patients with stroke. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of oxidants[8]. Oxidative stress plays an important role in the pathogenesis of acute ischemic stroke. This occurs through free radical (oxidant) injury. Free radical formation and subsequent oxidative damage may be a factor in stroke severity[9]. Attack of free radical on membrane lipid produces malondialdehyde (MDA) as one of the intermediate products formed during lipid peroxidation. High plasma levels of MDA have been previously described in patients with ischemic stroke attack[10]. Based on the above facts and increased burden of stroke in our society, this study was designed to determine the association between free radical injury, by measuring one of the intermediate products (malondialdehyde, MDA) of its attack on membrane lipid and some parameters of lipid profile; Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-Chol), Low Density Lipoprotein (LDL-Chol) and Triglyceride (TG).

MATERIALS AND METHODS

The study was carried out between July, 2013-February, 2014 at the Medical Outpatient clinic, Physiotherapy clinic and the Department of Chemical Pathology of Ladoke Akintola, University of Technology Teaching Hospital, Osogbo Nigeria. This study was approved by the Research Ethic Committee of the institution with protocol number; LTH/EC/2013/06/0145. A total of 100 subjects were assessed and informed consent was taken from each participant prior to inclusion in the study. Sixty of them were patients with stroke who were attending neurology clinic or physiotherapy clinic. Control subjects were 40 age- and sex-matched volunteers who visited the hospital during the course of the research. Both test and control subjects were selected based on inclusion criteria which were based on the clinical history and abnormal electroencephalography (EEG) diagnosis of stroke. The exclusion criteria included patients on lipid lowering drugs (statins). Patients who were suffering from other chronic disorders such as: Diabetes mellitus, renal failure, chronic liver disease, progressive organic brain syndrome due to some causes other than stroke and patients on antioxidant supplements were also excluded.

Analytical procedure: About 10 mL of venous blood sample was collected from each participant after an over-night fast (10-12 h) through the antecubital vein using aseptic techniques of phlebotomy. For plasma and serum extractions, it was dispensed into sodium ethylenediaminetetraacetic acid (NaEDTA) and plain specimen bottles, respectively. Each of the blood contained bottles was centrifuged at 3000 Xg for 5 min, the supernatant was aspirated and stored in another plain bottle at -20°C for a maximum period of 6 months until analysis. Fasting plasma sample was used for the analysis of lipid profile (TC, HDL-Chol and TG) and serum sample was used for the analysis of MDA.

Serum MDA content was determined spectrophotometrically by thio-barbiturate reactive substance formation as described by Tukozkan *et al.*[11]. Total cholesterol, TG and HDL-Chol were analyzed enzymatically using kits obtained from Randox Laboratories Limited, Crumlin, UK[12,13]. Plasma LDL-Chol was determined from the values of TC and HDL-cholesterol using the Friedewald's formula[14].

Statistical analysis: Statistical analysis was performed by Student's test using the SPSS software version 20.0. Results were expressed as Mean±SD, p-values<0.05 was considered statistically significant. Relationships among different variables of the test groups were compared using Pearson correlation coefficient.

RESULTS

Table 1 shows the socio-demographic data of the control group and patients with stroke. The study population

Table 1: Sociodemographic data of subjects and controls

	Stroke		Control	
Age distribution (years)	Males	Females	Males	Females
21-30	0	0	0	0
31-40	0	0	1	0
41-50	4	5	2	3
>50	6	45	4	30
Total/percentage	10 (16.7%)	50 (83.3%)	7 (17.5%)	33 (82.5%)
Grand total	60	40		

Table 2: Comparison of age and biochemical parameters in subjects and controls

Parameters	Control (n = 40)	Test (n = 60)	t-value	p-value
Age (years)	0.70 (±11.66)	0.69 (±11.95)	-0.304	0.762
TC (mmol L^{-1})	4.16 (±0.68)	4.37 (±0.93)	1.25	0.215
TG (mmol L^{-1})	0.84 (±0.23)	1.61 (±0.54)	9.80	**0.000
HDL (mmol L^{-1})	1.60 (±0.18)	1.10 (±0.38)	-8.49	**0.000
LDL (mmol L^{-1})	2.06 (0.48)	2.74 (±1.03)	4.48	**0.000
MDA (nmol mL^{-1})	7.82 (±1.80)	11.02 (±2.81)	7.00	**0.000

*Significant at the 0.05 level (2-tailed), **Correlation is highly significant at the 0.01 level (2-tailed)

Table 3: Correlations of biochemical parametres in stroke subjects using pearson's correlation coefficient (r)

	TC (mmol L^{-1})		TG (mmol L^{-1})		HDL (mmol L^{-1})		LDL (mmol L^{-1})		MDA (nmol mL^{-1})	
Parameters	r	p	r	p	r	p	r	p	r	p
TC (mmol L^{-1})	1		-0.42	0.750	-0.316	0.014	0.757**	0.000	0.258*	0.047
TG (mmol L^{-1})	-0.42	0.750	1		-0.110	0.402	0.071	0.587	-0.138	0.294
HDL (mmol L^{-1})	-0.316*	0.014	-0.110	0.402	1		-0.538**	0.000	-0.054	0.684
LDL (mmol L^{-1})	0.756**	0.000	0.071	0.587	-0.538**	0.000	1		0.195	0.135
MDA (nmol mL^{-1})	0.258**	0.047	-0.138	0.294	-0.054	0.684	0.195	0.135	1	

*Correlation is significant at 0.05 level (2-tailed), **Correlation is highly significant at 0.01 level (2-tailed), TC: Total cholesterol, TG: Triglyceride, LDL-Chol: Low density lipoprotein-cholesterol, HDL-Chol: High density lipoprotien-cholesterol, MDA: Serum level of malondialdehyde, r: Pearson's correlation coefficient, p: Significance value

included: 60 patients with stroke in the age group of 41-81 years whereas, the presenting age group of patients without stroke (control) was 40-80 years. In stroke subjects, 16.7% was male and 83.3% was female. The control group also included 17.5% male and 82.5% female. The mean level of age in stroke patients (69±11.95) did not differ significantly from that of the control group (70±11.66) at p>0.005.

Significant differences were observed in the parameters of plasma lipid profile and serum malondialdehyde between patients with stroke when compared with controls as shown in Table 2. Serum concentration of MDA (11.02 nmol mL^{-1}) and plasma concentrations of TG (1.61 mmol L^{-1}) and LDL-Chol (2.74 mmol L^{-1}) were significantly higher in patients with stroke when compared with that of control group (TG = 0.84 mmol L^{-1}, LDL-Chol = 2.06 mmol L^{-1} and MDA = 7.82 mmol mL^{-1}) at p<0.001. Serum HDL-Chol concentration, on the other hand was significantly lower in stroke patients (1.10 mmol L^{-1}) than the control group (HDL-Chol = 1.60 mmol L^{-1}) at p<0.001. No significant difference was observed between the means of plasma concentration of TC in stroke patients (4.37 mmol L^{-1}) and control group (4.16 mmol L^{-1}) at p>0.005.

On applying Pearson's correlation in subjects with stroke as shown in Table 3, TC was found to be positively correlated with LDL-Chol (r = 0.757, p<0.01) and MDA (r = 0.258, p<0.05), whereas, it is negatively correlated with HDL-Chol (r = -0.316, p<0.05). On the other hand, HDL-Chol was found to be negatively correlated with LDL-Chol (r = -0.538, p<0.01).

DISCUSSION

In the present study, all lipid parameters (except for total cholesterol) measured in plasma of patients with stroke showed significances when compared with controls. Total cholesterol concentration alone in plasma is considered as a non-sensitive parameter since it did not show any significant difference between disease and control group. This was also supported by Dey *et al.*[15] in their study on stroke patients.

The association of blood cholesterol with risk of stroke, a very important clinical and public health issue appears to be in dispute. Some studies found elevated total cholesterol levels in patients with stroke[16-18] while other studies found no

clear association[19,20]. However, no significant association between the level of total cholesterol and the occurrence of stroke was observed in the present study.

Association between concentrations of serum triglycerides and the risk of stroke is also over shadowed. Some studies reported negative results whereas others showed a positive association with high serum triglyceride concentrations[21-23]. Copenhagen City Heart Study showed a log linear association between serum triglyceride concentration and non-hemorrhagic stroke[24] while, no association was found of high plasma triglyceride concentration as a risk factor for both types of stroke in the study conducted by Mahmood *et al.*[25]. It was observed that stroke subjects have a significant increase in plasma triglyceride than the healthy control in our present study.

Serum HDL-Chol has anti-atherogenic properties with ability to trigger the flux of cholesterol from peripheral cells to the liver and thus having a protective effect[26]. Studies from various parts of the world reported an inverse relationship between HDL-Chol level and the incidence of stroke[27,28]. In this study, the HDL-Chol level was still significantly lower in stroke subjects compared to the controls. This confirms the fact that low HDL-Chol is a risk factor for stroke.

Stroke has been described as being associated with high levels of oxidative stress. Oxidative stress generally comes when free radicals being generated over whelm the available antioxidants. Free radicals attack and it damages membrane polyunsaturated fatty acids following a series of biochemical reactions (lipid peroxidation). One of the intermediate products of these reactions is MDA, a Thio-Barbituric Acid Reacting Substance (TBARS) which is now widely utilized as a marker of lipid peroxidation. It is widely measured just like in this present study to demonstrate the extent of free radical injury on membrane lipid. In the current study, MDA levels in the serum of the stroke patients were higher than those in the control group. This finding means there is increased oxidative stress as a result of free radical induced cerebral injury in patients with stroke. This was also reported by study of Jawalekar *et al.*[7]. A positive correlation of MDA was found with TC.

A significant positive correlation was observed between TC with LDL-Chol and MDA in patients with stroke, this is in support of the study conducted by Sreedhar *et al.*[29]. On the other hand, a significant negative correlation was seen between HDL-Chol with TC and LDL-Chol, an observation which is in agreement with the studies carried out by Lee *et al.*[30].

The mean age of participants shows that most were older adults. The most vulnerable age of stroke is 61 years and above for males and 51 years and above for females. Similar result was observed in this study, with the gender ratio of 1:5 for males to females. Because majority of the participants were postmenopausal, lacking protection from oestrogen to keep blood lipids levels low. During this period in women's lives, risk of atherosclerosis increases and one cause may be the marked increase in total cholesterol and LDL-Chol, as well as triglycerides levels. This finding requires further study which is beyond the scope of our current study.

CONCLUSION

This study suggests the presence of significance of parameters of plasma lipid profile (except for total cholesterol) and oxidative stress marker (MDA) in patients with stroke. It further emphasizes importance of screening for lipid abnormalities and oxidative stress (free radical injury) in patients with Transient Ischemic Attack (TIA). This may be a signal to commence the administration of lipid lowering and antioxidant agents to prevent the occurrence of stroke. In view of the significant positive association between malondialdehyde and total cholesterol, increased total cholesterol may be an indicator of increased free radical injury and vice versa in individuals with stroke.

SIGNIFICANCE STATEMENT

This study discovered correlation between MDA and abnormal lipid profile in stroke patients. Malondialdehyde may have effect on metabolism of lipids. Further study may be necessary to further ascertain this relationship. Malondialdehyde may be added to the routine test to assess risk factor for stroke in individuals.

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REFERENCES

1. Obiako, O.R., S.K. Oparah and A. Ogunniyi, 2011. Prognosis and outcome of acute stroke in the University College Hospital Ibadan, Nigeria. Niger. J. Clin. Pract., 14: 359-362.
2. Danesi, M., N. Okubadejo and F. Ojini, 2007. Prevalence of stroke in an urban, mixed-income community in Lagos, Nigeria. Neuroepidemiology, 28: 216-223.

3. Rosamond, W., K. Flegal, G. Friday, K. Furie and A. Go *et al*., 2010. Heart disease and stroke statistics-2007 update: A report from the American Heart Association statistics committee and stroke statistics subcommittee. Circulation, 122: e69-e171.
4. Hankey, G.J., 2013. The global and regional burden of stroke. Lancet Global Health, 1: e239-e240.
5. Roger, V.L., A.S. Go, D.M. Lloyd-Jones, R.J. Adams and J.D. Berry *et al*., 2011. Heart disease and stroke statistics-2011 update: A report from the American Heart Association. Circulation, 123: e18-e209.
6. Khan, N.I., L. Naz, S. Mushtaq, L. Rukh, S. Ali and Z. Hussain, 2009. Ischemic stroke: Prevalence of modifiable risk factors in male and female patients in Pakistan. Pak. J. Pharmaceut. Sci., 22: 62-67.
7. Jawalekar, S.L., U.J. Kulkarni, V.T. Surve and A. Deshmukh, 2010. Role of oxidants and anti oxidants in patients with cardiovascular diseases. Asian J. Med. Sci., 2: 181-184.
8. Tsai, N.W., Y.T. Chang, C.R. Huang, Y.J. Lin and W.C. Lin *et al*., 2014. Association between oxidative stress and outcome in different subtypes of acute ischemic stroke. BioMed Res. Int., Vol. 2014. 10.1155/2014/256879.
9. Ozkul, A., A. Akyol, C. Yenisey, E. Arpaci, N. Kiylioglu and C. Tataroglu, 2007. Oxidative stress in acute ischemic stroke. J. Clin. Neurosci., 14: 1062-1066.
10. Lorente, L., M.M. Martin, P. Abreu-Gonzalez, L. Ramos and M. Argueso *et al*., 2015. Serum malondialdehyde levels in patients with malignant middle cerebral artery infarction are associated with mortality. PLoS ONE, Vol. 10, No. 5. 10.1371/journal.pone.0125893.
11. Tukozkan, N., H. Erdamar and I. Seven, 2006. Measurement of total malondialdehyde in plasma and tissues by high-performance liquid chromatography and thiobarbituric acid assay. Firat Tip Dergisi, 11: 88-92.
12. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
13. Bucolo, G. and H. David, 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem., 19: 476-482.
14. Krishnaveni, P. and V.M. Gowda, 2015. Assessing the validity of Friedewald's formula and Anandraja's formula for serum LDL-cholesterol calculation. J. Clin. Diagn. Res., 9: BC01-BC04.
15. Dey, S.K., S. Ahmed, K.M. Rahman, M.J. Uddin and M.R. Alam *et al*., 2010. Lipid profile among ischemic and haemorrhagic stroke patients. Mymensingh Med. J., 19: 176-180.
16. Labreuche, J., P.J. Touboul and P. Amarenco, 2009. Plasma triglyceride levels and risk of stroke and carotid atherosclerosis: A systematic review of the epidemiological studies. Atherosclerosis, 203: 331-345.
17. Yaghi, S. and M.S. Elkind, 2015. Lipids and cerebrovascular disease: Research and practice. Stroke, 46: 3322-3328.
18. Wang, X., Y. Dong, X. Qi, C. Huang and L. Hou, 2013. Cholesterol levels and risk of hemorrhagic stroke: A systematic review and meta-analysis. Stroke, 44: 1833-1839.
19. Bowman, T.S., H.D. Sesso, J. Ma, T. Kurth, C.S. Kase, M.J. Stampfer and J.M. Gaziano, 2003. Cholesterol and the risk of ischemic stroke. Stroke, 34: 2930-2934.
20. Shahar, E., L.E. Chambless, W.D. Rosamond, L.L. Boland, C.M. Ballantyne, P.G. McGovern and A.R. Sharrett, 2003. Plasma lipid profile and incident ischemic stroke: The Atherosclerosis Risk in Communities (ARIC) study. Stroke, 34: 623-631.
21. Park, J.H. and H.M. Kwon, 2008. Association between metabolic syndrome and previous ischemic lesions in patients with intracranial atherosclerotic stroke. Clin. Neurol. Neurosurg., 110: 215-221.
22. Labreuche, J., D. Deplanque, P.J. Touboul, E. Bruckert and P. Amarenco, 2010. Association between change in plasma triglyceride levels and risk of stroke and carotid atherosclerosis: Systematic review and meta-regression analysis. Atherosclerosis, 212: 9-15.
23. Jain, M., A. Jain, N. Yerragondu, R.D. Brown and A. Rabinstein *et al*., 2013. The triglyceride paradox in stroke survivors: A prospective study. Neurosci. J., Vol. 2013. 10.1155/2013/870608.
24. Glasser, S.P., A. Mosher, G. Howard and M. Banach, 2016. What is the association of lipid levels and incident stroke? Int. J. Cardiol., 220: 890-894.
25. Mahmood, A., M.A. Sharif, M.N. Khan and U.Z. Ali, 2010. Comparison of serum lipid profile in ischaemic and haemorrhagic stroke. J. Coll. Physicians Surgeons Pak., 20: 317-320.
26. Zhang, Y., J. Tuomilehto, P. Jousilahti, Y. Wang, R. Antikainen and G. Hu, 2012. Total and high-density lipoprotein cholesterol and stroke risk. Stroke, 43: 1768-1774.
27. Zheng, J., Z. Sun, X. Zhang, Z. Li and X. Guo *et al*., 2019. Non-traditional lipid profiles associated with ischemic stroke not hemorrhagic stroke in hypertensive patients: Results from an 8.4 years follow-up study. Lipids Health Dis., Vol. 18, No. 1. 10.1186/s12944-019-0958-y.
28. Akande, J.O., A.A. Salawu, A.S. Atiba, E.O. Oke, R.O. Akande, D.P. Oparinde and P.S. Ogunro, 2019. Plasma levels of total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglyceride, Apo A-1 and Apo B in patients with Stroke in Ogbomoso, Southwestern Nigeria. J. Applied Biol. Biotechnol., 7: 29-35.
29. Sreedhar, K., B. Srikant, L. Joshi and G. Usha, 2010. Lipid profile in non-diabetic stroke-a study of 100 cases. J. Assoc. Physicians India, 58: 547-551.
30. Lee, J.S., P.Y. Chang, Y. Zhang, J.R. Kizer, L.G. Best and B.V. Howard, 2017. Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: The strong heart study. Diabetes Care, 40: 529-537.