VOLUME-7, ISSUE-10, OCTOBER-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Physiology

ASSESSMENT OF OXIDATIVE STRESS MARKERS AND LIPOPROTEIN(A) IN PATIENTS WITH STROKE IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To study the status of oxidative stress markers such as Superoxide Dismutase(SOD), Glutathione Peroxidase(GPx), product of lipid peroxidation Malondialdehyde(MDA) and serum Lipoprotein(a) concentrations in cases i.e. stroke subjects.

Materials and Methods: A total of 28 subjects, fourteen (14) cases and fourteen (14) controls were selected as per convenience keeping in mind the logistics and time constraints.Patient admitted to medicine wards with established diagnosis of stroke were taken as cases. The diagnosis was established on the basis of history, clinical examination and imaging studies. While age and sex matched controls were randomly selected from Medicine OPD. Unpaired T- test was used for statistical significance. A value less than 0.05 was considered as significant.

Results: Glutathione peroxidase(GPx) levels were lower in cases than in controls ($P < 0.05$).Superoxide dismutase(SOD) levels were very low in cases than in controls($P < 0.001$).Malondialdehyde(MDA) levels were higher in cases than in controls but relationship was not found to be significant($p > 0.05$). Lipoprotein (a) levels were very high in cases than in controls($P < 0.001$).

Conclusion: The present study reveals reduced GPx and SOD while MDA and Lipoprotein(a) levels were high in stroke subjects in first 24 hours.This indicates prominent role ofreduced GPx and SOD in increase of oxidative stress during early ischaemic period.Further it was also found that increased Lipoprotein(a) in early ischaemic period is associated with higher risk of stroke. Measurement of these biomarkers can quantify the risk of stroke.

KEYWORDS : Oxidative stress,Lipid peroxidation,Lipoprotein(a), Stroke

INTRODUCTION

Stroke or Cerebrovascular accident results when there is reduced supply of blood to the brain resulting in neuronal death. Cerebrovascular diseases include some of the life threatening disorders like is chaemic stroke, hemorrhagic stroke and cerebrovascular anomalies such as intracranial aneurysm and arteriovenous malformation. The clinical manifestations of stroke depends on the site of injury in the brain and its vasculature[1]. If neurological signs and symptoms resolves within 24 hours regardless of whether there is imaging evidence of new permanent brain injury, it is known as a transient ischemic attack (TIA)[1].Stroke has occurred if the neurological signs and symptoms lasts for more than 24 hours[1]. High blood pressure is the most common cause of stroke [2]. Other causes include tobacco smoking, obesity, high blood cholesterol, diabetes mellitus, previous TIA, and atrial fibrillation[2]. Several neuroprotective studies revealed the major role of oxidative stress in exacerbating the ischemic injury[3]. As the brain consumes about 20% of the body's total oxygen, neuronal cells are more vulnerable than other cells to oxidative damage, although brain constitutes less than 2% of the total body weight[4].The significance of oxidative damage as a component of many disease processes in the central nervous system (CNS) is being increasingly recognized. One of the major contributors of oxidative stress is free radicals; superoxide anion (O2–), hydroxyl radical (HO), lipid radicals (ROO–) and reactive nitrogen species (RNS) such as nitric oxide (NO).

During 1990 - 2010 total number of stroke cases fall by 10 percent in the developed countries but it increased by 10 percent in the developing countries[5].The average age for stroke cases in the developed countries is approximately 73 years[6]. The proportion of stroke in young population is significantly more in India than in developed countries;some of the more important causes for this are likely to be rheumatic heart disease, ischaemic strokes in peripartum period and arteriopathies as a sequale of CNS infections like bacterial and tubercular meningitis[7].

In 2013, stroke was the second most common cause of death after coronary artery disease, accounting for 6.4 million deaths (12% of the total)[8]. About 3.3 million deaths were a direct repercussion of ischemic stroke while 3.2 million deaths were aftermath from hemorrhagic stroke.[8]

One of the most demanding need is treatment after post stroke therapy. Patient has to be managed in a short span of time which often deteriorate medical care in time to minimize desolate and irremediable brain damage. Keeping these facts in mind this study was carried out in Medicine Department of J N Medical College with the following aims and Objective:

1) To study the status of antioxidants i.e. catalase, superoxide dismutase(SOD), glutathione peroxidase(GPX) in stroke subject.
2) To study status of lipid peroxidation i.e.Malondialdehyde in stroke subject.
3) To assess serum lipoprotein (a) concentrations in stroke subjects
4) To suggest protective /remedial measures if required

MATERIAL AND METHODS

This was a case control study carried out in Medicine department in collaboration with Physiology and Biochemistry department of J.N. Medical college, Aligarh after getting approval from ethical committee of college . A total of 28 subjects, fourteen (14) cases and fourteen (14) controls were selected as per convenience keeping in mind the logistics and time constraints.

Patient admitted to medicine wards with established diagnosis of

stroke were taken as cases. The diagnosis was established on the basis of history, clinical examination and imaging studies. While age and sex matched controls were randomly selected from Medicine OPD. Unpaired T- test was used for statistical significance. A value less than 0.05 was considered as significant

To test the oxidative stress level we measured the levels of four biomarkers: 1.Glutathione peroxidase (GPx), 2. Superoxide dismutase(SOD), 3.Malondialdehyde (MDA) and 4. Lipoprotein(a) For Glutathione peroxidase level testing we used Berge and Rust (1978) method . The reagents used were EDTA, sodium azide, NADPH, GSH, reduced glutathione, buffer solution and distilled water. Reaction mixture was vortexed and hydrogen peroxide was added. Optical density was read at 340nm for 2 min at a gap of 15 seconds. The value of GPx was calculated by using this formula:

$$\text{GPx(nmol)/mg Hb/ min} = \frac{x*1000}{6.22*\text{Hb/ml}*\text{sample volume}}$$

To test superoxide dismutase reagents used were Trissuccinate buffer, succinic acid and Pyragallol. Reaction mixture was Incubated at 25 degree centigrade for 20 minutes 0.1ml(100λ) pyragallol was added just before reading in cuvette and read at 412 nm, after each 30 seconds for 3 minutes.

To test Malondialdehyde stock reagent consisting of trichloroacetic acid, thiobarbituric acid and hydrochloric acid was used. The solution was heated to dissolve TBA In 1ml of sample 2ml of stock reagent was added and solution was heated for 15 minutes in boiling water bath. After cooling for 10 minutes the solution was read at 535 nm. The value of MDA was calculated by using the following formula:

$$\text{MDA Level (for serum/plasma)} = \frac{\text{Optical density nmol/ml}}{0.156}$$

Lipoprotein(a) was measured using ELISA kit

OBSERVATION AND RESULTS

Table 1: Status of oxidative stress enzymes and lipid peroxidation in stroke

ENZYME	Cases N=14	Controls N=14	P value
GLUTATHIONE PEROXIDASE (U/L)	6.4 ± 2.0	7.7 ± 1.1	< 0.05
SUPEROXIDE(U/ml) DISMUTASE	0.37 ± 0.11	0.58 ± 0.16	< 0.001
MALONDIALDEHYDE (nmol/ml)	16.5 ± 3.5	14.4 ± 3.4	> 0.05
LIPOPROTEIN(a) (mg/dl)	78± 4.5	31±2.5	<0.001

Table 1 reveals that Glutathione peroxidase levels were lower in cases than in controls (P <0.05).Superoxide dismutase levels were very low in cases than in controls(P <0.001).Malondialdehyde levels were higher in cases than in controls but relationship was not found to be significant(p>0.05). Lipoprotein (a) levels were very high in cases than in controls(P <0.001)

DISCUSSION

Oxygen is important to sustain life. It is relatively non reactive in a ground state. The cells use oxygen to produce ATP (Adenosine triphosphate). During the process free radicals are generated which are highly unstable and reactive[9].The tissues are protected against the oxidants by the presence of enzymatic and non-enzymatic antioxidant defense systems[10]. Enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase. Oxidative stress can be assessed by reduction in enzymatic and non-enzymatic antioxidant defense system and by assessing the product of lipid peroxidation by reactive oxygen species.

In our study we measured antioxidant enzymes Glutathione peroxidase,superoxide dismutase, product of lipid peroxidation (Malondialdehyde) and serum Lipoprotein(a)

A study by Jaspreetkaur et al[11] observed a significant increase total cholesterol,triglycerides, LDL cholesterol and MDA in stroke patients whereas a significant decline was observed in erythrocyte Superoxide dismutase and vitamin E levels as compared to control group. Another study by Cojucaru IM et al[12] reported significantly lower level of plasma GPx and SOD while high level of MDA in cases with acute ischemic stroke.

Dhamijaet al[13]studied the role of homocysteine and Lp(a) in ischemic stroke. They determined plasma Lp(a) concentration in 66 patients with ischemic stroke and 72 controls and found that these two parameters are independently associated with ischemic stroke with a significant positive correlation between them. Similar are the finding of our study and several other studies[14,15,16,17].

CONCLUSION AND RECOMMENDATIONS

The present study reveals reduced GPx and SOD while MDA and Lipoprotein(a) levels were high in stroke subjects in first 24 hours.This indicates prominent role of reduced GPx and SOD in increase of oxidative stress during early ischaemic period.Further it was also found that increased Lipoprotein(a) in early ischaemic period is associated with higher risk of stroke. Measurement of these biomarkers can quantify the risk of stroke. Risk factors identified as early as possible will help in implementing protective and remedial measures.

REFERENCES

1. Harrison principles of internal medicine 17th edition Page 2513, 17 edition
2. Rhoads, G. G., G. H. Dahlen, K. Berg, N. E. Morton, and A. L Dannenberg.1986. Lp(a) lipoprotein as a risk factor for myocardial infarction. JAMA (J.Am. Med. Assoc.) 256:2540-2544.
3. Sandholzer, C., D. M. Hallman, N. Saha, G. Sigurdsson, C. Lackner, A. Csaszar, E. Boerwinkle, and G. Utermann. 1991. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum.Genet. 86:607-614. (Abstr.)
4. Morton, N. E., K. Berg, G. H. Dahlen, R. E. Ferrell, and G. Rhoads. 1985.Genetics of the Lp Lipoprotein in Japanese-Americans. Genet. Epidemiol. 2:113-121
5. Miles, L.A., G.M. Fless, E.G. Levin, A. M. Scanu, and E. F. Plow. 1989. A potential basis for the thrombotic risks associated with lipoprotein(a). Nature(Lond.). 339:301-303.
6. Park pg 377 23rd edition
7. ICMR, 2004) [Assessment of Burden of Non-communicable Diseases, Final Report].
8. Edelberg, J. M., and S. V. Pizzo. 1991. Lipoprotein(a): the link between impaired fibrinolysis and atherosclerosis. Fibrinolysis. 5:135-143.
9. Valko, M., et al. (2007) Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. The International Journal of Biochemistry & Cell Biology, 39, 44-84.
10. Halliwell, B. (1996). Antioxidants in Human Health and Disease. Annual Review of Nutrition, 16(1), 33–50. doi:10.1146/annurev.nu.16.070196.000341Z
11. JaspreetKaur , SarikaArora , Bhawna Singh ,LC Thakur , J Gambhir , KM Prabhu; Role of Oxidative Stress in Pathophysiology of Transient Ischemic Attack and Stroke :Int J Biol Med Res. 2011; 2(3): 611-615
12. Cojocaru IM1, Cojocaru M, Sapira V, Ionescu A. Evaluation of oxidative stress in patients with acute ischemic stroke.Rom J Intern Med. 2013 Apr-Jun;51(2):97-106.
13. RajinderK.DhamijaP.GabaaS.AroraA.KainturaaM.KumaraJ.Bhattacharjeeb. Homocysteine and lipoprotein (a) correlation in ischemic stroke patients.Journal of the Neurological SciencesVolume 281, Issues 1–2, 15 June 2009, Pages 64-68
14. Warner DS, Sheng H, Batinić-Haberle I.; Oxidants, antioxidants and the ischemic brain.:Exp Biol. 2004 Aug;207(Pt 18):3221-31
15. Jürgens G, Költringer P.Lipoprotein(a) in ischemic cerebrovascular disease: a new approach to the assessment of risk for stroke. Neurology. 1987 Mar;37(3):513-5.
16. Milionis HJ, Winder AF, Mikhailidis DP ;Lipoprotein (a) and stroke Journal of Clinical Pathology: 2000;53:487-496.
17. Rigal, M., Ruidavets, J. B., Viguier, A., Petit, R., Perret, B., Ferrieres, J., &Larrue, V. (2007). Lipoprotein (a) and risk of ischemic stroke in young adults. Journal of the Neurological Sciences, 252(1), 39–44. doi:10.1016/j.jns.2006.10.004