ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIO CHEMISTRY

EVALUATION OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN PATIENTS WITH EPILEPSY

RAKESH MUDARADDI[*1], RAMESH [2], SAMEER [3], AMARESHWARA M [4], RAVINDRA MARADI [5], ANIL B [6] AND BEENA V. SHETTY [7]

[1] Department of Biochemistry, SDMCMSH, Dharwad, Karnataka, India.
[2] Department of Biochemistry, RIMS, Raichur, Karnataka, India.
[3] Department of Biochemistry, Sikkim Manipal IMS, Gangtok, Sikkim, India.
[4] Department of Biochemistry, VIMS, Bellary, Karnataka, India.
[5] Department of Biochemistry, Manipal University, KMC, Manipal, India.
[6] Department of Biochemistry, SDMCMSH, Dharwad, Karnataka, India.
[7]Department of Biochemistry, Manipal University, KMC, Mangalore, India.

RAKESH MUDARADDI
Department of Biochemistry, SDMCMSH, Dharwad, Karnataka, India.

*Corresponding author

ABSTRACT

The main aim of this study was to evaluate the role of Plasma advanced oxidation protein product (AOPP), protein carbonyl as indicator of oxidative stress and reduced glutathione, total thiols, albumin as antioxidant status among epileptics. 25 patients with history of epilepsy on treatment in the age group of 20-60 years were compared with 25 normal healthy controls of same age group. There was highly significant increase in AOPP (P=0.002) and protein carbonyl (p=0.001), with significantly decreased antioxidants such as reduced glutathione (p=0.007), total thiols (p=0.023) and albumin (0.01) were noted among epileptics when compared to healthy controls. From this study it could be concluded that increased oxidative stress and reduced antioxidants may be associated with pathophysiology of epilepsy.

KEYWORDS

Epilepsy, AOPP, Protein Carbonyl, Antioxidants

INTRODUCTION

A seizure is paroxysmal event due to abnormal, excessive, hypersynchronous discharges from an aggregate of central nervous system neurons[1]. Annual incidence of epilepsy in the developed countries is approximately 50 per lakh of general population and in the developing countries it is nearly double. Around 50 million people in the world have epilepsy at any one time[2]. The interaction between reactive oxygen species (ROS) and host antioxidant defense systems is a part of normal life and appears to play an important role in normal and abnormal functioning of the central nervous system[3]. The generation of ROS in normal cells, including neurons is under tight homeostatic control. Biological antioxidants and antioxidant enzymes detoxify H_2O_2 by converting it to O_2 and H_2O. However, when ROS levels exceed the antioxidant capacity of a cell, a deleterious condition known as oxidative stress occurs[4]. Free radicals preferentially attack myelin, which contains easily peroxidizable phospholipids. The basal ganglia seems to be an area that is especially susceptible to radical damage which is possibly related to the synthesis of neurotransmitters. The clinical picture of the resulting cell death is dominated by convulsions[5]. So, the present study was planned to evaluate the role of oxidants and antioxidants in epileptics in comparison with normal control subjects.

MATERIALS AND METHODS

Our study comprised 25 epilepsy patients between the age group of 20 to 60 years who were on treatment, selected from the department of medicine. Diagnosis and selection of the patients were made on the basis of detailed history from patients, interview of an eye witness who observed attacks, thorough neurological examination, EEG findings of an interictal record showing bursts of abnormal discharge containing spikes or sharp waves, CT and MRI to exclude a structural cause for seizures were considered.

The control group included 25 normal healthy subjects of same age group. None of the cases and controls had diabetes mellitus, hypertension, rheumatoid arthritis and other disorders known to cause oxidative stress. Smokers, tobacco chewers and alcohol drinkers were excluded from both the study groups and none of them were on antioxidant therapy.

Under aseptic conditions about 5.0 ml of venous blood was drawn and collected in a heparinized vaccutainer, after taking informed consent and study was approved by the ethical committee of the institution. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at room temperature and following parameters were estimated in the plasma.

The present study was conducted to assess oxidative stress in terms of advanced oxidation protein product (AOPP) and protein carbonyl, where as reduced glutathione, total thiols and albumin to evaluate antioxidant status in epilepsy patients.

Protein carbonyl was evaluated by method based on Levin et al. Reaction of the carbonyl group with 2,4-dinitrophenyl hydrazine (DNPH) forms a yellow coloured 2,4-dinitrophenylhydrazone which was measured spectrophotometrically at a wavelength of 360 nm. Values were expressed as micromoles/ml[6].

AOPP was determined by modified Witko's method. Concentration of AOPP was measured by absorbance in acidic conditions at 340 nm in the presence of potassium iodide and total thiol was estimated by G. L. Ellman's

procedure. Both AOPP and total thiols were expressed as mmol/L[7,8].

Reduced glutathione activity was measured by Ernest Beutler method. Dithiobis 2-nitrobenzoic acid (DTNB) is a disulfide, readily reduced by sulphydryl compounds to an intensely yellow coloured compound. The absorbance of reduced chromogen is measured at 412 nm and is directly proportional to the glutathione concentration which is expressed in terms of mg/dl[9].

Total protein and albumin were measured in plasma by Biuret method[10]. Globulins were calculated from the difference between total protein and albumin.

The statistical analysis was done by Mann Whitney 'U' Test.

RESULTS

The plasma AOPP values found in cases showed a highly significant increase (p=0.002) compared to control groups. There was statistically very highly significant increase in protein carbonyl (p=0.001) level in epilepsy cases when compared to controls. There was a highly significant decrease in the plasma reduced glutathione (p=0.007) in epileptics when compared to healthy controls. Plasma total thiols (p=0.023), total proteins (p=0.036), albumin (p=0.01) were also found to be significantly decreased in cases in this study. But difference in the plasma globulin did not show any statistical significance (Table 1).

Table No – 1

comparison of plasma protein oxidation products, antioxidants in epileptics and normal healthy controls.

PARAMETERS	CONTROL (n=25)	TEST (n=25)	P VALUE
AOPP (mmol/L)	0.0672±0.03903	0.1092±0.04893	0.002 (HS)
PROTEIN CARBONYL (µmol/ml)	2.8000±0.63246	9.4200±1.51383	0.001 (VHS)
REDUCED GLUTATHIONE (mg/dl)	10.8760±4.13112	7.5344±3.87024	0.007 (HS)
TOTAL THIOLS (mmol/L)	0.5472±0.17167	0.4160±0.19664	0.023 (S)
TOTAL PROTEIN (g/dl)	6.9220±0.66192	6.2840±0.84911	0.036 (S)
ALBUMIN (g/dl)	4.5020±0.45150	4.0840±0.70751	0.01 (S)
GLOBULIN (g/dl)	2.3752±0.61329	2.2000±0.85440	0.393 (NS)

(n= number of subjects, SD= Standard Deviation, p=Test of significance, S= Significant, HS= Highly Significant, VHS= Very Highly Significant, NS= Not Significant.)

DISCUSSION

Oxidative stress has been implicated in a wide variety of degenerative processes and diseases which includes central nervous system disorders like seizures[11]. Neurons are especially vulnerable to free radical attack and impaired defenses or exposure to excess free radicals can lead to neuronal death [12]. Seizures disrupt normal redox homeostasis in the cell. This may be important for seizure related cell death and have significant functional consequences in surviving neurons[13].

So, the present study emphasizes on an increase in ROS as indicated by elevated AOPP and protein carbonyl in the plasma of epileptics when compared to normal healthy subjects.

These observations are in consistent with that of Jeffrey Lopej et al[14]. AOPP are novel markers of oxidative damage and considered reliable for the estimation of degree of oxidant mediated protein damage. Formation of AOPP could be induced in control plasma by chlorinated oxidants such as chloramines or hypochlorous acid. AOPP resulted from the interaction between such oxidants and plasma proteins. Neutrophils which constitute the most important source of chlorinated oxidants due to their high content in myeloperoxidase might be involved in plasma AOPP formation[15].

In this study we observed increased levels of protein carbonyls in case group. Free radicals produced during oxidative stress can damage the peptide back bone, resulting in generation of protein carbonyls. The process is initiated by hydrogen abstraction from the α-carbon in a peptide chain. If two protein radicals are in close proximity, they may cross link with one another by radical coupling. Alternatively, O_2 can attack the α-carbon centered radical to form peroxide intermediates, leading to rearrangement and subsequent cleavage of the peptide bond to form carbonyl containing peptides. As a marker of oxidative damage to proteins, protein carbonyls have been shown to accumulate during aging and age related diseases in variety of organisms. Levels of protein carbonyls are therefore, a potentially useful indicator of intracellular redox status[16].

Cellular antioxidants known as ROS scavengers protect cells against toxic free radicals[17]. This is supported by the lack of similar increase in antioxidants in the form of reduced glutathione, total thiols and albumin thus leading to oxidative damage.

This study is in accordance with Liao KH et al[18], who reported a decreased glutathione reductase activity in blood of epileptics than in controls, which may lead to a decrease in reduced glutathione. The ratio of reduced glutathione to oxidized glutathione in normal cells is kept high because of reduction of oxidized glutathione back to reduced glutathione by glutathione reductase. Shifting of this ratio towards oxidizing state activates several signaling pathways, there by reducing cell proliferation and increasing apoptosis. The resulted oxidative stress plays a key role in the pathogenesis of many diseases including seizures, Alzheimer's disease and Parkinson's disease[19].

In this study we found low levels of total thiols in epileptics. Ganesan Murali et al[20] observed an associated increase in ROS and decline in the levels of total thiols in epilepsy induced rats. Total thiols comprise a dynamic system called redox thiol status, which is important for normal physiological function. The concept of plasma redox status postulates that, there are dynamic interactions in between the different redox forms of thiols realized through redox reactions, including thiol disulfide exchange. Changes in thiol redox gradient across the cells could adversely affect any transport or cell signaling processes, which are dependent on formation and rupture of disulfide linkages in membrane proteins. Therefore, change in the redox status of thiols significantly influences the structure and function of cellular and extracellular proteins[21].

In the present study we have observed significant decreased plasma total protein and albumin levels in epileptics compared to controls. Albumin may provide antioxidant protection by functioning as a

serum peroxidase in the presence of reduced glutathione and may also help to stop copper ions from peroxidation, by inhibiting copper ion dependent lipid peroxidation and $OH^{\cdot}$ radical formation[22,23]. But, according to P Garzon et al[24] there were no differences with regard to total proteins, albumin and albumin/globulin ratio in serum of epileptics receiving antiepileptic therapy.

From this study it could be concluded that antioxidant status is impaired in epileptics as a consequence of elevated oxidative stress and better treatment with antioxidants, might improve antioxidant status as well as may slow down the progression of the condition. However, large number of studies are required for better understanding of antioxidant role in preventing further future attacks of seizures.

REFERENCES

1. Kasper, Braunwald, Fauci, Hauser, Longo, Jameson. Harrison's Principles of Internal Medicine: seizures and epilepsy. 16th Ed, vol 2, Mc Graw Hill publisher: 2357-2372.
2. Epilepsy: aetiology, epidemiology and prognosis. Programs and Projects. WHO Feb 2001.
3. Byung Ho Choi. Oxygen, Antioxidants and Brain Dysfunction. Yonsei Medical Journal, 34(1):1-10, (1993).
4. Jeffrey A, Klein, Susan L, Ackerman. Oxidative stress, cell cycle and neurodegeneration. The journal of clinical investigation, 111(6): 785-793, (2003).
5. Weber GF. The pathophysiology of reactive oxygen intermediates in the central nervous system. Ann Neurol, 32: 16-21, (1992).
6. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG et al. Determination of carbonyl content in oxidatively modified proteins. Meth Enzymol, 186: 464-78, (1990).
7. Witko V, Nguyen AT, Descamp S, Latscha B. Microtitre plate assay for phagocyte derived taurine chloramines. Journal of Clinical Lab Anals, 6: 47-53, (1992).
8. GL Ellman. Measurement of total thiols in plasma. Archives of Biochemistry and Biophysics, 82: 70, (1959).
9. Ernest Beutler, Olga Duron, Barbara Milius Kelly. Improved method for the determination of blood glutathione. Journal of Clinical Lab Medicine, 61: 883-887, (1963).
10. Harold V. Proteins. Text book of Practical Clinical Chemistry, 5th Ed, vol 1: 963-967, (2004).
11. Davies KJ. Oxidative stress: the paradox of aerobic life. Biochem Soc Symp, 61: 1-31, (1995).
12. Jesberger JA, Richardson JS. Oxygen free radicals and brain dysfunction. Int J Neuroscience, 57(1-2): 1-17, (1991).
13. Cock, Hannah R. Mitochondrial dysfunction and oxidative stress in epilepsy: 105-137.
14. Jeffrey Lopez, Maria E, Gonzalez, Lourdes Lorigados, Lilia Morales, Gretel Riveron, Jose Y. Bauza. Oxidative stress markers in surgically treated patients with refractory epilepsy. Science Direct Clinical Biochemistry, (2007 Jan).
15. Melike Erol Demir Bilek, Nedret Kilic, H.Ferhan Komurcu, K. Okhan Akin. Advanced oxidation protein products in aged with dementia. American J of Immunology, 3(2): 52-55, (2007).
16. Lawrence J, Marnett, James N, Riggins, James D West. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. The journal of Clinical Investigation, 111(5): 583-593, (2003).
17. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione Metabolism and its implications for Health. J Nutr, 134: 489-92, (2004).
18. Liao KH, Mei QY, Zhou YC. Determination of antioxidants in plasma and erythrocyte

in patients with epilepsy. Zhong Nan Da Xue Bao Yi Xue Ban, 29(1): 72-4, (2004).
19. Guoyao Wu, Yun-Zhong Fang, Sheng Yang, Joanne R, Lupton, Nancy D Turner. Glutathione metabolism and its implications for health. J. Nutr, 134: 489-492, (2004).
20. Ganesan Murali and chinnakkannu Panneerselvam. Age associated oxidative macromolecular damages in rat brain regions: role of glutathione monoester. The Journals of Gerontology series A: Biological science and medical sciences, 62: 824-830, (2007).
21. Deneke S M. Thiol based antioxidants. Current topics in cellular regulation, 36: 151-180, (2000).
22. Barry Halliwell, JMC Gutteridge. The antioxidants of human extracellular fluids: Archives of Biochemistry and Biophysics, 280(1): 1-8, (1990).
23. Charles A, Thomas Jr, Mark F. McCarty. Biochemical Health Profiling: Antioxidants. The Linus Pauling Institute, Oregon. Spring/Summer (1999).
24. P Garzon, S Gonzalez-Cornejo, G Hernandez- Hernandez, L Aguirre-Portillo, C Almodovar-Cuevas, A Navarro-Ruiz. Effects of antiepileptic drugs on concentration of serum proteins and immunoglobulins of epileptic patients. Gen Pharmacol, 17(1): 49-55, (1986).