



RESEARCH ARTICLE

RELATION OF OXIDATIVE STRESS AND SEIZURES IN EPILEPSY

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ABSTRACT

Background: Epilepsy is a neurological disorder of brain. Oxidative stress may contribute to seizures noticed in epilepsy. The present study was taken to relate the oxidative stress role in epilepsy and also possible mechanism which may lead to further seizures and finally neuronal death noticed in Epilepsy.

Aims and objectives: A total of 150 patients and 100 cases were taken in the study. All patients were diagnosed as epileptic by neurologist. Patients were categorized as Group-I (GTCS) 50 patients, group-II (JME) 50 patients and Group-III controls 50. All patients were on treatment with Anti Epileptic Drugs (AED) monotherapy and none of them are on Polytherapy i.e., 1 or 2 AEDs. Biochemical parameters MDA were analyzed by TBARS method and Total Thiols by Ellman's method. Continuous variable with a normal distribution were expressed as means with Standard error of mean. The intergroup difference was tested using one way ANOVA test. The categorical variables were expressed as numbers with percentage. All statistical analysis was performed by using SPSS (15.0) package. A value of $P < 0.05$ was considered as statistically significant.

Results: The MDA levels were significantly increased in cases compared to controls, whereas Total Thiols were significantly decreased compared to controls with P -value < 0.001 .

Conclusion: study reports increased oxidative stress (OS) and decreased antioxidant status, suggesting that OS has relation with seizure generation and pathophysiology of epilepsy. Treatment of Epilepsy with antioxidants, along with anti-epileptic drugs (AEDs) may help to scavenge the excessive free radicals generation in epilepsy.

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INTRODUCTION

Epilepsy history intervened with the history of humanity. Epileptic seizure description can be traced back to 2000 B.C. It is the neurological disorder of the brain affecting 1-2% of the population, and around 70 million worldwide affecting the quality of life (Ngugi *et al.*, 2010). In India, the prevalence contributes to one-sixth of the global burden (Senthil Amudhan *et al.*, 2015). Epileptic Seizures is a brief episode due to abnormal excessive synchronous neuronal activity. It is a disease of brain characterized by an enduring predisposition to generate epileptic seizures (Fisher *et al.*, 2005; Fisher *et al.*, 2014). Several factors like genetic, excitotoxicity-induced mitochondrial dysfunction, altered cytokine levels,

and oxidative stress have been implicated in epileptogenesis (Ferriero, 2005). Recently, much attention was paid to find the oxidative stress role by analysing the biochemical alteration levels to study the pathological mechanisms in epilepsy. Studies reported that oxidative stress plays a vital role in epilepsy, but the exact mechanism of oxidative stress during seizures remains unclear (Ho *et al.*, 2015). Brain prone to more oxidative stress as it utilizes a high amount of oxygen, and it contains high concentrations of polyunsaturated fatty acids, that are prone to peroxidation, and metals, such as iron, which can catalyze hydroxyl radical formation and is relatively deficient in antioxidant enzymes is more susceptible to the damaging effect of oxidative stress. Therefore, oxidative injury in the brain was one of the main causes of a number of neurologic conditions and neurodegenerative disorders, including epilepsy (Manmohan *et al.*, 2012). It was demonstrated that status epilepticus cause oxidative damage to biomolecules, change redox potential decreasing ATP levels,

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which can lead brain to suffer with lack of energy supply (Wasterlain *et al.*, 1993; Liang *et al.*, 2006). Evidences from seizure induced animal models, Knockout animal models and genetic studies also proposed that increase ROS production, mitochondrial dysfunction might be the reasons leading to neuronal death observed in the brain regions like hippocampus after persistent seizures showing that connection exists between oxidative stress and Epilepsy (Shin *et al.*, 2011; Milder *et al.*, 2011).

The existing literature report that oxidative stress may contribute to Epilepsy and this mechanism might lead to seizures. Therefore, the present study was taken to relate the oxidative stress role in epilepsy and also possible mechanism which may lead to further seizures and finally neuronal death noticed in Epilepsy.

MATERIALS AND METHODS

Subjects

A total of 150 patients 100 cases and 50 age matched controls were recruited for the present study. All the patients were diagnosed as epileptic by neurologist from Girija Neuro center Vijayawada. Patients were categorized into two groups. Group-I 50 patients with Generalized Tonic Clonic seizures (GTCS), Group- II 50 patients with Juvenile Myoclonic Epilepsy (JME) (Myoclonic seizures) with an age group of 5-40 years were taken to the study, and Group-III 50 age and sex matched healthy volunteers were taken as controls from same geographic area. All Epilepsy patients taken in study were on treatment with Anti Epileptic Drugs (AED). In Group-I (n=30) are on Carbamazepin (CBZ), (n=10) on Phenobarbitol (PB) and (n=10) on Eptoin. In Group- II (n= 10) are on Carbamazepin (CBZ), (n=39) are on sodium Valoprate (SV), (n=1) on Phenobarbitol (PB). All the patients recruited in the study are on monotherapy and none of them are on Polytherapy i.e., 1 or 2 AEDs. Ethical Approval for this study was obtained from the ethical committee of "Institutional Human Ethical Committee, Bidar Institute of Medical Sciences", Bidar, Karnataka and a written informed consent was obtained from all the participants.

Selection of participants

The Detailed information on the study was given to each patient and all subjects gave their written consent to attend this study.

For each patient, the following information were obtained: complete medical history of patient was taken, interview of an eye witness who observed attacks was taken and clinical examination with special emphasis on age, sex, type and frequency of seizure, duration of illness, AED(s) used with their doses, and age of starting treatment and control of seizures. EEG findings of an interictal record showing bursts of abnormal discharge containing spikes or sharp waves, CT and MRI to exclude astructural and other neurological disorders which may lead to cause for seizures were excluded. Smokers, tobacco chewers were excluded from both the study groups and none of them were on antioxidant therapy.

Collection of blood samples

Around 3ml of blood samples was collected from all the patients and controls in plain bottles and allowed to clot for one hour and centrifuged at 2000rpm for 10 min serum obtained is collected and stored at -40°C until analysis.

Biochemical Analysis

Serum MDA levels were measured by TBARS method. Total thiols analysed by Levine *et al.* Method using DNPH (Ellman's reagent) spectrophotometric method.

Statistical Analysis

The Statistical software namely SPSS 15.0 was used for the analysis. Results were expressed as Mean \pm SEM and Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, Post-Hoc Tukey test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Significance is assessed at 5 % level of significance.

RESULTS

Table 1 showing the age distribution in the study group and also in the control group focusing on Mean \pm SEM and percentages which shows that most of the patients age group in all three groups were between 21-30 where in group-I it is 46%, Group-II 36% and controls 52%.

Table 1. Age distribution in study groups and control group

Age in years	Group I (GTCS)		Group II (JME)		Group III (controls)	
	No	%	No	%	No	%
<10	3	6.0	5	10.0	2	4.0
10-20	11	22.0	28	56.0	5	10.0
21-30	23	46.0	17	34.0	26	52.0
31-40	13	26.0	0	00.0	17	34.0
Total	50	100.0	50	100.0	50	100.0
Mean \pm SEM	24.04 \pm 1.42		19.06 \pm 1.29		26.66 \pm 0.84	

Table 2. Gender distribution in patient group and control group

Gender	Group I (GTCS)		Group II (JME)		Group III (controls)	
	No	%	No	%	No	%
Female	22	44.0	28	56.0	24	28.0
Male	28	56.0	22	44.0	26	52.0
Total	50	100.0	50	100.0	50	100.0

Table 2: Representing the gender distribution in all the three groups. In GTCS Group-I and in Controls the male % was more compared to female % whereas in Group-II Female % was more.

Table 3. Comparison of study variables in three groups

	Group I (GTCS)	Group II (JME)	Group III (CONTROLS)	P value
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	
MDA (nmol/ml)	4.61 \pm 0.24	4.79 \pm 0.27	2.79 \pm 0.19	<0.001**
Total Thiols (μ moles/L)	120.13 \pm 9.12	118.76 \pm 8.76	247.67 \pm 5.51	<0.001**

Table 4. ANOVA Table of MDA (nmol /ml) for group wise

ANOVA					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	121.531	2	60.765	21.416	<0.001
Within Groups	417.086	147	2.837		
Total	538.617	149			

P value <0.001, There is a significance difference in MDA between the three group.

Table 5. ANOVA Table of TOTAL THIOLS for group wise

ANOVA					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	548149.500	2	274074.750	86.483	<.001
Within Groups	465860.207	147	3169.117		
Total	1014009.708	149			

P value <0.001, There is a significance difference in TOTAL THIOLS between the 3 group.

ANOVA test was used to find the significance difference in markers between three groups. The MDA levels were significantly increased in cases compared to controls, whereas Total Thiols were significantly decreased compared to controls with P-value <0.001.

DISCUSSION

Oxidative stress was one the important mechanism in the development and progression of many neurological disorders, including epilepsy (Ramalingam *et al.*, 2013). Seizure generation may be related to the homeostatic imbalance of antioxidants and oxidants. The present study results indicate increased levels of oxidants MDA (4.61 \pm 0.24, 4.79 \pm 0.27) and decreased levels of antioxidants (120.13 \pm 9.12, 118.76 \pm 8.76) in both GTCS and JME patients compared with controls. Our study was in line with the studies Rakesh Mudaraddi *et al.*, 2011; Pandey *et al.*, 2012 Mahmut Abuhandan *et al.*, 2013 who reported that increased lipid peroxidation might be one of the causes of brain injury and may contribute to neuronal cell death. The Increased influx of calcium observed in epileptic seizures can trigger mitochondria to generate increased reactive oxygen species (ROS) for a prolonged period can cause neurodegeneration. Neurons are especially vulnerable to free radical attack and impaired defences or exposure to excess free radical can lead to neuronal death (Jesberger *et al.*, 1991). One of the important concepts which attracted the attention of research in epilepsy was the increased oxidative stress observed in epilepsy can lead to mitochondrial dysfunction. As mitochondria the main site to regulate the intracellular calcium homeostasis, may get effected by the increased ROS generation which can lead to neuronal excitability and synaptic transmission making neurons more vulnerable to oxidative stress, which may lead to energy failure in brain, which mainly dependent on mitochondria for ATP generated by oxidative phosphorylation. This energy depletion possibly can lead to neuronal death in

epilepsy and increased oxidative stress might be the reason for the repeated onset of further seizures in epilepsy and also its severity (Waldbaum *et al.*, 2010; Chang *et al.*, 2010). The study also reported decreased total thiol levels in both groups GTCS and JME (120.13 \pm 9.12; 118.76 \pm 8.76) compared with controls. Total thiols consists glutathione (GSH) and protein thiols (PSH), Glutathione and its oxidized disulfide GSSG are generally considered as thiols and indicators of oxidative stress, but in total thiols there was a contribution of other Non protein thiols like Cysteine which was mostly ignored. Recent studies done on animal brain tissue shows non protein oxidized thiols were much varied compared to main thiols GSH, CSH against oxidative stress (Patsoukis *et al.*, 2004; Akerboom *et al.*, 1981; Giles *et al.*, 2001). Among the total thiols GSH and cysteine were most studied in epilepsy as GSH main thiol in total Redox system, abundant thiol act as antioxidant defense mechanisms, and also GSH has role in release of glutamate, which mainly observed in epilepsy (Janaky *et al.*, 1999; Dringen *et al.*, 2000). Modification of intracellular levels of GSH has also been shown to regulate seizure susceptibility and neuronal survival (Nikolaos *et al.*, 2005). Our study results correlate with studies of Sudha *et al.*, 2001; Mueller *et al.*, 2001; Rakesh Mudaraddi *et al.*, 2011; reported both GSH levels and GR activity are lowered in brain regions and plasma of epileptic patients. Another important non protein thiol gained importance in epilepsy was Cysteine. As neurons exchange extracellular cysteine with intracellular excitotoxic glutamate a protective antioxidant mechanism which utilizes cysteine for GSH synthesis and glutamate converted to glutamine by astrocytes and microglia which connects antioxidant defense with neuronal excitability. Whereas increased levels of glutamate impairs this mechanism which mainly observed in increased oxidative stress leading to hyperexcitability of neurons and, further seizures along with neuronal death (Albrecht *et al.*, 2010; Aissouni *et al.*, 2012). The role of ROS balance was important in neurological disorders because neurons are more vulnerable to the harmful

effects of ROS and also CNS had a poor antioxidant defense mechanism indicating that oxidative stress play role in the pathological mechanism of epilepsy. Evidences from experimental and human epilepsy proposed that as brain contains more mitochondria any dysfunction to mitochondria can lead to neuronal death and can prolong the process of epilepsy (Divya et al., 2013). Martinc et al., 2012 explained that increased ROS generation causes changes in calcium levels noted in epilepsy may lead to increased release of glutamate by altering GABA receptor. The glutamate can again contribute to OS and hyperexcitability cell death and further seizures.

Mitochondria a sub cellular organelle performs many important functions as calcium homeostasis, ATP generation by oxidative phosphorylation, regulation of cell death, and it was a main site for ROS generation. The ROS generated normally mediated signal transduction, but increased ROS levels leads oxidative damage which can cause further seizures which was associated with epilepsy. Evidences from human and experimental studies explained to some extent the mitochondrial dysfunction in epilepsy but, the clear, detailed role of mitochondria and its relation to seizure induced neuronal death remains unclear (Divya et al., 2013). In conclusion, the present study reports increased oxidative stress (OS) and decreased antioxidant status, suggesting that OS has relation with seizure generation and pathophysiology of epilepsy. Treatment of Epilepsy with antioxidants, along with anti-epileptic drugs (AEDs) may help to scavenge the excessive free radicals generation in epilepsy.

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