Original Article

ID: JCDR/2012/4228:0011

# The Lipid Peroxidation Product as a Marker of Oxidative Stress in Epilepsy

Biochemistry Section

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## ABSTRACT

**Context:** Free radicals have been implicated in the development of acute and chronic diseases of the brain e.g. Epilepsy, Cerebrovascular diseases, Parkinson's disease, Alzheimer's disease, etc. The present study was designed to assess the oxidative stress in epilepsy, since very few of such studies have been designed in human beings.

**Aims:** To study the level of the lipid peroxidation products i.e. malondialdehyde (MDA) as a marker of oxidative stress in epilepsy patients.

**Settings and Design:** This case control study had 170 samples which comprised Group I(n-90) patients of epilepsy as the cases, which were compared with Group II (n-80) which were age and sex matched controls.

**Methods and Materials:** The lipid peroxidation product i.e. MDA formation was estimated by assessing the levels of thio-barbituric acid reactive substances (TBARS) by using spectrophotometry.

**Statistical Analysis Used:** The statistical analysis was done by using the SPSS software and the results were described by using the unpaired T test and p values.

**Results:** In the present study, the mean MDA value in Group I (2.38 ±0.31) was significantly raised ($p<0.01$) than in Group II (2.15±0.36), thus suggesting that the MDA values were raised in epilepsy. The mean MDA level after 1 year of therapy was 2.25±0.25, with a p value of $<0.05$, thus suggesting a reduction in the oxidative stress with therapy.

**Conclusions:** The level of the lipid peroxidation is significantly higher in epilepsy as compared to the control and oxidative stress increases which were found with the duration of the epilepsy. The oxidative stress had no significant difference in the males and females. The oxidative stress was found to reduce on adequate antiepileptic therapy.

**Key Words:** Malondialdehyde (MDA), Thio- Barbituric Acid Reactive Substances (TBARS), Lipid Peroxidation Product (LPP)

## INTRODUCTION

Lipids are important components of the cell membrane. Lipid peroxidation is implicated in the pathogenesis of a number of diseases and clinical conditions. These include diabetes, adult respiratory distress syndrome, premature birth disorder, aspects of shock, Parkinson's disease, Alzheimer's disease, pre-eclampsia and eclampsia, various chronic inflammatory conditions, ischaemia, reperfusion mediated injury to organs which include the heart, brain and the intestine, atherosclerosis, organ injury which is associated with shock and inflammation, fibrosis, cancer, inflammatory liver injury, anthracycline induced cardiotoxicity, silicosis and pneumoconiosis [1,2,3,4]. It has been suggested that an increase in the free radicals may cause neuronal degeneration through lipid peroxidation and a decrease in the glutathione peroxidase levels. The lipid peroxidation product, malondialdehyde (MDA) is commonly used as a measure of the oxidative stress in cells. Lipid peroxidation, being a free radical reaction, it occurs when the hydroxyl radicals, possibly oxygen, react with the unsaturated lipids of the bio-membranes, resulting in the generation of lipid peroxide radicals (ROO•), lipid hydroperoxide (ROOH) and fragmentation products such as MDA [5,6]. This aldehyde is a highly toxic molecule and it should be considered as more than just a marker of lipid peroxidation. Its interaction with the DNA and proteins has often been referred to as potentially mutagenic and atherogenic. Researchers have evaluated the role of oxidative stress in epilepsy in several animal models (Lores et al., [7]; Uoda et al., [8]; and its correlation in human beings was done by Rao and Rao [9], Wilson [10], and Roktya et al., [11]. Our study was planned with the following aims and objectives:

- To study the level of the lipid peroxidation product i.e. MDA as a marker of oxidative stress in epilepsy patients.
- To compare this with the level in the controls (i.e. without epilepsy)

## SUBJECTS AND METHODS

This cross-sectional study was conducted at a tertiary care hospital in north India. An informed consent was obtained from all the participants (from parents if participant was minor) and the institutional ethics committee approved the study. Each case of epilepsy was asked to give a detailed clinical history or the attendants of the patients or witnesses were asked to give it.

### Study Cases

The cases were divided in two groups:

Group-1: Epileptic cases (n-90)
Group-II: Control group (n-80)

### Inclusion Criteria

1. Age between 10 to 60 years, thus ensuring a representative sample of the epileptics, which was comparable with the standardization samples of the questionnaire which was used.

2. The diagnosis of epilepsy was corroborated on the basis of the clinical history or a definitely abnormal electroencephalogram (EEG).
3. A minimum period with epilepsy for one year, during which five or more epileptic attacks had occurred.
4. Both new and follow-up patients were taken for the study.
5. No clinical evidence of a drug overdose and a postictal effect at the time of the assessment.

### Exclusion Criteria

1. Patients who were more than 60 years and less than 10 years of age.
2. Patients who were suffering from any other chronic serious physical illness or an organic brain syndrome due to some cause other than epilepsy.

The control groups were persons who visited the hospital during the same period, who were not suffering from epilepsy and were age and sex matched.

**Methods Estimation of MDA:** MDA was estimated by assessing the levels of Thio- Barbituric Acid Reactive Substances (TBARS). The TBARS assay was performed by using MDA equivalents which were derived from tetra-ethoxy-propane. MDA was identified as a product of lipid peroxidation which reacted with TBA to give a pink coloured species that gave an absorbance at 532 nm. The method involved heating of the separated platelets of the patients with the TBA reagent which contained Tri-chloro Acetic acid (TCA), Thio-Barbituric Acid (TBA) and Hydrochloric Acid (HCl). After cooling the solution, it was centrifuged at 2000 rpm and the precipitate which was obtained was removed .The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents minus the platelets. The MDA equivalents of the sample were calculated by using an extinction of $1.56 \times 10^5 M^{-1}cm^{-1}$ [12]. The level of MDA was read as nmoles MDA per $10^9$ platelets.

44 patients (30 males and 14 females) were given carbamazepine 600 to 1200 mg per day as per the weight of the case and their MDA levels were measured after one year.

## RESULTS

The total number of cases which were included in this study were 170, out of which 105 (61.76%) were males and 65(38.23%) were females. The male to female ratio in this study was 1.6:1. The mean value of MDA in Group I was 2.38±0.31 and in Group II, it was 2.15±0.36 (p<0.01), thus suggesting the occurrence of oxidative stress in epilepsy [Table/Fig-1]. A maximum of 29 patients (32.22%) in Group I fell in the age group of 21 to 30 years, followed by 25 in the age group of 15 to 20 years. The mean age of the patients in Group I was 29.72±10.53 and that in Group II was 29.72±10.53, thus suggesting that epilepsy was a disease of the young population [Table/Fig-2]. The duration of the epilepsy was compared with the oxidative stress marker in Group-I. Patients with a longer history of epilepsy had higher levels of MDA [Table/Fig-3]. 44 patients of Group I were followed up after 1 year of antiepileptic (carbamazepine) treatment according to the weight of the patients. These patients had no seizure for one year. The mean MDA level after one year of therapy was 2.25±0.25 (p value<0.05), thus suggesting a significant difference which showed reduction in the oxidative stress with therapy [Table/Fig-4]. It had been already seen in previous studies that carbamazepine itself had an antioxidant effect [13]. The most common seizure type in the present study

| | Group | I | II |
|---|---|---|---|
| Male | No. | 60 | 45 |
| | Mean | 2.38 | 2.18 |
| | SD | 0.35 | 0.42 |
| | T | 2.59 | |
| | P | p<.05 | |
| Female | No. | 30 | 35 |
| | Mean | 2.36 | 2.11 |
| | SD | 0.18 | 0.27 |
| | T | 4.44 | |
| | P | p<.01 | |
| Total | No. | 90 | 80 |
| | Mean | 2.38 | 2.15 |
| | SD | 0.31 | 0.36 |
| | T | 4.43 | |
| | P | p<.01 | |

**[Table/Fig-1]:** Average levels of MDA

| Age | | I | II |
|---|---|---|---|
| 10-20 | No. | 25 | 20 |
| | Percent | 27.78 | 25.00 |
| 21-30 | No. | 29 | 26 |
| | Percent | 32.22 | 32.50 |
| 31-40 | No. | 13 | 17 |
| | Percent | 14.44 | 21.25 |
| 41-50 | No. | 21 | 16 |
| | Percent | 23.33 | 20.00 |
| 51-60 | No. | 2 | 1 |
| | Percent | 2.22 | 1.25 |
| Total | | 90 | 80 |
| Mean | | 29.72 | 29.72 |
| SD | | 10.53 | 10.53 |

**[Table/Fig-2]:** Age Distribution of Cases

| Duration | 0-5 | 6-10 | 11-15 | 16-20 | 2-25 |
|---|---|---|---|---|---|
| Group I | 2.26 | 2.42 | 2.8 | - | 2.94 |

**[Table/Fig-3]:** MDA Level & Duration of Epilepsy

| | Group –I | Group I after 1 year of therapy |
|---|---|---|
| N | 90 | 44 |
| Mean MDA level | 2.38 | 2,25 |
| SD | 0.31 | 0.21 |
| 't' | 2.5 | |
| P | <0.05 | |

**[Table/Fig-4]:** MDA –After One Year of Carbamazepine Therapy

| Type of Seizure | No. | Percent |
|---|---|---|
| Generalized | 32 | 35.56 |
| Partial | 40 | 44.44 |
| Partial with sec. generalization | 11 | 12.22 |
| Mixed Type | 3 | 3.33 |
| Atypical | 4 | 4.44 |
| Total | 90 | |

**[Table/Fig-5]:** Seizure Types in Epilepsy

was partial seizures (44.44%), followed by the generalized type of seizures (35.56%) [Table/Fig-5].

## DISCUSSION

Membrane lipids which contain unsaturated fatty acids are particularly sensitive to oxidative stress, and peroxidation of the membrane lipids leads to a disturbance of the membrane integrity [14,15]. The normally damaged membranes are repaired and one important repair mechanism is reacylation of the phospholipids in the membrane. There are reports that lipid peroxidation inhibits this reacylation process [14]. The nervous system is more susceptible to the damaging effect of oxidative stress, due to the high content of polyunsaturated fatty acids that are susceptible to lipid peroxidation [16]. It receives a large percentage of oxygen and is relatively deficient in antioxidant enzymes. It has been suggested that an increase in the free radicals may cause neuronal degeneration through per-oxidation and decrease in the glutathione peroxidise levels. These free radicals have been implicated in the development of many acute and chronic diseases of the brain, like epilepsy, cerebrovascular disease, Alzheimer's disease, etc. In the human brain, there is a distinct regional distribution of thio-barbituric-acid (TBA) positive materials in the endogenous pool, with higher levels in the cerebellar vermis and lower levels in the thalamus, cortical regions, substantia nigra, caudate nucleus, pallidum, putamen, thalamus and the pineal gland [17]. Oxidative stress exacerbates the aetiology of epilepsy [18].

The present study signified the higher levels of oxidative stress in epilepsy than in the age and sex matched controls. This oxidative stress was decreased in patients who took regular antiepileptic medication for one year. The present study was supported by evidences which were provided by the past studies.

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**FINANCIAL OR OTHER COMPETING INTERESTS:**

None.

Date of Submission: **Mar 04, 2012**
Date of Peer review: **Apr 14, 2012**
Date of Acceptance: **Apr 18, 2012**
Date of Publishing: **May 31, 2012**