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CYTOGENETICS AND MOLECULAR STUDIES ON EPILEPTIC CHILDREN

Medical Science

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ABSTRACT

Epilepsy is a neuronal disorder that is observed globally but still it is not explored very well in most parts of the world. This disease is linked to different provocative causes and affects almost all generation, ethnicity and age population. There are 50 million people living with epilepsy worldwide. The last decade has witnessed rapid advances in understanding the role of factors in epilepsy. The aim of the present study was to evaluate cytogenetic and molecular studies on epileptic children. Forty one study subjects with epilepsy and twenty healthy control subjects were taken for this study. The extent of DNA damage was quantified by Cytokinesis-Block Micronuclei (CBMN) assay. Detailed demographic, clinical and lifestyle characteristics were compared with subjects. The micronuclei frequency was significantly elevated in study subjects as compared with that of control subjects. Various risk factors such as demographic, clinical and biochemical characters of subjects showed increased micronuclei. Treatment can reduce or prevent seizures with improving the quality of life. Controlling epilepsy also lowers the risk of falling and other complications.

KEYWORDS

Epilepsy, Seizure, DNA damage and Cytokinesis-Block Micronuclei (CBMN) assay

INTRODUCTION

Epilepsy is a chronic disorder, hallmark of which is recurrent, unprovoked seizures. Epilepsy affects the central nervous system and as a result nerve cell activity in the brain becomes distorted and causes seizures. Seizures occur due to a sudden rush of electrical activity in the brain. Basically there are two types of seizures, focal seizures and generalized seizures. Adults and children have same type of seizure. Seizures beginning from one area of the brain are called focal seizures whereas generalized seizures affect both the cerebral hemispheres. Some children develop epilepsy as a result of any injury caused to brain, For example meningitis, difficulties at birth, severe head injury etc. Epilepsy with a likely genetic cause is called idiopathic epilepsy. Infectious diseases play an important role in seizures and long term burden causing both new onset epilepsy and status epilepticus (Nandanavana, 2014).

There are 50 million people living with epilepsy worldwide, and most of them reside in developing countries. About 10 million persons with epilepsy are there in India. The last decade has witnessed rapid advances in understanding the role of genetic factors in epilepsy. Mutations and chromosomal defects underlying many inherited symptomatic epilepsies have now been identified, and several genes have been associated with rare idiopathic epilepsies transmitted in a mendelian manner. However, the genetic factors underlying inherited susceptibility to idiopathic epilepsy remain to be identified (Dimitri, 2002).

Epilepsy has an impact on many aspects of a child's development and functioning. It affects about one in 11 persons experiencing at least one seizure at some point (William et al., 1998). Nowadays research demonstrate that families of children with epilepsy function less well, experience more maternal stress and depression, particularly when the child has a comorbid condition such as behavior disorder or intellectual disability. Since today's children are tomorrow's future, understanding about the disorder will help in finding out better management options. Genetic testing may assist with understanding the progress or outlook of epilepsy and provides a basis for further genetic counseling for the family, if required.

MATERIALS AND METHODS

Forty one study subjects with epilepsy and twenty healthy control subjects were taken for this study. All these study subjects were referred from Sree Chitra Tirunal Institute for Medical Sciences and Technology to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala for genetic testing. Detailed socioeconomic, demographic and other relevant clinical information were recorded using proforma. 5 ml of venous blood was collected in sodium heparinized vacutainers for quantifying the extent of somatic DNA damages by cytokinesis-block micronuclei (CBMN) assay.

Two ml blood was added to a culture tube containing 10 ml RPMI 1640 medium supplemented with 100units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 10µg/mL phytohaemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) at 44th hours of incubation. Cells were harvested after 72nd hr incubation, and they were treated with a hypotonic KCl solution (0.075M) for 10 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

OBSERVATIONS AND RESULTS

Distribution of mean CBMN frequency among study and control subjects

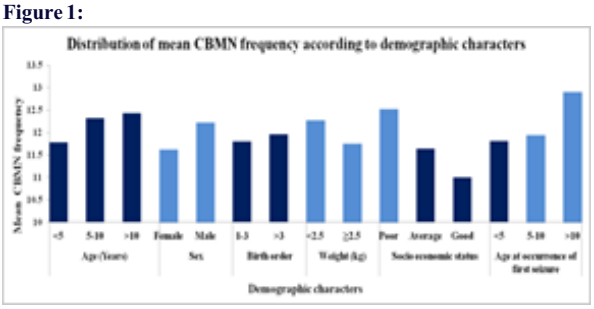
Table 1:

Subjects	Number	Mean CBMN frequency
Study subjects	41	11.96
Control subjects	20	10.11

Distribution of mean CBMN frequency of the study and control subjects was given in table 1. The mean CBMN frequency of study subjects was 11.96 and control subjects was 10.11. The mean CBMN frequency of the study subjects was comparatively higher than that of control subjects.

Distribution of mean CBMN frequency according to various demographic characteristics of the study subjects

Figure 1:

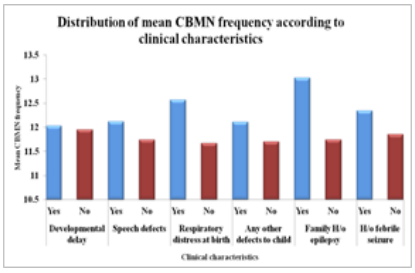


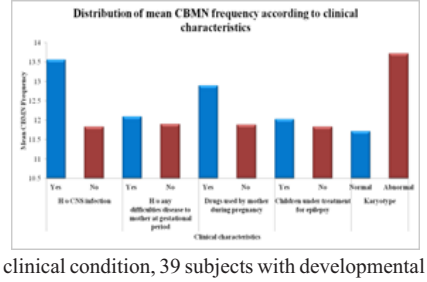
The age of the study subjects were ranged from few months to 15 years with a mean age of 7.6 years and the age of the control subjects ranged from 2 to 15 years with a mean age of 7.9 years. 15 (36.59%) study subjects belonged to the age range of >10 years showed high mean CBMN frequency of 12.43. Male subjects were showed highest mean CBMN frequency. This indicates that male subjects are more susceptible to epilepsy. Subjects with low birth weight (<2.5kg) were showed higher mean CBMN frequency of 12.27. Subjects with poor social status were showed mean CBMN frequency of 12.52 and lowest mean CBMN frequency was observed in subjects with good socio economic status. The study subjects were grouped according to their age at occurrence of first seizure. The mean CBMN frequency (12.9) was shown to be high in subjects whose age ranged >10 years compared to the age range <5 years having mean CBMN frequency (11.81).

Distribution of mean CBMN frequency according to various clinical characteristics of the study subjects

Table 2:

Category	Variables	Number (Percentage %)	Mean CBMN frequency
Karyotype	Normal	36 (89%)	11.71
	Abnormal	5 (12.19%)	13.72





Based on clinical condition, 39 subjects with developmental delay and their mean CBMN frequency was 12.03. Subjects having speech defects were observed with a mean CBMN frequency of 12.12. Subjects with respiratory distress have high mean CBMN frequency (12.57). Family H/o epilepsy were observed in 7 (17%) subjects with high mean CBMN frequency of 13.02 and those without family H/o epilepsy had a mean CBMN frequency of 11.74. This indicates family H/o epilepsy has a correlation with mean CBMN frequency. 9 (21.95%) study subjects with H/o febrile seizures had a mean CBMN frequency of 12.34 and those without H/o febrile seizures was 38 (92.68%) and had a mean CBMN frequency of 11.85.

H/o CNS infection was observed in 3 (7.31%) subjects and had a mean CBMN frequency of 13.56. 11 subjects having H/o any difficulties/disease during gestational period of mother and observed with highest mean CBMN frequency (12.09). Some mothers were observed to be using certain drugs during pregnancy period and their mean CBMN frequency was high (12.89). Karyotype of subjects was also analyzed based on mean CBMN frequency. 5 subjects had abnormal karyotype and they showed high mean CBMN frequency of 13.72.

Distribution of mean CBMN frequency according to biochemical characteristics

Table 3:

Category	Variables	Number (Percentage %)	Mean CBMN frequency
RBS (mg/dl)	70-130	24 (58.54%)	11.86
	>130	17 (41.46 %)	12.09
Blood urea (mg/dl)	5-20	31 (75.6%)	11.79
	>20	10 (24.3%)	12.47

The mean CBMN frequency was analyzed based on the biochemical characteristics. 17 study subjects were having blood sugar >130 mg/dl and they showed high mean CBMN frequency of 12.09. The study subjects were also analyzed by blood urea level and the highest mean CBMN frequency (12.47) was observed in 10 (24.3%) study subjects with blood urea level >20 mg/dl.

DISCUSSION

Many studies report a higher incidence of epilepsy in males than female in both developed and developing countries (Noronha et al., 2007). The difference in incidence may be due to some sex hormones which has some association with epilepsy. Present study also showed majority of epileptic subjects were males and having highest mean CBMN frequency.

According to Gajana (1995) and Bale (1993), there is good evidence that children in developing countries are exposed to higher rates of events that can cause epilepsy such as CNS infection. Present study also revealed that children with the H/o CNS infection had increase in the mean CBMN frequency.

Bharucha, (2003) suggests that a variety of risk factors are thought to be significant in developing countries to cause epilepsy. These include neurocysticercosis, other nervous system infections, head trauma, perinatal factors, genetic problems caused by consanguinity, and febrile seizures. In the present study H/o febrile seizure to the child clearly indicates an increase in the mean CBMN frequency. So the present study also proves febrile seizure is a risk factor for causing epilepsy.

Sawhney et al., (1999) found in addition that head injury, developmental delay, and a family history of epilepsy were significant risk factors. According to the present study those having developmental delay and a family H/o epilepsy had a higher mean CBMN frequency. This result suggests a strong correlation between the DNA damage and epilepsy.

According to Carl (2003), abnormal glucose levels, whether too high or too low, can cause seizures. Clinical studies show that adults with hyperglycemia have an increased predisposition to experiencing seizures. The present study indicates that CBMN frequency was very high in subjects with glucose level >130 mg/dl. This suggests that high blood glucose level can cause seizure.

CONCLUSION

From the study it is clear that, there are several factors which lead to epilepsy. Malnutrition is one of the major underlying causes of epilepsy worldwide. Many studies also show that genetic factor plays an important role in epilepsy. If there is a family history of epilepsy there is higher chance for an individual in that family to be epileptic. Burden of epilepsy could be reduced by alleviating poverty and by reducing the preventable causes, viz. perinatal insults, parasitic diseases, and head injuries. Empowering primary health care workers to diagnose and start treatment might significantly reduce the treatment gap.

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