

CORRELATION OF DNA DAMAGE IN SEIZURE AND ORGAN DYSFUNCTION OF PERINATAL ASPHYXIAA. Manoj*¹, B. Vishnu Bhat², C. Venkatesh², Z. Bobby³

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ABSTRACT

The present study was conducted to evaluate the level of DNA Damage in Seizure and Organ Dysfunction in Perinatal Asphyxia and its correlation of DNA damage and Organ dysfunction. Eighty term asphyxiated babies were included in this study in which fifty nine babies had seizure. Blood samples were collected within 24 hours of birth. Comet assay was used to detect the level of DNA damage. Oxidative stress was assessed by estimation of Serum MDA level. Seizure was found in three, four and five system with % DNA in Tail of Comet as 44.39868 ± 6.018107 , 46.02007 ± 3.899824 and 73.19868 ± 4.359216 respectively. MDA level in seizure was positively correlated with Multiorgan Dysfunction (r value 0.7210). Positive correlation between % DNA in tail of comet and MOD (r value 0.8408). Therefore Comet Assay and Estimation of Serum MDA level are best Parameters to determine the level of DNA damage and oxidative stress respectively in Perinatal Asphyxia.

KEYWORDS: Seizure, Hypoxic Ischemic Encephalopathy (HIE), Multiorgan Dysfunction (MOD), % DNA in Tail of Comet, Serum MDA level.

INTRODUCTION

Perinatal Asphyxia results deprivation of oxygen to newborn infants multi organs viz. Brain, heart, lungs, liver and kidney. Hypoxia causes formation of free radicals in the affected organs leads to oxidative stress which endangered nucleic acids, resulting hampering of production of enzymes and proteins required for the existence of the normal development of the baby.^[1] Involvement of brain in hypoxia leads to neonatal encephalopathy which is the major cause of neurodevelopmental disability in term infants. Seizures are observed in 20% to 50% of infants with HIE and usually starts between 6 and 24 hours after the insult.^[2] They are most often seen Sarnat stage-2 HIE and rarely in stage-3 and never seen in stage1 HIE. Seizure in Hypoxic Ischemic Encephalopathy usually subtle, tonic or multifocal clonic.^[3] As a result of neurodevelopmental deficit some baby dies during newborn period, another sustains permanent deficits of motor and cognitive functions. DNA damage is assessed by Comet Assay for which extension of tail and % of DNA in tail indicates the severity of damage. Lipid Peroxidation is estimated by serum MDA.

MATERIALS AND METHODS

The present study was conducted at Cytogenetic unit of Department of Anatomy in collaboration with division of Neonatology of Department of Paediatrics and Department of Biochemistry of JIPMER Pondicherry from February 2008 to July 2010. The study was approved by Institute Research Council and Ethical committee. Asphyxiated babies were enrolled based on the inclusion criteria as the following (1) Apgar score less than 6 at 5 minutes (2) Meconium stained liquor (3) Change in fetal heart rate (4) Clinical evidence of Hypoxic Ischemic Encephalopathy (5) Multiorgan Dysfunction.^[4] Preterm or post term babies, large (LGA) or small (SGA) for gestational age babies, those with congenital malformations and delivered of mothers with significant illness were excluded. Comet assay was used to detect the DNA damage as per the protocol of Singh et al.^[5] Thiobarbituric acid reactive substances (TBRAS) which measure MDA present in the serum was estimated for assessment of oxidative stress.^[6]

STATISTICAL ANALYSIS

Multiple comparison was ascertained by using One way Anova. Correlation between different variables had assessed by Carl Pearson correlation Coefficient. Data

was analysed by Graph Pad (InStat, San Diego, USA) and P value <0.05 was taken as significant.

RESULTS

Fifty nine seizure babies had multiorgan dysfunction for which nineteen had three system (21%), twenty each (34%) had four and five system involvement. The percentage of DNA in tail of comet was significantly increases with involvement of more systems ($p < 0.05$). Serum MDA level in seizure was significantly associated

with DNA damage in organ dysfunction (P value <0.0001). The extension of tail of comet was significantly increases with involvement of more system (P value <0.0001). Hypoxic ischemic encephalopathy was positively correlated with multiorgan dysfunction (P value <0.0001). There was significant association between Seizure and HIE (P value <0.0001). Apgar score was inversely proportional with DNA damage (P value <0.0001).

Table-1: Exhibiting Significant increase of Comet tail Length, % of DNA in tail of Comet and serum MDA level in Seizure and Multiorgan Dysfunction.

MOD	Comet Tail length In seizure	% DNA in Tail In seizure	Serum MDA level
Three systems	55.19305±11.61641	44.39868±6.018107	6.867±0.464285
Four Systems	56.05219± 3.877912	55.02007±3.899824	7.866833±0.599565
Five systems	90.51936±2.291733	73.19868±4.359216	8.399375±0.561155
	P value <0.0001	P value <0.0001	P value <0.0001

Table-2: Showing Co-efficient Co-relation (r) between parameters (*Negative correlation P value <0.0001); (Positive Correlation P value <0.0001).**

PARAMETER	VARIABLES					
	HIE	MOD	% DNA	TAIL LENGTH	MDA	SEIZURE
APGAR	-0.8172*	-0.785*	-0.6371*	-0.7383*	-0.7701*	-0.7584*
HIE		0.8237**	0.873**	0.947**	0.779**	0.9599**
MOD			0.8408**	0.8625**	0.7210**	0.8588**
% DNA				0.8123**	0.7287**	0.8520**
TL					0.7921**	0.8890**
MDA						0.8765**

DISCUSSION

The most common cause of neonatal seizures is hypoxic ischemic encephalopathy (HIE), in fact about two-thirds of cases of neonatal seizures are due to HIE. In the case of HIE, these seizures usually occur within the first 1–2 days of birth and often remit after a few days, but carry with them a risk of long-term neurological deficits. Our study aimed the correlation between DNA damage in seizure with multiorgan dysfunction. We found the DNA damage in seizure was significantly associated with organ dysfunction. The Percentage of DNA in tail of comet was significantly increases with more involvement of organs. We observed that the DNA in the tail of comet was more than 45%, 55% and 73% with three, four and five systems respectively. Involvement of more organs is directly proportional to DNA damage in seizure. Seizure was positively correlated with MOD which indicates that above 45% DNA in tail of comet leads to adverse neurodevelopmental implications. Our observations has been agreeing by Glass *et al* whose data suggested that clinical neonatal seizures and their treatment are associated with adverse long-term cognitive and neuromotor outcomes in children at risk for perinatal asphyxia.^[7] Severe oxidative stress DNA damage leads to alteration in the secretion of neurotransmitter which manifests convulsions in Perinatal Asphyxia. Miller SP

et al reported that clinical seizure occurred in 33 of 90 infants which are independently associated with brain injury with diminished N-acetylaspartate/ choline.^[8] In the current study among the 80 asphyxiated babies, 59 (74%) had seizure. We checked whether the clinical association between Sarnat and Sarnat score^[9] with DNA damage in Seizure and Multiorgan dysfunction, for which MOD was positively correlated with HIE and seizure. Among the 143 babies enrolled in the study of Hannah *et al*, 16 (11.18%) were died with wide spectrum of HIE and severe seizure which affects the basal ganglia. In our study 28(47%) babies were expired with severe HIE. It is agreed by Tekgul H *et al* as the overlapping of adverse effects of hypoxic-ischemic brain injury and early post injury seizures have hindered determination of the independent neurodevelopmental consequences of neonatal seizures in humans with high risk mortality.^[10] DNA damage in seizure was significant association between MOD and Comet tail length. The neurodevelopmental deficit was due to lipid peroxidation in seizure, which was positively correlated with % DNA in tail and Organ dysfunction which was strengthened earlier reports of same author.^[11] Thus there was significant increase of DNA damage in seizure and MOD.

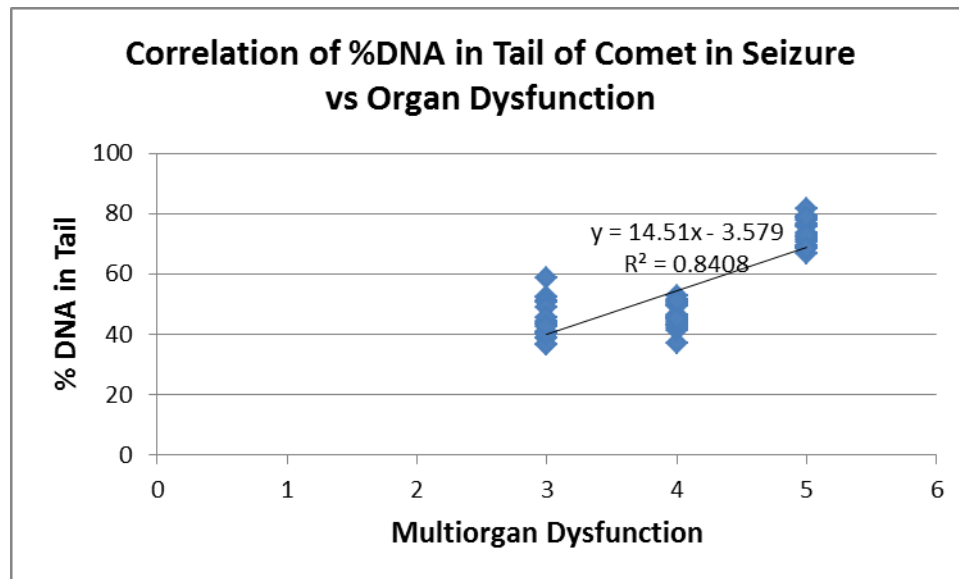


Figure-1: Correlation Coefficient between % DNA in Tail of Comet and Multiorgan Dysfunction (r value 0.8408 and P value- <0.0001).

CONCLUSION

To my best of my knowledge this is the first study documenting correlation between DNA damage in Seizure and MOD. DNA damage in Seizure of Perinatal Asphyxia leads to adverse neurodevelopmental outcome which was significantly associated with multiorgan dysfunction.

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