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Correlation of DNA damage and oxidative stress with organ dysfunction in perinatal asphyxia.

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Abstract

The current study was conducted to correlate the level of DNA damage with multiorgan dysfunction (MOD) in perinatal asphyxia. Among the 80 cases with MOD, 25% each of the babies had five and four organ system involvement , while 23.8% of babies had involvement of 3 systems and 26.3% had 2 organ system dysfunction. The DNA damage increased with the number of systems involved. The comet tail length increased from 24.76±3.203, 42.192±4.685, 57.40±4.246 and 90.734±2.485mm with two, three, four and five organ system dysfunction respectively. The percentage of DNA in tail of comet was also significantly higher with increase in number of system involved (p <0.001). There was significant positive correlation between %DNA in tail of comet and serum MDA level. It is concluded that oxidative stress and DNA damage have a linear correlation to MOD in perinatal asphyxia.

Keywords: Perinatal asphyxia, Multiorgan dysfunction(MOD),Comet Assay, Malondealdehyde(MDA).

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Introduction

Multiorgan dysfunction (MOD) is defined as derangement in the function of two or more organ systems in the body. Perinatal asphyxia is an important contributor to MOD in the neonatal period(1,2). The diving reflex activated by asphyxia results in shunting of blood from less important organ to vital organs such as heart, adrenal and brain to protect them from damage due to hypoxic ischemic encephalopathy(1). Insufficient perfusion of blood to organs in perinatal asphyxia leads to a cascade of biochemical reactions involving neurotransmitters, free radicals and other inflammatory mediators that ultimately promote cellular dysfunction or cell death. If there is an imbalance between free radicals (oxidants) and antioxidants, excess oxygen free radicals interact with biological molecules to bring about disruption in the cellular integrity as well as functional impairment leading to organ dysfunction(3). Comet assay is a sensitive method to evaluate the level of DNA damage. Oxidative stress can be assessed by the estimation of serum MDA level. The present study was conducted to correlate the DNA damage with oxidative stress and organ dysfunction in perinatal asphyxia.

Material and Methods

This study was carried out in the Cytogenetic unit, Department of Anatomy in collaboration with Division of Neonatology (Department of Pediatrics), and the Department of Biochemistry from February 2008 to July 2010. The study was approved by the Institute Research and Ethics (human) Committee. Term babies with asphyxia diagnosed based on Apgar score less than 6 at 5 minutes, cord blood pH <7, evidence of multiorgan dysfunction, and clinical manifestation of hypoxic ischemic encephalopathy were included as cases. The inclusion criteria were adopted based on a previous study from our own centre (4). Babies with major congenital malformation or delivered before term were excluded. Two ml of cord blood was collected from babies meeting the inclusion criteria. Serum was separated and stored at -80 deg C for estimation of malondealdehyde (MDA). Babies were examined for congenital malformations, gestation and development of organ dysfunction. All babies were managed as per standard guidelines and monitored regularly till discharge. Comet assay was carried out based on the guidelines of Singh et al (8). Serum MDA level was estimated as per recommendations Satoh K et al (6).

Statistical Analysis

Comparison between groups were done using one way ANOVA(Turkey Kramer Multiple Comparison Test). The association between percentage of DNA in tail of comet and oxidative stress was carried out by Carl Pearson correlation coefficient. Data was analysed by Graph Pad (Instat San Deigo USA) and p value <0.05 was considered significant.

Results

Apgar score was inversely related to the number of organ dysfunction in perinatal asphyxia. The common organ dysfunctions identified include CNS (100%), Pulmonary (100%), CVS (73.8%), Renal (50%) and GIT (25%). Among the 80 cases investigated, 20 infants (25%) had dysfunction of five organ systems (CNS, Renal, CVS, GIT and Pulmonary), 20 infants (25%) had dysfunction of 4 organ systems (CNS, Renal ,CVS and Pulmonary), 19 infants (23.8%) had dysfunction of 3 organ systems (CNS, CVS and Pulmonary) and 21 infants (26.3%) had dysfunction of 2 organ systems (CNS and pulmonary). Twenty eight babies (35%) expired and they suffered from dysfunction of four or more organ systems. Tail length of comet increased significantly with dysfunction of more organ systems with a mean tail length of 24.76±3.203 micrometer for two organ dysfunctions to 90.734±2.485 for five organ dysfunction(Table1). The tail length of comet was found to be small in those with pulmonary and CNS dysfunction only in comparison to other organ system dysfunctions. The percentage of DNA in tail was also significantly higher among those who had more number of organ dysfunctions. Oxidative stress, as estimated by measurement of MDA, was shown to be high in those with five organ dysfunction (8.488±0.591 micromol/litre) as compared to those with two organ dysfunction (3.88±0.348 micromol/litre). There was good correlation between percentage of DNA in tail and serum MDA level with r value 0.8391and p value <0.000 (Table2). Multiorgan dysfunction was positively associated with %DNA in tail of comet (Fig).

Table 1. *Tail length, percentage of DNA in the tail of comet and serum MDA level with organ dysfunction*

No. of systems involved	Tail length of comet (µ meter)	%DNA in Tail of comet (1-100%)	Serum MDA level (µmol/lit)
Two	$24.76\pm3.203^{T-2}$	$25.98\pm4.332^{D-2}$	$3.88\pm0.348^{M-2}$
Three	$42.192\pm4.685^{T-3}$	$44.39\pm6.183^{D-3}$	$4.628\pm0.931^{M-3}$
Four	$57.40\pm4.246^{T-4}$	$46.02\pm4.01^{D-4}$	$7.11\pm0.515^{M-4}$
Five	$90.734\pm2.485^{T-5}$	$73.199\pm4.472^{D-5}$	$8.488\pm0.591^{M-5}$

T-Tail length, D-%DNA in tail and M- MDA
T_5 vs T_4 , T_5vsT_3, T_5 vs T_2,T_4 vsT_3, T_4 vs T_2 and T_3 vs T_2 (p value<0.0001)
D_5 vs D_4, D_5 vs D_4, D_5 vs D_3, D_5 vs D_2, D_3 vs D_2 (p value<0.001) D_4 vs D_3 (p value>0.05)
M_5 vs M_4, M_5 vs M_3, M_5 vs M_2, M_4 vsM_3 and M_3 vs M_2 (p value <0.001)

Table 2. *Correlation coefficient (r) between various parameters*

Parameter	Variables				
	HIE (n=80)	MOD (n=80)	Tail length (n=80)	% DNA in Tail (n=80)	MDA (n=80)
Apgar score	-0.7315**	-0.6301**	0.6667**	-0.7162**	-0.5045**
HIE Staging		0.9235*	0.9061*	0.9044*	0.8070*
MOD			0.9075*	0.9169*	0.9341*
Tail length				0.9331*	0.8989*
% DNA in Tail					0.8391*

Positive correlation (p value<0.001)* * Negative correlation (p value<0.0001)*

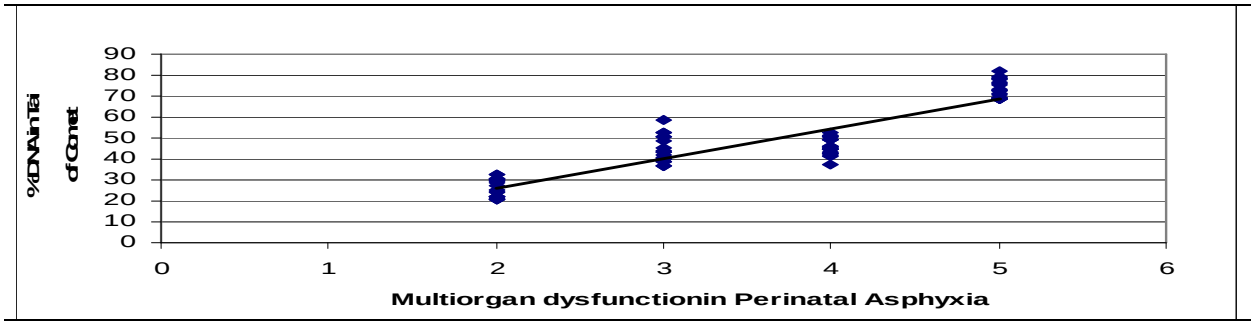

Figure depicting Correlation Coefficient (Pearson) between % DNA in tail of comet and serum MOD (p <0.0001 and r = 0.9169).

Discussion

The present study focused on the dysfunction of different organs in perinatal asphyxia and its correlation with lipid peroxidation and DNA damage. Our study assumes significance in that there are hardly any studies looking at correlation of multiorgan dysfunction with DNA damage and oxidative stress. From our study, we find that low Apgar score was inversely associated with number of organ dysfunctions which is similar to the study done by Martin-Ancel A et al which showed that low five minute Apgar is predictive of organ system dysfunction (2). The common organ dysfunction observed in our study were that of CNS and pulmonary followed by CVS and Renal dysfunction whereas in the Martin- Ancel et al study , CNS dysfunction was the commonest followed by Renal, Cardiac, Pulmonary and G.I.T dysfunction. In our study, MOD was seen in 100% of the study population. This observation is similar to that of a study by Shah et al, in which all study subjects had evidence of MOD (1). There was significant positive correlation between MOD and HIE staging.The pulmonary involvement in the study by Shah et al was 86% in comparison to 100% reported in our study. The mortality in our study was found to be 35% and was found to be associated with dysfunction of four or more organ systems. It is known that risk of death increases with increasing number of failing organs or increasing severity of organ dysfunction(7). The amount of DNA in comet tail is indicative of the extent of DNA damage/ repair (8). In our study, we found the comet tail length is increased with increasing organ dysfunctions. There was also a similar trend observed in serum malondialdehyde levels. Increasing levels of MDA was seen with increasing organ dysfunction in our study and MDA levels correlated well with percentage of DNA in comet tails. We found the level of serum MDA level was significantly associated with muliorgan dysfunction. Oxidative stress markers like MDA and protein carbonyl are shown to be high in perinatal asphyxia. It can cause tissue injury and is significantly associated with morbidity and mortality(9,10). Hence it is clear from our study that perinatal asphyxia is significantly associated with DNA damage and oxidative stress, which inturn is associated with multiorgan dysfunction.

Conclusion

Significant DNA damage and oxidative stress occurs in perinatal asphyxia. The extent of DNA damage and oxidative stress is proportional to the severity of organ dysfunction. Increasing organ dysfunction is associated with increased mortality.

Reference

1. Shah P, Riphagen S, Beyene J,PerlmanM. Multiorgan dysfunction in infants with post-asphyxial hypoxic ischemic encephalopathy. Arch Dis Child Fetal Neonatal Ed 2004; 89:F152-F155.
2. Martin-Ancel A, Garcia-Alix A, Gaya F, Cabanas F, Burgueros M, Quero J. Multiple organ involvement in perinatal asphyxia. J Pediatr 1995; 127: 786-793.
3. Thorat VN , Surykumar AN , Sardeshmukh AS , Sarwade SS . Oxidants and antioxidants in hypoxicischemic encephalopathy. Indian Journal of Clinical Biochemistry 2004; 19 (2): 32-35.
4. Bharadwaj SK, Bhat BV. Therapeutic hypothermia using gelpacks for term neonates with hypoxic ischemic encephalopathy in resource limited settings: a randomized controlled trial. J Trop Pediatr 2012; 58(5): 382-388.
5. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple techanique for quantification of low levels of DNA damage in individual cells. Experimental Cell Research1988; 175: 184-191.
6. Satoh K. Serum lipid peroxide in cerebrovascular disorder determined by anew calorimetric method. Clin Chem Acta 1978; 90: 37-43.
7. Marshall JC. The multiple organ dysfunction syndrome. In: Holsheimer RG, Mannick JA, editors. Surgical Treatment: Evidence- Based and Problem-oriented. Munich: Zuckschwerdt; 2001. Available from: http://www.ncbi.nlm.nih.gov/books/NBK6868/
8. Singh SK, Dua T , Tandon A, Kumari S, Ray G , Batra S. Status of lipid peroxidation and antioxidant enzymes in hypoxic ischemic encephalopathy. Indian Pediatr 1999; 2: 659-668.
9. Mondal N, Bhat VB, Banupriya C, Koner BC. Oxidative stress in perinatal asphyxia in relation to outcome. Indian J Pediatr 2010; 77: 515-517.
10. Kumar A, Ramakrishna SV, Basu S, Rao GR. Oxidative stress in perinatal asphyxia. Pediatr Neurol 2008; 38(3): 181-185.

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