

DNA Analysis in Predicting the Outcome in Perinatal Asphyxia

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Abstract

This study was conducted to evaluate the level of DNA damage in perinatal asphyxia and correlate it with severity and outcome. Eighty term neonates with asphyxia and sixty term control babies participated in this case control study. Blood samples were collected within 24 hours of birth for comet assay, micronucleus assay, karyotyping and serum malondeadehyde (MDA) estimation. There was significant difference in tail length and percentage of DNA in tail of comet between expired and survived cases (75.857 ± 6.568 vs 37.541 ± 11.8). Micronucleus score was significantly different in expired and survived cases (6.341 ± 2.563 vs 2.542 ± 1.416). There was significant difference in mean frequency of chromosomal aberration and serum MDA level in cases and controls (5.058 ± 1.349 vs 2.699 ± 1.330) and (8.268 ± 0.7265 vs 5.782 ± 1.458) respectively. Receiver operating characteristic (ROC) curve of tail length, % DNA in tail of comets, micronucleus score, chromosomal aberration and serum MDA level showed area under curve 0.888, 0.876, 0.876 and 0.890 with cut off value of $61.33 \mu\text{m}$, 52.46%, 4.5 MN, 4.50 CA and 7.48 $\mu\text{mol/lit}$ respectively that can predict the death due to perinatal asphyxia.

Key words: DNA damage, Comet assay, Micronucleus (MN), Chromosomal aberration (CA), Malondealdehyde (MDA)

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Introduction

Perinatal asphyxia causes significant oxidative stress which leads to DNA damage. DNA is precious and potent molecule which acts as the blue print of our life and it controls and coordinates all the cellular activities. Derangement of DNA causes widespread changes in genotypic and phenotypic constitution of the baby. Among the numerous lesions in DNA, unrepaired strand breaks lead to double strand breaks which results in chromosomal aberration or genomic instability. DNA damage studies like comet assay can detect the degree of DNA damage, micronucleus assay and karyotyping analysis can assess the chromosomal aberrations and serum MDA level can be used to evaluate oxidative stress [1].

Material and methods

This study was carried out in the cytogenetic unit of department of Anatomy in collaboration with departments of Pediatrics and Biochemistry from February 2008-December 2010. This study was approved by institute research council and human ethical committee. There were 80 term asphyxiated babies and 60 nonasphyxiated infants in this case control study. The gestational age, sex and weight were matched between cases and controls. Babies with Apgar score <6 at 5 mts, evidence of multi-organ dysfunction and clinical evidence of hypoxic ischemic encephalopathy (HIE) were included as cases. Babies whose mothers had significant illness, pre or post term babies and those with multiple congenital anomalies were excluded from the study. Clinical scores like Apgar score [2] and Sarnat and Sarnat score [3] were used for evaluation of severity of asphyxia and HIE respectively. Heparinized blood samples were collected within 24 hours of birth and immediately processed. Blood samples were mixed with

histopaque and after centrifugation the interface leucocytes were isolated for comet assay and supernatant plasma was used for estimation of serumMDA. Whole blood was inoculated for CBMN assay and karyotyping. Comet assay was carried out as per the recommendations of the protocol of expert panel of comet assay [4]. CBMN assay was done by the standard methods of International collaborative project on micronucleus frequency in human population (HUMN) project [5]. Karyotyping was done based on the standard protocol of International system of human cytogenetic nomenclature(ISCN) [6]. Serum MDA level was assessed based on the protocol of Satokh et al method [7].

Statistical Analysis

Difference between the groups was analysed by means of analysis of variance and student's t-test. The association between the groups was analysed by Pearson correlation coefficient. All the data except ROC were analysed using Graph Pad (InStat, San Diego, USA). ROC curve was analysed bySPSS (SPSS Inc., Chicago, USA).

Results

There was significant difference in Apgar score between cases and controls(4.9 ± 1.624 vs 8.63 ± 0.604). However birth weight and gestational age were not significantly different (2.88 ± 0.394 vs 3.128 ± 0.5) (39.45 ± 1.490 vs 39.32 ± 1.440). Among the 80 asphyxiated infants 21(26%), 40 (50%) and 19 (24%) had stage-1,2 and 3 hypoxic ischemic encephalopathy respectively. Fifty nine (74%) babies developed seizures, 28 (35%) expired and 2 left against medical advise. However 50 (62.5%) infants survived. There was significant difference in tail length and % DNA in tail of comet between expired and sur-vived infants($p < 0.0001$). Micronucleus score was statistically different between expired and survived infants ($p < 0.0001$). It has been observed that there was significant difference in chromosomal aberration between expired and survived groups ($p < 0.0001$). There was significant difference in serum MDA level between expired and sur-vived children.

The detailed correlation coefficient of various parameters in expired children is given in Table:2. There was significant positive correlation between tail length and % DNA in tail of comet with serum MDA level. It was found that there was significant correlation between micronucleus score and encephalopathy. Chromosomal aberration was significantly correlated with serum MDA level.

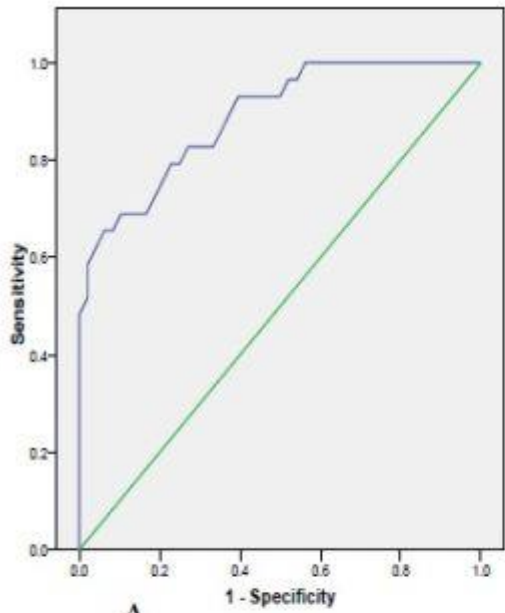
Table 1. Mean \pm SD of variables in expired and survived infants

| Variables | Expired | Survived |
|------------------------|---------------------|---------------------|
| Tail length | 75.857 \pm 16.568 | 37.541 \pm 11.8 |
| % DNA in tail | 60.943 \pm 4.882 | 39.960 \pm 22.025 |
| Micronucleus score | 6.341 \pm 2.563 | 2.542 \pm 1.416 |
| Chromosomal aberration | 5.058 \pm 1.349 | 2.699 \pm 1.330 |
| Serum MDA level | 8.268 \pm 0.7265 | 5.782 \pm 1.458 |

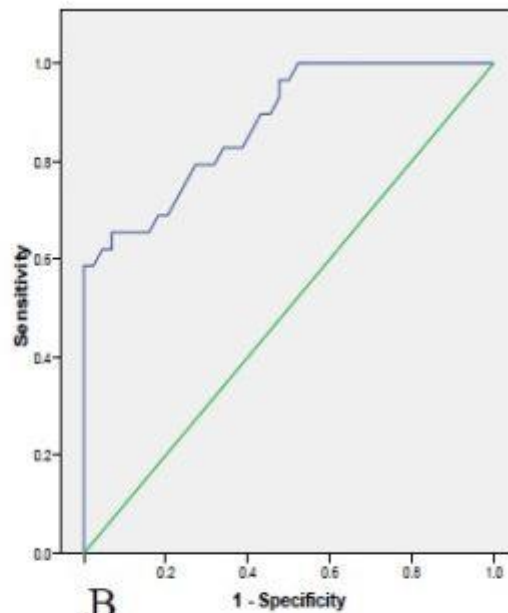
Table 2. Correlation between different parameters of expired babies in perinatal asphyxia

| | Variables | | | | | | |
|------------------|------------|---------|-------------|---------------|---------|------------------|---------|
| | HIE stages | Seizure | Tail length | % DNA in tail | MN | Chrom.aberration | MDA |
| Apgar Score | -0.8788 * | - | -0.8732* | -0.8572* | - | -0.8591* | - |
| HIE stages | | 0.8805* | 0.9694 | 0.9642 | 0.9749* | 0.6972 | 0.9285* |
| Seizure | | | 0.8204 | 0.9619 | 0.8856 | 0.6289 | 0.9191 |
| Tail length | | | | 0.9405 | 0.8738 | 0.5627 | 0.8785 |
| % DNA in tail | | | | | 0.884 | 0.6577 | 0.9552 |
| MN | | | | | | 0.7509 | 0.8846 |
| Chrom.aberration | | | | | | | 0.8951 |
| | | | | | | | 0.8963 |

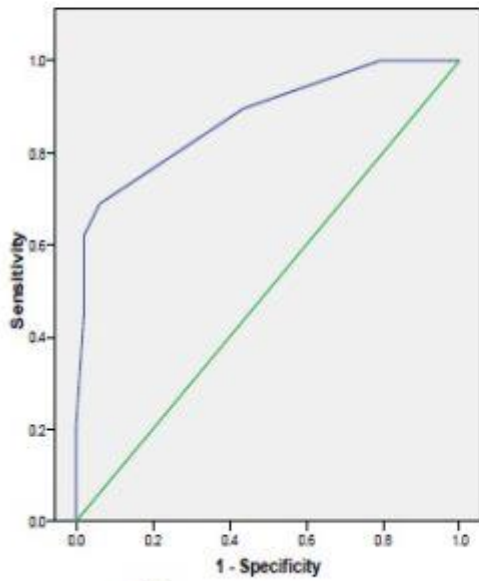
**Negative correlation*



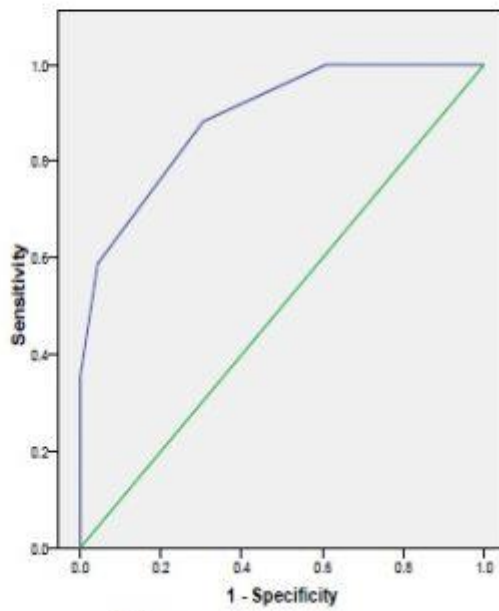
A



B



C



D

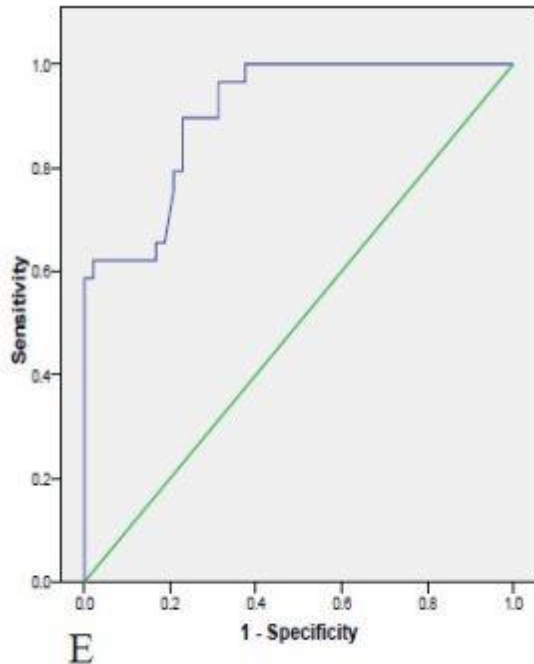


Figure 1: Receiver operating characteristic (ROC) curve of comet tail (A), percentage of DNA in tail (B), micronucleus score(C), chromosomal aberration (D) and serum MDA level(E)

Receiver operating characteristics (ROC) curve of tail length (Fig.1A) showed area under curve (AUC) of 0.888 ($p < 0.05$) with cut of value of 61.33 micrometer with sensitivity (69%) and specificity (89.6%). ROC curve of % DNA in tail (Fig.1B) showed AUC of 0.876 ($p < 0.05$) with cut off value 52.46 with 58.6% sensitivity and 97.7% specificity. ROC curve of micronucleus score (Fig.1C)and chromosomal aberration (Fig.1D) showed AUC of 0.876 ($p < 0.05$) and 0.890 ($p < 0.05$) with cut off value of 4.5 MN (69% sensitivity and 93.7% specificity) and 4.50 CA (58% sensitivity and 95.7% specificity) respectively. ROC curve of serum MDA level (Fig.1E) showed AUC 0.908 ($p < 0.05$) with cut of value 7.48 micromol/lit (76% sensitivity and 79.2% specificity) for predicting death in Perinatal asphyxia

Discussion

Perinatal asphyxia is a universal problem resulting in high mortality and long term morbidity. Insufficient circulation to the vital organs culminates in the formation of excess ROS with reduction in scavenging mechanism which leads to alteration in the integrity of biological molecules. DNA damage is an effect of hypoxia and if it is unre-paired leads to cell death However, misrepaired DNA leads to irreversibility in replication resulting in chromo-somal aberrations and genomic instability with severe sequelae. The molecular mechanism of oxidative DNA damage in Perinatal asphyxia has not been fully evaluated till date. Therefore this study has been undertaken to look into the genome and its alteration that can predict the im-pending death in perinatal asphyxia. This is probably the first study to report prediction of outcome in asphyxia based on DNA damage.

There was significant DNA damage and lipid peroxida-tion in asphyxiated babies than unasphyxiated infants. A retrospective analysis concluded that the 5 minute Apgar score was a valid predictor of neonatal mortality, but us-ing it to predict long term outcome was inappropriate [8]. In our study 35% of severely asphyxiated infants expired before discharge with low Apgar score at 5 mts. In ex-pired babies there was significant increase in tail length,%DNA in tail and chromosomal aberration compared with survived babies. There was significant negative correla-tion between Apgar score and DNAdamage. Earlier workers also noted increased risk of mortality and cere-bral palsy where the Apgar score is low at 5 mts [9,10]. The risk of poor neurologic outcomes increase when Apgar score is 3 or less at 10,15 and 20 minutes [11].

Many studies like the present one recorded usefulness of the Sarnat HIE stages in predicting outcome. We found that the DNA damage in mild HIE was less compared to moderate and severe cases [12]. Therefore the DNA damage positively correlated with severity of HIE. Babies with mild HIE had normal outcome compared with severe sequelae or death among 96% of those who had stage-III HIE. About 25% of neonates who have moderate HIE expired or had adverse outcome [13]. Among the infants who expired 17 (60.7%), 10 (35.7%) and 1 [3.6%] had HIE stage 3, 2 and 1 respectively. Earlier reports showed that EEG investigation in hypoxic ischemic encephalopathy had the best predictive ability at 6 hrs of age (95%) and 48 hrs (93%) by receiver operating characteristic curve [14].

To predict the death in perinatal asphyxia we have drawn receiver operating characteristic (ROC) curve for DNA damage parameters and serum MDA level (fig.1A-E). At the cut off level of 61.33 micro meter of tail length the sensitivity and specificity of predicting the death were 69% and 89.6% respectively. In the current study there was significant elevation of serum MDA level between the expired and the survived groups. Therefore death and survival rate can be predicted based on serum MDA level. This is agreement with another study by Mondal et al who reported that outcome can be predicted based on the elevated levels of serum MDA level and protein carbonyl at birth, and at 48 hours of life [15]. ROC of serum MDA level had an area under curve of 0.908 ($p < 0.05$). The cut off level of serum MDA level for poor outcome was 7.48 micro mol/ lit with a sensitivity of (76%) and specificity (79.2%). Our results are in agreement with earlier report documented by Banupriya et al which showed that urinary excretion of uric acid, MDA and proteins is higher in asphyxia and have the potential biochemical markers for severity and death prediction in perinatal asphyxia [16]. Because of the significant association between DNA damage and serum MDA level, DNA damage is also a valuable predictor of death in birth asphyxia. The results of ROC for micronucleus score and chromosomal aberrations documented in this study were also good predictors of impending death. There was significant negative correlation between Apgar score with comet tail length and % DNA in tail. There was significant positive correlation between encephalopathy and DNA damage in expired groups. Since the area under curve (AUC) of tail length, % DNA in tail, MN score, chromosomal aberrations and serum MDA of ROC curve was significant and the cut of values were optimally sensitive and specific, we suggest that DNA damage can act as a good predictor of death in perinatal asphyxia. Treatment aimed at oxidative stress probably will reduce the DNA damage and outcome in perinatal asphyxia.

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