Chromosomal aberrations in Perinatal Asphyxia.

A. Manoj, K.Ramachandra Rao, B.Vishnu Bhat*, C. Venkatesh*, Zachariah Bobby**

Departments of Anatomy, Paediatrics* and Biochemistry**, Jawaharlal Institute of Post-graduate Medical Education and Research (An Institution of National Importance), Pondicherry, India.

Abstract

This study was conducted to evaluate the level of chromosomal damage in perinatal asphyxia and correlate it with severity of asphyxia. Forty term neonates with asphyxia and twenty term babies without asphyxia were included in this case control study. Blood samples were collected within 24 hours of birth for karyotyping and oxidative stress by serum malondialdehyde (MDA) estimation. The degree of chromosomal damage and MDA level were significantly associated with severity of hypoxia. There was significant difference in mean frequency of chromosomal aberration and MDA level in controls (3.4 ± 0.280 vs 0.95±0.198) and cases (6.749±1.818 vs 3.687±0.156) respectively. Chromosomal aberrations also significantly correlated with Apgar score, HIE stages, seizure and serum MDA level.

Key words. Chromosomal aberration, DNA damage, Malondialdehyde (MDA), Perinatal asphyxia.

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Introduction

Perinatal asphyxia is a major cause for neonatal mortality and morbidity around the world especially in developing countries like India [1]. Oxygen deprivation and excess accumulation of CO\textsubscript{2} results in metabolic acidosis. This can alter the ionic exchange and cause defect in the liberation of ATP eventually leading to energy failure. Thus cations accumulate inside the cell causing cytotoxic edema. The reduction of O\textsubscript{2} results in the generation of reactive oxygen species which interact with nucleic acid and make alteration in the structure and functioning of the genome [2]. Peripheral blood lymphocyte culture technique has been found to be useful in analysing the morphology and aberrations of human chromosome. Serum malondialdehyde is the end product of lipid peroxidation and a useful marker for the evaluation of oxidative stress.

Material and Methods

The study was carried out in the Cytogenetic unit of Department of Anatomy in collaboration with departments of Paediatrics and Biochemistry from February 2008 to July 2010. The study was approved by the Institute Research and Human Ethics Committee. Asphyxiated appropriate for gestational age babies were taken as cases. Gestational age and weight matched babies without asphyxia were taken as controls. Asphyxia was diagnosed when 3 or more of the following features were present:-(i) Apgar score less than 6 at 5 mts. (ii) meconium stained liquor (iii) changes in the fetal heart rate (iv) clinical evidence of HIE (v) evidence of multiorgan dysfunction [3]. Karyotyping was carried out based on recommendations of International system of human cytogenetic nomenclature (ISCN) [4] and analysis was done with metasystem IKROS software, Germany. There were 40 asphyxiated and 20 control babies with metaphase spreads of good quality and included in this study. Thiobarbituric acid reactive substances (TBRAS) which measure MDA present in the serum was estimated for assessment of oxidative stress [5].

Comparison of groups was done using unpaired student t-test and one way ANOVA (Kruskall Walli’s test). The association between variables has been assessed using with Karl Pearson’s correlation coefficient. P value < 0.05 was considered significant. Data was analysed by Graph Pad (InStat, San Diego, USA).

Results

Birth weight and gestational age of asphyxiated babies were not significantly different from controls. The mean Apgar score [6] of cases was significantly different from the controls (4.9±1.624 vs 8.633±0.604). Using Sarnat and Sarnat score [7] the asphyxiated babies were categorised into HIE stages 1, 2, and 3 with 12, 14 and 14 neonates respectively in each group. Among the cases, 28 developed seizure, 17 (42.5%) expired and one left against medical advice. All the controls were discharged.
Table 1. Comparison of chromosomal aberration and serum MDA level between cases and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chromosomal aberrations</th>
<th>Serum MDA (µmol/L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=20)</td>
<td>0.95±0.198 a</td>
<td>3.687±0.1561 b</td>
</tr>
<tr>
<td>Hypoxia (n= 40)</td>
<td>3.40±0.280 a1</td>
<td>6.749±1.818 b1</td>
</tr>
<tr>
<td>HIE-1 (n=12)</td>
<td>2.083±1.311 c</td>
<td>4.588±0.3220 d</td>
</tr>
<tr>
<td>HIE-2 (n=14)</td>
<td>3.250±1.45 c1</td>
<td>7.065±0.1779 d1</td>
</tr>
<tr>
<td>HIE-3 (n=14)</td>
<td>4.78±1.311 c2</td>
<td>8.600±0.1751 d2</td>
</tr>
<tr>
<td>Seizure Present(n=28)</td>
<td>4.07±0.763 e</td>
<td>7.832±0.9419 f</td>
</tr>
<tr>
<td>Seizure Absent (n=12)</td>
<td>2.083±1.311 e1</td>
<td>4.582±0.9661 f1</td>
</tr>
</tbody>
</table>

a – a1 p value <0.05; b-b1 p value<0.05; b-d p value>0.05
 c-c1 p value>0.05; c-c2 p value<0.05; a-c p value >05
 c1-c2 p value<0.05; e-e1 p value<0.05, f-f1 p value<0.05

The chromosomal aberration and serum MDA level of asphyxiated cases and controls were compared (Table 1). The mean frequency of the total aberration in asphyxiated and control babies was observed to be 3.40 ± 0.280 and 0.95 ± 0.198 respectively. Chromosomal aberration was significantly increased with severity of asphyxia. After post-hoc test chromosomal aberrations between control vs HIE stage 1 and HIE stage 1 vs HIE stage 2 were not statistically significant (Table 1).

It was found that serum MDA level significantly more in asphyxiated babies than controls (6.709±1.695 vs 3.683±0.536) and it correlated with severity of asphyxia (Table 1).

Table 2. Correlation between different parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variables</th>
<th>HIE</th>
<th>Seizure</th>
<th>Chromosomal Aberration</th>
<th>Serum MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apgar Score (n=40)</td>
<td></td>
<td>-0.6314**</td>
<td>-0.7580**</td>
<td>-0.5606**</td>
<td>-0.8325**</td>
</tr>
<tr>
<td>HIE Staging (n=40)</td>
<td></td>
<td>0.9599*</td>
<td></td>
<td>0.5901*</td>
<td>0.9004*</td>
</tr>
<tr>
<td>Seizure (n=28)</td>
<td></td>
<td>0.3962*</td>
<td></td>
<td>0.7702*</td>
<td>0.7441*</td>
</tr>
<tr>
<td>Chromosomal aberrations (n=40)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

*P <0.05 – positive correlation; **p - negative correlation

Figure 1. Images of Giemsa –trypsin stained 100X karyotype of severely asphyxiated infant exhibiting chromatid breakage sporadically in 4th 6th 8th and X chromosome, chromatid interconnection in 5th and 10th chromosomes (Analysed by metasystem IKROS software).
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\[ y = 0.3619x + 5.5748 \]
\[ R^2 = 0.1375 \]

Figure 2. Chromosomal aberrations in perinatal asphyxia illustrating Chromatid breakage (CB) with Ideogram, Chromatid Interconnection (ICC), Dicentric Chromosome (DC), Ring Chromosome (RC) and Acentric chromosome (AC).

Figure 3. Correlation coefficient (Pearson) between Chromosomal aberration and serum MDA level (P value <0.05 and r value 0.7441).

In seizure babies there was significant increase in chromosomal aberration compared with non-seizure babies. The serum MDA level was also significantly more in seizure than non seizures, babies (Table 1).

The occurrence of chromosomal aberration like Chromosomal breakage (CB), Acentric chromosome (AC), Chromatid interconnection (CI) or sister chromatid exchanges, Dicentric chromosome (DC) and Ring chromosome (RC) is depicted in Figs.1 and 2.

There was a significant correlation between chromosomal aberration and serum MDA level (p <0.05) (Table 2 and Fig. 3). Also chromosomal aberration has significant positive correlation with HIE stages, seizure and negative correlation with Apgar score.

Discussion

According to Maxomo Vento et al [8] DNA damage results in structural alterations such as strand breaks, deletions, base changes and even chromosomal aberrations. In normal course the adduct can be repaired by nucleotide excision repair pathway. Unrepaired adduct can interfere in the formation of RNA polymerase and block the production of vital genes leading to cell death [9].

In the current study it has been observed that the chromosomal aberrations occurred spontaneously and sporadically, but not in recognized points like fragile sites. It was further observed that chromatid breakage was significantly higher in cases compared with controls (3.40 ±
The chromosomal breakage was visible in all severe cases and it was due to unrepaired double strand break (DSB). Other chromosomal aberrations like acentric fragments were produced due to failure of rejoining of strands of DNA, interchromatid exchange resulting sister chromatid exchanges and its later modifications like dicentric chromosome.

Neilson et al reported the percentage of chromosomal aberration in neonatal asphyxia as 4.2% in their study [10]. They further observed 1.1% chromosomal aberration in respiratory distress syndrome. However in the present study the percentage of chromosomal aberration was 3.4%. Children with cerebral palsy with double hemiplegia had high percentage of chromosomal aberrations (7.63±0.51) compared to those with hemiparesis and controls [11]. The results of the present study are in agreement with previous reports dealing with DNA damage and chromosomal aberration in perinatal asphyxia [10,12]. These reports suggest that genomic instability is present in perinatal asphyxia.

MDA is the end product of lipid peroxidation and it was significantly more in asphyxiated babies than controls in the present study. Earlier studies have reported increased level of serum MDA in asphyxiated babies [13-15]. It was also reported that MDA level can predict the outcome in perinatal asphyxia [16,17]. Further we observed that the chromosomal aberration was directly proportional to the level of serum MDA.

There was positive correlation between chromosomal aberration and HIE stages. The Apgar score was inversely correlated with chromosomal aberration and serum MDA level. From the above findings it could be concluded that perinatal asphyxia leads to oxidative stress which results in DNA damage which increases with severity of asphyxia. The irreversibility of double strand break leads to chromosomal aberration which alter the gene production. If the oxidative stress can be prevented in perinatal asphyxia by early administration of antioxidants, the outcome may be better.

**Reference**


**Correspondence to:**
B.Vishnu Bhat
Department of Pediatrics and Neonatology
JIPMER, Pondicherry 695006, India.