ORIGINAL RESEARCH PAPER

Volume-7 | Issue-5 | May-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSOCIATION OF DEOXY-RIBONUCLEIC ACID DAMAGE AMONG THE RISK FACTORS ATTRIBUTING TO DELAYED MILESTONES

Anatomy

Surraj S* Senior Resident, Department of Anatomy, JIPMER *Corresponding Author

Parkash Chand Senior Professor (retired), Department of Anatomy, JIPMER

Vishnu Bhat B Senior Professor and Director, Department of Neonatology, JIPMER

Nandeesha H Associate Professor, Department of Biochemistry, JIPMER

Raveendranath V Associate Professor, Department of Anatomy, JIPMER

ABSTRACT

Background and aims: Delay in milestones can be either due to syndromes or due to other non-syndromic causes with risk factors. An undoubtedly marked DNA damage occurs in developmental delay due to non-syndromic causes, just as it occurs in chromosomal syndromic anomalies leading to delayed development but there is an element of uncertainty whether this damage to the DNA is actually associated with the various risk factors that are the prominent causes for non-syndromic developmental delay or whether it is occurring spontaneously . Hence the present study aims to just find out if there is a certain degree of DNA damage associated with the major risk factors that contribute to delayed development non-syndromically. However this study does not test the causational role of the risk factors in damaging the DNA.

Methodology: In this observational study, the DNA damage was assessed in the lymphocytes of 30 clinically diagnosed children with non-syndromic developmental delay using the comet assay. The levels of DNA damage has been assessed for some of the major risk factors like low birth weight, low socioeconomic status, asphyxia, microcephaly, preterm birth and increased duration of hospital stay. The levels of DNA damage has also been assessed for the various modes of delivery and with the severity of developmental delay.

Results: Significantly elevated levels of DNA damage was found only in children with low birth weight ($p<0.001$), but not among the other risk factors ($p>0.05$), though there were marginal elevations in DNA damage among the other risk factors.

Conclusion: Non-syndromic risk factors are associated with an underlying genetic basis of DNA damage in attributing to developmental **delay**

KEYWORDS

Risk factors, Delayed milestones, DNA damage, Comet assay

Introduction

Development revolves around various domains linked to either motor or adaptive skills or language or cognitive fields. If a child fails to achieve the desired milestones in one or more domains then this ensues as developmental delay. If the milestones in all the domains are delayed in a child the delay is said to be global, which usually occurs in children below five years of age (1,2). Hence the study has been carried out in children under five years of age.This developmental delay can be either syndromic or non-syndromic. It is obvious that there is an underlying genetic defect leading to DNA damage in children with syndromic anomalies leading to developmental delay. However, in the recent past it is also now proven that in developmental delay due to other causes that are non-syndromic in origin like perinatal asphyxia, neonatal stressors, etc, DNA damage does occur. However it is not fully known if it is the risk factors that cause the non-syndromic variety of developmental delay that are associated with DNA damage or whether the DNA damage just occurs spontaneously without any involvement of risk factors(1-3). There are a lot of strand breaks occurring sporadically in the neurons that cause DNA damage resulting in delayed development, without any syndromic manifestations(3). Hence the present study was undertaken to see if the major non-syndromic risk factors that caused developmental delay were associated with DNA damage or not. Chen et al., had shown that majority of the risk factors contributing to developmental delay were of non syndromic origin that included prematurity, low birth weight, neonatal insults that occurred before, during or after delivery like low apgar scores, infantile spasms, severe hyperbilirubinemia, CNS lesions like hydrocephalus, intracranial hemorrhage, hypoxic encephalopathy, infections, seizures, trauma and idiopathic causes. A minor proportion were due to genetic defects with congenital anomalies(2). Asphyxia during the perinatal period affects the neurons causing them to die and alters the interaction of proteins with ubiquitin (4).

A longer duration of hospital stay for a preterm child in the intensive care unit, is directly proportional to the amount of damage imparted to the brain of the developing child, because it indirectly indicates that some sensory insult had occurred when the child was in the mother's uterus that has affected the neural development(5). Microcephaly, which is basically a reduction in the circumference of the skull upto three times lesser than the mean adjusted deviation from the standard is frequently associated with reduced volumes of the brain as a result of disturbances in the proliferation of existing neuronal cells and impairment in the production of new neuronal cells, thereby attributing as a factor for developmental delay(6). Though there a few studies pointing out the occurrences of DNA damage in these various risk factors, there are hardly any studies highlighting the severity of DNA damage in children with these risk factors, especially in the southern part of India. Hence in the present study, the levels of DNA damage among various risk factors in children with non-syndromic developmental delay has been compared, using the comet assay. The comet assay is used in this study as it is unique in assessing DNA damage when compared to other techniques like PCR, FISH, etc, because it is quicker, economical and sensitive(7).

Methodology

After getting approval from the Institute Research Council and Institute Human Ethics Committee , this study was carried out in the research laboratory in the Department of Anatomy at JIPMER, Puducherry, in collaboration with the Paediatrics and Biochemistry departments for a duration of 13 months. Since it was an observational study, the subjects for the study were selected from the out-patient department of Pediatrics. The hospital case sheets of the children were viewed and 30 children with development delay, under five years, without any evidence of syndromes or dysmorphic features were chosen at random and screened for the presence of risk factors. Accordingly they were categorized based on the risk factors as shown in table 1. The subjects were chosen for the study only after a proper written, informed consent was obtained from their parents/guardians.

The sample size of 30 was calculated using the N-masters software.The primary variable for assessing the outcome was DNA damage. The standard deviation of DNA damage was 5 micrometer in a previous study on Down's syndrome (8). We estimated that 28 patients would be required to detect a mean difference of 3 micrometre in DNA damage based on the previous standard deviation. Calculations were based on 80% power; $\alpha=0.05$ and 2 sided 95% confidence interval.

Lymphocytes were separated from the heparinized blood (2ml), that was withdrawn under sterile conditions, from the peripheral veins of children, by centrifugation method using Histopaque (SIGMA). They were then subjected to the comet assay, wherein the buffy coats

containing the lymphocytes were removed and the lymphocytes were then meshed between two layers of agar gels and subjected to lysis using a buffered solution (Nacl,Triton X, EDTA,TRIS). Electrophoresis was carried out in an alkaline medium followed by neutralization of slides.The slides were then stained using silver nitrate and observed under a light microscope.The images were captured by a digital camera and the comet parameters were scored and analysed using the Image J image quantification software. The data on clinical and socio-demographic parameters such as severity of developmental delay, birth weight, duration of hospital stay, presence or absence of associated risk factors, age, socioeconomic status, etc were analyzed and compared with comet parameters.

Statistical analysis:

Descriptive and inferential statistics were used to analyze the data. The baseline characteristics were analyzed by descriptive statistics. The data on the level of comet parameters were expressed as mean with standard deviation or median with range whichever was appropriate. The tests for normality were applied to check for the Gaussian nature of the continuous data. To present the normally distributed data, mean with standard deviation was used and for non normal data, median with interquartile range was used. The data on subject characteristics such as gender, clinical factors, socio-demographic factors, etc which represent the categorical data were described using frequencies and percentages.The students t-test was used to compare the continuous variables between the groups; while the Mann Whitney U test was used in case of a non-parametric distribution.All statistical analyses were carried out at 5% level of significance and the p value <0.05 was considered significant. The SPSS software, version 20, was used for statistical analysis. The graphs were plotted using Microsoft office excel work sheet.

Results

The distribution of various risk factors among children with non-syndromic developmental delay is shown in table 1

Table 1: Distribution of risk fsactors among cases

Risk factors	Children with non-syndromic developmental delay (N=30)	
	n	%
Low socioeconomic status (SES class IV)	14	46.7
Preterm birth	19	63.3
Low birth weight (<2.5kg)	21	70.0
Increased hospital stay (≥6days)	11	36.7
Microcephaly	12	40.0
Low apgar score (< 3)	14	46.7
DQ < 70 (severe delay)	16	53.3

From the above table it is evident that most of the children with non-syndromic developmental delay were associated with a low birth weight .

The comet images with severe DNA damage and moderate DNA damage among the children are shown in Fig 1:

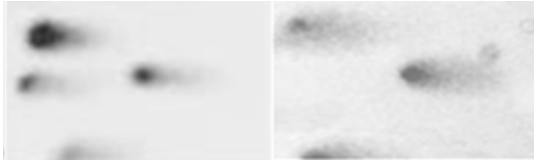

Figure moderate DNA damage (left), severe DNA damage (right)

The comparison of DNA damage among the various factors that possibly contribute to non-syndromic developmental delay is shown in table 3

Table 2: Comparison of comet parameters among various risk factors

Comet parameters						
Factors	**Total comet length**	**Head diameter**	**Comet tail length**	**% of DNA in tail**	**% of DNA in head**	**p value**
Low socioeconomic status	51.91±8.04	29.83±2.91	22.07±7.94	72.77±4.25	34.52±14.11	>0.05
Better socioeconomic status	50.06±6.99	30.50±4.07	19.56±7.53	70.32±8.73	33.99±14.36	
Preterm	52.83±5.99	30.54±3.83	22.29±7.35	71.81±7.90	36.17±12.67	>0.05
Term	47.63±8.75	29.58±3.03	18.05±7.87	70.86±5.41	30.89±16.13	
Low birth weight	53.28±4.35*	30.10±3.86	23.17±4.92*	71.60±6.33	37.84±12.96	*<0.05, >0.05
Normal birth weight	45.43±10.21*	30.29±2.81	15.05±10.10*	71.14±8.80	25.83±13.30	
Increased ICU stay	51.73±8.00	29.60±3.50	22.13±8.49	72.11±7.35	33.65±15.72	>0.05
Decreased ICU stay	50.46±7.26	30.53±3.60	19.93±7.31	71.09±6.97	34.58±13.35	
Microcephaly	51.67±7.83	29.86±3.08	21.80±7.56	70.46±8.28	38.59±13.64	>0.05
Normocephaly	50.43±7.33	30.41±3.87	20.02±7.92	72.13±6.17	31.34±13.85	
Apgar <3	52.41±6.62	30.58±4.16	21.84±6.88	71.51±8.35	37.30±15.73	>0.05
Apgar ≥3	49.62±8.05	29.85±2.97	19.77±8.44	71.42±5.87	31.56±12.17	
DQ<70	52.18±6.91	30.09±3.81	22.09±7.12	71.74±8.30	37.12±12.61	>0.05
DQ ≥70	49.49±7.99	30.30±3.33	19.18±8.29	71.15±5.45	30.94±15.23	

Discussion

It is well known that a low socioeconomic status coupled with a poor education among parents can adversely affect their child's development(9). The socioeconomic status in this study was assessed by using the revised Kuppusamy's scale for children from urban areas and by the modified Prasad's scale for those from rural areas. In this study, it was found that 46.7% of the cases were from a low socioeconomic background. The comet parameters of tail length, total comet length and percentage of DNA in tail were slightly higher in them, when compared to those cases with a better socioeconomic background. This shows that the severity of DNA damage is more in children with a poor socioeconomic background that could have indirectly contributed to non-syndromic developmental delay

According to I-Chun Chen et al, 55% of children with developmental delay were born of a spontaneous normal delivery and 45% of them due to caesarean delivery, when they analyzed 1048 Chinese children. They inferred that there was no significant difference between the modes of delivery in the causation of developmental delay(2). In this study, it was found that 36.7% of the cases were born of a normal delivery, while 43.3% of them were born of a caesarean section and 20% with the use of instruments.

It was found that the levels of DNA damage were higher in those children born of a Caesarean section or due to the use of instruments, when compared to those born of a normal delivery. Furthermore, it was evident that the levels of DNA damage were slightly more in instrumental deliveries though these differences were not significant

statistically, indicating that the severity of DNA damage is more when a child is under a perinatal stress due to abnormal modes of delivery, that could also contribute to a delay in development. However, the findings in this study are not consistent with the findings of I-Chun Chen et al(2), but it would be inaccurate in concluding that the mode of delivery has a definitive contributive role in causing DNA damage in developmental delay as far as this study is concerned because this study has a minimum sample size.

Preterm birth, low birth weight, increased duration of hospital stay and microcephaly are known to significantly contribute to non-syndromic developmental delay (4,5,6,10). Literature states that the motor performance in a child gets reduced to twice or thrice the existing levels, if the child is born of a low birth weight accompanied by a lower gestational age(2). I-Chun Chen et al had shown in their study that children with either a motor or a global delay had a much lower birth weight and gestational age compared to children with other kinds of delay(2).

In this study, 63.3% of the cases were preterm, 70% were of low birth weight and up to 40% of the children had a head circumference below thrice the normal deviation of the standard age and 36.7% of the cases had a prolonged hospital stay in the intensive care unit for more than 5 days. On analyzing the comet parameters for these risk factors in our study, it was found that all the comet metrics were increased in preterm children compared to term children with developmental delay, but the strength of significance was weak. This supports the findings stated in literature that preterm births cause brain damage and affect development, as the brain is more vulnerable to the effects of DNA damage due to hypoxic stress(3,5).

A marked increase in the levels of total comet length and tail length among cases with a low birth weight, with a good strength of significance was seen in this study, indicating that as the birth weight reduces, the severity of DNA damage is more. Since, birth weight reflects upon the growth status of the child(1), it can be concluded from the above results that a poor birth weight adversely affects the child's development, and so growth and development are intimately related to each other

In the present study, the total comet length and tail length were slightly elevated in children with microcephaly when compared to those with a normal head circumference. This indicates that the severity of DNA damage is more when the diameter of the skull is reduced. These findings are supportive of the evidences in literature, which state that microcephaly results in a poor volume of the brain accompanied by a reduced growth of neurons, that hampers development. The brain in turn is more vulnerable to DNA damage (6).

Manoj et al, had shown in their study that children with perinatal asphyxia had increased levels of DNA damage when compared to normal children. They had shown in their study that the comet parameters of tail length and percentage of DNA in tail were significantly elevated in severely asphyxiated infants(11). In the present study, the cases were classified based on the Apgar score as those with severe asphyxia (where the score was below 3) and those with moderate asphyxia (if the score was 3 and above). It was found that the percentage of DNA in tail, tail length and total comet length were slightly elevated in children with severe asphyxia, but the elevation was not significant enough. This shows that the severity of DNA damage increases with the severity of asphyxia. Our findings are consistent with the findings of Manoj et al. The present study also reveals that the severity of DNA damage increases with the duration of hospital stay.

Genetic defects have been found in non-syndromic developmental delays, and up to 50% of those delays ranging from a moderate to severe grade result from a genetic defect (12). The developmental quotient was used to grade the severity of delay in this study. Children with a DQ between 70 and 85 were grouped as having a less severe delay and those with a DQ less than 70 as having severe delay(1). It was found in this study that the total comet length, tail length and percentage of DNA in tail were increased in children with severe developmental delay compared to the less severe ones, though the increase was not statistically significant. This finding can have a prognostic significance, as early detection of DNA damage can help in early intervention and rehabilitative measures. Since DNA damage is more severe in children with a low DQ, it also indirectly indicates that neuronal growth has been compromised as a result of DNA damage.

Conclusion

The levels of DNA damage are marginally increased among the various risk factors in children with non-syndromic developmental delay, though not statistically significant. However, in children with a low birth weight, DNA damage is significantly increased. Since birth weight directly reflects upon the growth status of the child, this severity of DNA damage in low birth weight indicates that growth and development are closely related to each other. The small sample size in this study is a major limitation of this study, because of which precise conclusions cannot be drawn as to whether the other risk factors that have slightly increased levels of DNA damage are definitely associated in the causation of developmental delay. However, it can be said that DNA damage does occur among the various non-syndromic risk factors attributing to developmental delay, apart from sporadic causes.

References

1) Kumar AS. Handbook of Pediatrics. 5th ed. New Delhi: All India Publishers and Distributors;2010.Chapter 1, Growth and Developmen;p.2-30. Chapter 4, Neonatology;p.100-102
2) Chen I-C, Chen C-L, Wong M-K, Chung C-Y, Chen C-H, Sun C-H. Clinical analysis of 1048 children with developmental delay. Chang Gung Med J. 2002; 25(11):743–50.
3) Thanan R, Oikawa S, Hiraku Y, Ohnishi S, Ma N, Pinlaor S, et al. Oxidative Stress and Its Significant Roles in Neurodegenerative Diseases and Cancer. Int J Mol Sci. 2014 Dec 24; 16(1):193–217.
4) Kiss P, Vadasz G, Kiss-Illes B, Horvath G, Tamas A, Reglodi D, et al. Environmental Enrichment Decreases Asphyxia-Induced Neurobehavioral Developmental Delay in Neonatal Rats. Int J Mol Sci. 2013 Nov 13; 14(11):22258–22273.
5) Ramachandran S, Dutta S. Early developmental care interventions of preterm very low birth weight infants. Indian pediatr. 2013;50(8):765–70.
6) Reynolds JJ, Stewart GS. A single strand that links multiple neuropathologies in human disease. Brain. 2013 Jan 1; 136(1):14–27.
7) Vidya G, Gladwin V, Chand P. A Comprehensive Review on Clinical Applications of Comet Assay. J Clin Diagn Res. 2015;9(3):GE01-GE05.
8) Jayaprakash T, Rao KR, Bhat V, Chand P, Nandhakumar S. DNA damage studies in cases of trisomy 21 using comet assay. Curr Pediatr Res.2010; 14(1):1-4.
9) Ali SS. A brief review of risk factors for growth and developmental delay among preschool children in developing countries.Adv Biomed Res.2013 Nov 30;2:91.
10) Koul R, Al-Yahmedy M, Al-Futaisi A. Evaluation of children with global developmental delay: A prospective study at Sultan Qaboos University Hospital, Oman. Oman Med J. 2012; 27(4):310.
11) Manoj A, Rao KR, Bhat BV, Venkatesh C, Bobby Z, KT H. DNA Analysis in Predicting the Outcome in Perinatal Asphyxia. Curr Pediatr Res. 2011; 15(2):121-125
12) Wu Y, Ji T, Wang J, Xiao J, Wang H, Li J, et al. Submicroscopic subtelomeric aberrations in Chinese patients with unexplained developmental delay/mental retardation. BMC Med Genet. 2010; 11(1):72.