

## **Evaluation of DNA damage in babies with Neural Tube Defects.**

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### **Abstract**

DNA damage in cases with neural tube defects occurs due to many causes like genetic mutations, metabolic derangements. The level of DNA damage can be evaluated by single cell gel electrophoresis method (comet assay). The present study included 30 clinically diagnosed Neural tube defect cases, and 30 controls matched for age and sex. The cases were categorized into three groups according to the severity of the disease as mild, moderate and severe. The mean total length of comet ( $71.9 \pm 19.2 \mu\text{m}$  vs  $49.0 \pm 10.7 \mu\text{m}$ ) ( $p < 0.05$ ); mean tail length ( $33.6 \pm 15.1 \mu\text{m}$  vs  $4.7 \pm 2.4 \mu\text{m}$ ) ( $p < 0.05$ ); mean % of DNA in tail ( $20.3 \pm 7.1$  vs  $8.5 \pm 3.9$ ) ( $p < 0.05$ ) were significantly more in cases than in controls. As the severity increases the DNA damage also increases among the cases. The mean % of DNA in tail were  $11.2 \pm 7.1$ ;  $21.4 \pm 6.5$ ;  $22.9 \pm 5.0$ ; in mild, moderate and severe cases respectively. It is concluded that there is significant DNA damage in Neural tube defect cases and also the damage increases with severity of the disease.

**Keywords:** Neural tube defect, Comet assay, DNA damage.

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### **Introduction**

Neural Tube Defects (NTD) are one of the common congenital anomalies. The incidence of NTDs has been estimated to be 16/10,000 live births in the eastern United States. There are various types of neural tube defects e.g; cranium bifidum with meningocele, holoprosencephaly, meroencephaly or anencephaly, spinabifida occulta, meningocele, meningoencephalocele(1). Population based study in remote areas in India showed the incidence of NTD's as 6.5 to 8.2 per 1000 live birth which is among the highest worldwide(2). These NTDs may result from genetic mutations, nutritional and environmental factors, teratogenic drugs or combination of all these factors(1). A variety of genetic mutations and abnormal chromosomal pattern can occur in Neural tube defect cases. A pattern of 46XX, r(13) (p11 q32); 45XX, -r(13) was reported in a fetus with anencephaly(3). 45X/46X, r(X) (p11.22q12) pattern was observed in anencephaly and dorsal rachischisis associated with diaphragmatic hernia(4). Other studies showed three different chromosomal patterns like Trisomy 13, 47,XXX, and 92,XXXX(5-7). Single cell gel electrophoresis(SCGE) or Comet assay is one of the commonly used, easy to perform and sensitive method to evaluate the DNA damage(8). So this study has been undertaken to evaluate the DNA damage in neural tube defect cases by comet assay.

### **Material and Methods**

The study group consisted 30 clinically diagnosed Neural tube defect(NTD) cases and 30 age and sex matched controls. The study was approved by Institute Ethics Committee and informed written consent was obtained from the parents of study and control groups. Congenital disorders other than NTD and Down's syndrome cases were excluded from this study. Based on the types and severity of the disease, the cases were categorized into mild (n=5), moderate(n=6) and severe(n=19) disease. Mild form of cases include spina bifida, lipomenigocele, hydromyelia; moderate form include encephalocele and meningocele; while exencephaly, anencephaly and meningocele are the severe forms of Neural tube defects [9].

Blood samples were collected by venepuncture in a heparinised syringe. Lymphocytes separated from those blood samples were subjected to single cell gel electrophoresis (comet assay) as described by Singh et al . Comet pictures were captured by using Olympus BX51 trinocular microscope and scoring was done by using comet score software. Independent student's t' test was used to compare the data between cases and controls by Graph-Pad InStat 3 software and the analysis was carried out at 5% level of significance.

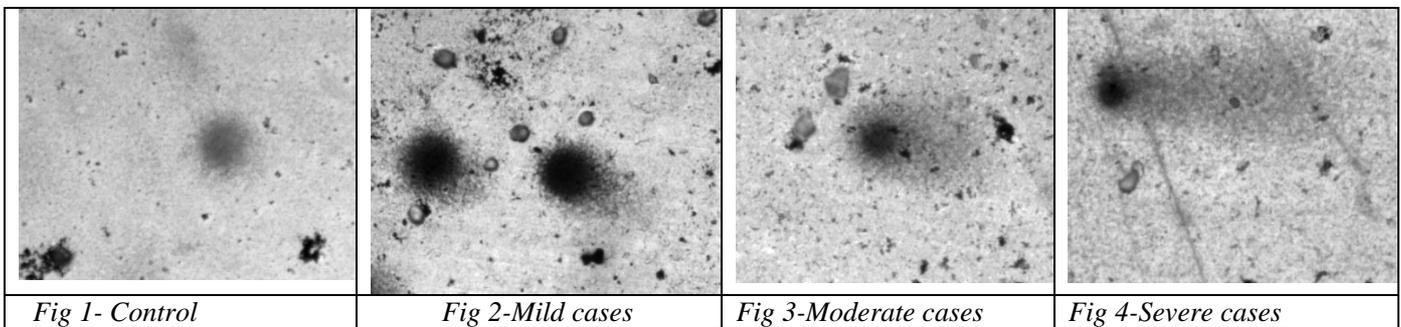
**Results**

Comet parameters like total length of comet, head diameter, tail length, % of DNA in head and % of DNA in tail of comet were tabulated for cases and controls(table1). In the comet, head diameter and % of DNA in head refers to normal or undamaged DNA; length of the comet's tail

and % of DNA in tail refers to damaged DNA(8). The comet parameters were compared according to the severity of the Neural tube defects (table 2). The tail of the comet is increasing in length from mild to severe form of cases (fig 2-4) and in controls there is no tail formation (Fig 1).

**Table 1.** Mean values of comet parameters in cases and controls.

Category	Total length (µm)	Head diameter (µm)	Tail length (µm)	% of DNA in head	% of DNA in tail
Cases (n=30)	71.9	44.8	33.6	79.7	20.3
Controls (n=30)	49.0	44.5	4.7	91.5	8.5
P value	<0.05	>0.05	<0.05	<0.05	<0.05



**Figures 1-4.** Comet appearance in relation to severity of the defect

**Table 2: Percentage of DNA in tail according to the severity of the disease.**

Category	Mean (%) of DNA in tail	S.D
Mild (A)	11.2	± 7.1
Moderate (B)	21.4	±6.5
Severe (C)	22.9	±5.0

A Vs B - P <0.05; A Vs C - P <0.05; B Vs C - P >0.05:

**Discussion**

Neural tube defects occur due to failure of closure of neuropores from rostral to caudal direction(1). According to Pangilinan et al., there are common genetic variation in 82 candidate genes as risk factors for the development of NTDs(10). MTHFR gene mutation is the most common cause for NTDs(11). The proper neural tube development requires the co-operation of Tr p53 and Gadd 45a genes(12). DNA is constantly subjected to chemical modifications. Several types of DNA damage such as single strand break(SSB), double strand break(DSB), cyclobutane pyrimidine dimers(CPD), 6-4 photoproducts and their Dewar valence isomers have been identified that

results from alkylating agents, hydrolic deamination, free radicals and reactive oxygen species formed by various photochemical processes including UV radiation. Breaks in DNA may also result from damaged DNA replication forks or from oxidative destruction of deoxyribose residues. Double strand breaks are lethal as they affect both the strands of DNA and lead to the loss of genetic information(13).

Most of the previous studies were done to identify the chromosomal abnormalities and genetic mutations as the causes of NTD. But our study was intended to estimate the DNA damage and correlate the severity of DNA damage with the types of NTD. Manoj et al., reported that unrepaired strand breaks in DNA leads to double strand breaks, which results in chromosomal aberrations or genomic instability(14). Tice et al., and Nandhakumar et al., suggested that the comet assay is one of the most sensitive and specific method to estimate the DNA damage(15,8). Mozaffarieh et al., used comet assay in glaucoma patients to estimate the DNA damage among them(16). Seidel et al., evaluated the radiation induced DNA damage by alkaline comet assay(17). Kaur et al.,

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used comet assay to assess DNA damage in agricultural workers exposed to pesticides(18).

In the present study the comet parameters like total length of comet, comet tail length, % of DNA in head, % of DNA in tail showed significant DNA damage in NTD cases than in controls. Severe form of the NTDs like anencephaly and meningomyelocele showed increased percentage of DNA damage compared with less severe type like spina bifida occulta. This may be due to more number of abnormal incorporation of base pairs in DNA strands of patients with severe type of NTDs than the mild and moderate types of cases. From this study it is evident that all types of neural tube defects are associated with DNA damage. The damage is mild in milder form of the disease. In moderate and severe form of NTDs the damage is more. The percentage of DNA damage increases with the severity of the disease. So the most severe cases show extensive DNA damage.

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