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Comparison of DNA damage with the clinical manifestations of children with Congenital Heart Diseases.

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Abstract

DNA damage in relation to the commonly ascribed clinical profile among forty clinically/3D Doppler Echocardiogram diagnosed children with various types of congenital heart disease (CHD) was studied and compared with equal number of age and sex matched controls. Using *Density Gradient Separation Technique,* the lymphocytes were isolated from the whole blood with histopaque, followed by submarine gel electrophoresis in high alkaline buffer media targeting single tailed Comets was assessed. The anthropometric measurements of the children with congenital heart disease were comparable to the normal control children. While comparing the cyanotic with acyanotic heart disease, only the *% DNA in tail* in the cyanotic group was found statistically increased. There was a marginal increase in the DNA damage among the children suffering from various severity of illness (mild, moderate and severe) which was not statistically significant. Children with CHD have increased DNA damage and relatively more among those with cyanotic heart disease.

Keywords: Congenital Heart Disease, DNA damage, Severity of illness, Oxidative stress.

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Introduction

Extensive literature regarding the role of nutritional status [1-4] and bio-chemical profile [5,6] among children suffering from CHD do exist. The developmental delay and interruption in achievements of milestones have been correlated with congenital heart defects. [6,7] The cardiac compatibility with that of anomaly involved was also expressed variously in terms of compensated and decompensated cardiac failure. [7-9] Literature on occurrence of hypoxia induced oxidative stress and DNA damage in CHD is scanty. [10,11] Hence the relevance of association of anthropometry and severity of congenital cardiac malformations with the level of DNA damage have been put forth in the current study.

Methods

Forty children with congenital heart disease diagnosed clinically and confirmed by echocardiography, were included as cases and age and gender matched healthy children were included as the control group in the current study. Written and Informed consent was obtained from the concerned parents pertaining to the investigation. Both the cases and controls were screened thoroughly by clinical examination and echocardiography (Philips iE-33). Their lymphocytes were separated by Density Gradient Separation Technique using histopaque and it was subjected to Conventional Comet Assay. [12-14].

The nutritional status of the children were assessed by recording the anthropometric parameters which includes height, weight and mid-arm circumference; the values of which were compared using the standard WHO growth chart. Milestones of both cases and controls were assessed using Trivandrum Developmental Scale. Based on the presence or absence of cyanosis the study group was divided into acyanotic (n=24) & cyanotic groups (n=16). Children with CHD were interrogated for hospitalization due to complaints of breathlessness with the cyanotic spells and the numbers of episodes were taken into consideration. Based on the number of such visits the severity of the condition was designated as mild (with single hospitalization; n=12), moderate (2 visits; n=17) and severe (>3 admissions; n=11).

The parametric Unpaired 't' test and One-way ANOVA test through SPSS-version 19 statistical software were used and $p < 0.05$ was taken as significant.

Results

The average height, weight and mid arm circumference were almost equal in both cases and controls. There was no statistically significant difference in the anthropometric measurements between cases and controls. However, the %DNA in Tail was increased in cases when compared to controls which was statistically significant (Table 1). Among the comet metrics, only the *% DNA in tail* in the cyanotic group was found to be significantly increased when compared with acyanotic patients (Table 2). While relating the severity of illness such as ***mild, moderate and severe*** with the extent of DNA damage, a marginal increase in the DNA damage was observed with increasing severity but not statistically significant (Table 3).

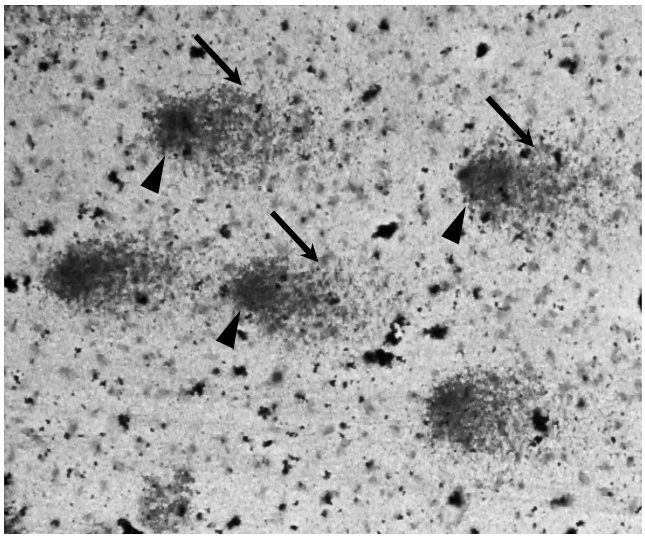

Figure 1. *Comet assay showing head with minimal tail formation among Control*

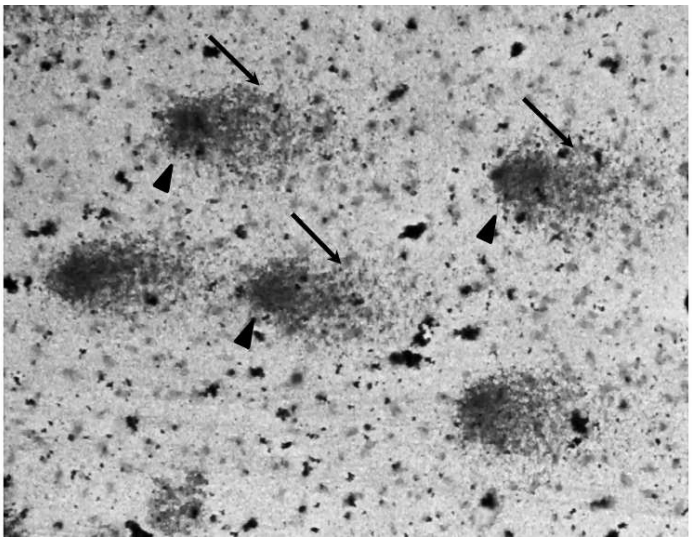

Figure 2: *Comets assay showing longer tail indicating increased DNA damage in case (black arrow heads-comet head and black arrow showing comet tail)*

Table 1. *Comparison of anthropometric measurements and comet metrics between Cases & Control*

	Anthropometric Parameters (cm)			**Comet Metrics**
	Height	Weight	Mid-arm Circumference	%DNA in Tail
Case Mean ± SD (n=40)	76.65±16.12	10.09±4.62	12.14±2.04	13.32±5.01
Control Mean ± SD (n=40)	78.33±16.18	11.03±4.57	12.36±2.09	10.98±7.61
p Value	p > 0.5	p > 0.5	p > 0.5	**p < 0.5***

Table 2. *Comparison of the comet metrics with the type of CHD among the cases*

		Comet Metrics				
		Total Comet Length (µm)	Head Diameter (µm)	Comet Tail Length (µm)	%DNA in Head	%DNA in Tail
Type of CHD	Cyanotic (n=16)	69.91±15.57	47.28±7.57	22.65±11.34	84.53±5.45	15.47± 5.45
	Acyanotic (n=24)	66.95± 14.28	48.94±10.48	18.07±11.12	90.33±12.34	11.89±4.22
p Value		p >0.05	p >0.05	p >0.05	p >0.05	**p < 0.05***

Table 3. *Comparison of the comet metrics with the severity of illness*

		Comet Metrics				
		Total Comet Length (µm)	Head Diameter (µm)	Comet Tail Length (µm)	%DNA in Head	%DNA in Tail
Severity of Illness	Mild (n=12)	62.0±14.9	44.6±7.8	17.3±7.9	88.6 ±3.5	11.4±3.5
	Moderate (n=17)	73.8±12.5	50.9±10.3	23.0±13.4	89.7±15.1	13.4±5.2
	Severe (n=11)	66.1±15.5	48.2±8.7	17.9±10.6	84.7 ±5.6	15.3±5.6
	p Value	p >0.05	p >0.05	p >0.05	p >0.05	p > 0.5

Discussion

Anthropometric measurements in children with CHD cases and controls will be comparable provided the factors such as timely vaccination, balanced diet regimen and constant check of haemogram are taken care of. [15] In the current series, a similar trend between the cases and controls with regards to anthropometric profile was observed. However there are emphatic reports regarding occurrence of the cases with CHD being severely undernourished in cyanotic category and mild degree of under nourishment in acyanotic. [2]

The developmental milestones were observed to be within the normal range based on the clinical examination of the children. There are reports regarding the occurrence of neuronal injury and impairment of neurotransmitters in children suffering from cyanotic heart defects especially due to low oxygen saturation of blood circulating via great vessel anomaly and septal defect. As a result of hypoxia there is a predisposition to impairment of neuronal growth and development leading to brain injury / impaired brain maturity as evidenced by altered ratio of N-acetylaspartate / choline and lactate/ choline which were found to be elevated in infants with CHD compared to the controls.[16] Non-invasive studies through proton magnetic resonance spectroscopic imaging and Diffusion tensor imaging also showed further evidence of brain injury as revealed by significant increase in average diffusivity and decrease in white matter fractional anisotropy. [16] Therefore, infants and children with CHD below two years of age require constant monitoring through regular follow up pertaining to neural development. Under these circumstances, children investigated in the current series who appeared to be within the normal limits need to be periodically scanned by the above said tools to evaluate the neurodevelopmental status. There is neurodevelopmental deficit in infants with complex cardiac malformations which is due to altered hemodynamic and hemostatic factors in individuals of CHD and hence are more prone for increased risk for brain injury. [17] But in the present study, there were no indications of impairment of neurological development in cyanotic and acyanotic group of children with CHD as evidenced by normal motor, verbal and social milestones achievement.

In the current study, the extent of DNA damage was increased in children with CHD when compared with the controls as evidenced by the statistically significant increase in the %DNA in Tail in cases. In addition, the extent of DNA damage was increased in children with cyanotic heart disease in respect to %DNA in tail when compared to acyanotic. These data are in concordance with the findings of oxidative DNA damage estimated by Srujana et al., with reference to the comet tail length in TOF and biochemically estimated oxidative stress put forth by Ercan et al., in children with CHD [18].

While comparing the severity of the illness of the cases investigated with that of the extent of DNA damage there is no statistical significance. However a gradual increase in Total Comet Length (TCL), Head Diameter (HD) and % DNA in Head (%DH) was observed from mild to moderate severity of illness only, whereas a gradual increase (minimal) with reference to % DNA in Tail (%DT) to all grades of severity of illness was observed. There is no continuous or sustained significant elevation of the data of comet metrics – an indicator of oxidative stress with the progressive disorder of the condition probably because of intervention by supplements of anti- oxidants like; multi vitamins which are commonly administrated as adjunct. The reason for persistent elevated DNA damage with reference to the gradation of the stages of severity with reference to % DT could be due to no medication either for the cardiac failure or non-administration of normal maintenance dose of multi-vitamins as anti-oxidants.

Conclusion

The DNA damage estimated through comet assay in cases with CHD was found to be significantly high when compared to control. In addition the clinical profile, the severity of the congenital heart disease causes a marginal increase in the DNA damage. This increase in DNA damage is probably caused by increase in oxidative stress. Hence early detection and prompt management of the disease can reduce the morbidity of the children with congenital heart disease.

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