

Micronuclei as A Biomarker in Monitoring Genetic Damage in Down Syndrome

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INTRODUCTION:

Down syndrome (DS) is a most frequent cause of mental retardation in humans, with frequency of 1 in 800 live births.¹ It is due to the inheritance of an extra copy of chromosome 21 as a result of chromosomal non-dysjunction during meiosis.

Although the chromosomal basis of DS have been known for 50 years, there is still a surprising lack of knowledge about the methods of monitoring genetic damage in DS individuals. Micronucleus

ABSTRACT:

Background: Down syndrome (DS) is a most common cause of mental retardation in humans. Monitoring genetic damage in DS individuals helps in evaluating genetic damage. Micronucleus assay is potentially an excellent tool to serve as a biomarker which detects chromosomal loss or malfunction.

Aim of the study: The objective of the study is to evaluate micronuclei (MN) frequency in exfoliated buccal cells in DS individuals.

Materials and Methods: The study subjects consisted of clinically diagnosed cases of Down syndrome. Healthy subjects without any medical conditions formed the control group. The cyto-smears were stained with Papanicolaou stain.

Results and Conclusion: The presence of increased number of micronuclei in Down syndrome ($P < 0.05$) patients may be useful in identifying individuals with increase risk of mental retardation.

Key words: Down syndrome; Micronucleus; Genetic damage

assay is potentially an excellent tool to serve as a biomarker which detects chromosomal loss or malfunction of mitotic spindles caused by aneugenic mechanism.² The objective of the study is to evaluate micronuclei (MN) frequency in exfoliated buccal cells in DS individuals.

MATERIALS AND METHODS:

The study sample comprised of 28 Down syndrome individuals and 28 healthy controls, matched according to the age and gender (Table 1).

All the Down syndrome individuals or their guardians signed post-informed consent for the study.

CELL COLLECTION, SLIDE PREPARATION AND SCORING:

Smears were taken from clinically normal buccal mucosa of the patients using wooden spatula moistened in distilled water. The scrapings were then transferred to clean glass previously marked with patient's reference number, and spread thinly and uniformly over the slide. After air drying, they are fixed and stained with Papanicolaou (PAP) stain. Wholeslide is screened for counting MN at 400X and 1000X magnifications. Micronuclei (Figure 1) are counted using Tolbert et al criteria.³

STATISTICAL ANALYSIS:

Statistical analysis was carried out using Student t test to compare the frequency of MN cells between DS and healthy controls, age groups and gender in DS. Values are expressed as means \pm standard deviation. The level of significance is 5% ($P \leq 0.05$).

RESULTS:

Micronucleus frequency was significantly higher in DS than controls (about 2 fold; $P \leq 0.05$) and higher in male DS compared to females DS (1.75 fold; $P \leq 0.05$). Significant increase of MN was observed in older DS age group compared to younger DS age group (1.5 fold; $P \leq 0.05$) (Graph 1).

DISCUSSION:

Micronuclei are small extra nuclear bodies formed by chromosome fragments or whole chromosome which did not reach spindle poles during mitosis and remained encapsulated at telophase in separate nucleus. They reflect structural or numerical chromosomal aberration.²

In the present study DS individuals showed 2 folds increase in MN frequency compared to that of controls. In agreement with the present results, Ferreira et al showed that MN frequency in buccal cells of DS individuals was about 3.25 fold higher than control group.⁴ Thomas et al also reported 7 folds higher MN frequency in DS individuals when compared with healthy controls and 2 folds higher

when compared with older individuals.⁵ In contrast Scarfi et al observed lower incidence of MN in cytokinesis blocked lymphocytes in DS individuals than controls.⁶ However Maluf and Erdtmann found no significant difference between DS individuals and controls as evaluated by MN frequency in cytokinesis blocked lymphocytes.⁷ The difference in the frequency of MN in buccal mucosa and lymphocytes could be explained by their metabolism. Simko et al demonstrated that different cell lines respond differently in terms of micronucleus induction and apoptosis in response to genotoxic insults.⁸

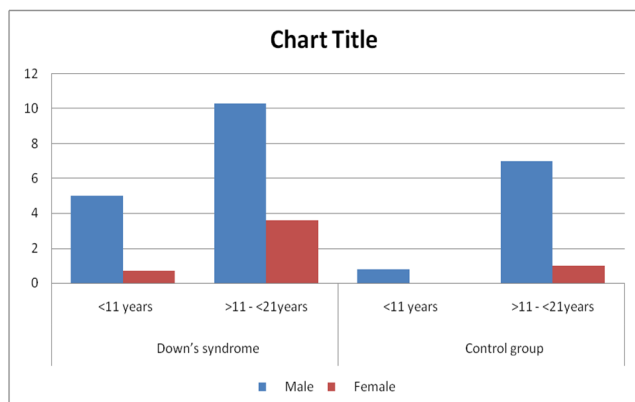
Several mechanisms are likely to be involved in formation of micronucleus in tissues of individuals affected by DS. These depend on the origin of micronucleus itself i.e., from chromosomal breakage or from chromosomal mal-segregation.

Oxidative stress ensues when the normal balance between production of reactive oxygen species and antioxidant ability of the target cell is upset. This leads to cellular dysfunction resulting in elevated genomic instability such as MN formation and eventual cell death. In DS, Superoxide dismutase 1 (SOD-1), an antioxidant enzyme encoded on chromosome 21(21q22.1) causes dismutation of superoxide anion into hydrogen peroxide and oxygen resulting in oxidative stress.^{9, 10}

Another plausible mechanism of MN formation is folic acid deficiency. Folate is an essential nutrient acting as methyl donor for conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate required for DNA synthesis and repair. Folate is also required for production of S-adenosylmethionine, the methylating agent required for maintenance of cytosine methylation patterns essential for gene expression regulation and for chromatin structure stability in critical areas such as centromeres. Under conditions of folate deficiency, dUMP accumulates causing breaks in DNA strand as a result of base removal by glycosylases following uracil misincorporation into DNA in place of thymine leading to MN formation. And accumulation of homocystein also occurs due to folate deficiency.¹¹ Impairments in folate/ homocystein metabolism have been suggested as a risk factor for having

Table 1: Age and gender of subjects studied

Group	Down's syndrome		Control group	
	<11 years	>11 - <21years	<11 years	>11 - <21years
Male	7	7	7	7
Female	7	7	7	7



Graph 1: Frequency of micronuclei in buccal mucosal cells of Down syndrome and control individuals according to gender. Statistical significance according to the Student 't' test (P<0.05).

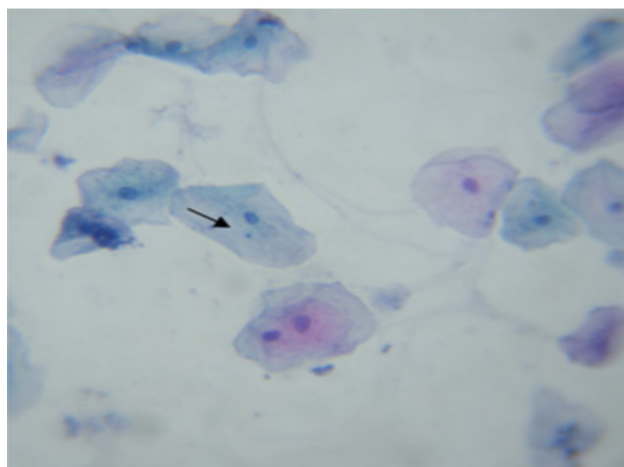


Figure 1: Photomicrograph showing the Miconuclei (100X)

infants with DS. It is based on the assumption that they could result in aberrant methylation of pericentromeric regions as well as of regions involved in chromosome recombination, thereby affecting chromosome 21 segregation.¹²

In the present study, DS individuals aged >11 to ≤21 years showed increased frequency of micronuclei than younger DS individuals ≤ 11 years. In agreement, Ferreira et al also found positive correlation of MN with age.⁴ DS is associated with lower DNA repair efficiency and accelerated decline in DNA repair capacity with age.¹³ Increasing age with DS is also associated with increase rates of depression, thyroid dysfunction and sensory impairment.¹⁴⁻¹⁶

DS individuals usually develop alzheimer's disease by fourth decade of life. It is because of constitutive trisomy 21 which results in over expression of genes mapping to chromosome 21, in

particular that coding for amyloid precursor protein (APP).¹⁷ Alterations in frequency of MN have also been found in alzheimer's disease.^{18, 19} So, determination of MN frequency might be applied to detect alzheimer's disease risk. Further insight is necessary to better understand the relation.

DS individuals have increased risk of leukaemia, retinoblastoma, lymphomas and germ cell tumors.²⁰ MN frequency has already been suggested for identifying individuals with high risk for cancer.²¹ So, determination of MN frequency can probably also be applied for identifying DS individuals with high risk cancer.

Further studies should be conducted for better understanding the non-dysjunction process. The ultimate goal should be development of preventive treatment strategies. This can only be developed on the basis of clear understanding of pathological process involved.

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