Micronucleus Assay as Biomarker for Chromosome Malsegregation in Young Mothers with down syndrome Children

Thesis

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Abstract

We observed an increased frequency of binucleated micronucleated lymphocytes in mothers who had a Down syndrome (DS) child before 30 years of age and the fluorescence in situ hybridization analysis revealed that micronuclei were mainly originating from malsegregation chromosome 21.The present study included 62 Egyptian young mothers (age < 30 y) ,free from any chronic disease, not taking any regular drugs, there were divided into 2 groups: group 1; 22 mothers of classic Down syndrome children and group 2; 40 healthy Egyptian matched females as control group. Cases were recruited from the Genetics outpatient clinic of National Research Centre and Children's Hospital, Cairo University.

Statistical analysis for malsegergation of chromosome 21 using FISH probe revealed high susceptibility of malsegergation (p=0.0001) in young mother of Down syndrome children compared to controls mothers.

Key words:

(Down syndrome, Chromosome, Micronucleus, fluorescence in situ hybridization, Malsegregation).

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Mohamed Badie Taher

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LIST OF ABBREVIATIONS

AD	Alzheimer Disease
AAI	Atlantoaxial Instability
AD	Atopic Dermatitis
ADHD	Attention Deficit Hyperactivity Disorder
APE	Adapted Physical Education
CBMN	Cytokinesis-Blocked Micronucleus
CD	Coeliac Disease
CHD	Congenital Heart Disease
CVS	Chorionic Villus Sampling
DS	Down Syndrome
DSCR	Down Syndrome Critical Region
FISH	Fluorescence In Situ Hybridization
HUMN	Human Micronucleus
MDS	Mothers of Down Syndrome
MI	Meiosis I
MII	Meiosis II
MN	Micronuclei
PCP	Pentachlorophenol
PD	Parkinson Disease
PPHN	Persistent Pulmonary Hypertension of the Neonate
PUBS	Percutaneous Umbilical Blood Sampling
PVN	Predictive value negative
PVP	Predictive value positive
RSV	*Respiratory Syncytial Virus*
SCE	Sister Chromatid Exchange
SCGE	Single-Cell Gel Electrophoresis
UTAs	Urinary Tract Anomalies

INTRODUCTION

Down syndrome (DS) or trisomy 21 is by far the most common genetic syndrome of chromosomal origin. It affects up to 1 in 800 live births worldwide **(Karaman, 2010)**. Clinical presentation of DS is complex and variable. Non-disjunction trisomy 21 (classic Down syndrome or primary Down syndrome) leading to DS is caused by the failure of normal chromosome 21 segregation during meiosis and accounts for 91% of total DS cases. In 95% of cases the extra 21 chromosome is maternal in origin **(Egan et al., 2011).**

In the last ten years, some researchers documented an increase in the percentage of DS babies born by young mothers (< 35 years of age). An Indian study revealed that DS births were increased among the young Indian mothers especially in the rural areas **(Maryl et al., 2010).** In USA, a demographic study of live-births DS from 1989 to 2006, detected an increase in DS births among females < 34 years of age in the last years **(Egan et al., 2011).**

Investigators started to use micronucleus (MN) assay as a biomarker to genetic damage in the cell. Biologically, micronuclei are the chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division. The MN scoring is an indication of DNA damage (e.g. cancer, heart attacks, Alzheimer disease) and susceptibility to chromosome malsegregation. Hence, it is used as a biological test in various diseases. Some researchers used FISH analysis or flow cytometry with MN assay, which increased the specificity and broaden the spectrum of use of MN assay **(Walitza et al., 2009).**

Migliore and her co-workers studied the susceptibility to chromosome 21 malsegregation in young mothers (< 35y) with DS children **(Migliore et al., 2009).** They documented an increased frequency of MN which points to an increased aneuploidy in peripheral lymphocytes among young mothers of individuals with DS. They also reviewed the recent mechanisms and risk factors for chromosome 21 non-disjunction; and suggested that human non-disjunction (especially in trisomy 21) is a multifactorial trait, where the maternal susceptibility to chromosome malsegregation play an important role. MN assay is increasingly used as a method of choice for evaluation of genetic damage in cell, because of its affordability and efficiency **(Benedetti et al., 2013).**

AIM OF THE WORK

1- Investigating and analyzing (using the micronucleus assay) the cytogenetic characteristics and predisposition to chromosome malsegregation of peripheral blood lymphocytes in a group of young women (age < 30 y), who have a child with classic Down syndrome (non-disjunction trisomy 21).

2- Applying the fluorescence in situ hybridization (FISH) using LSI 21 FISH probe to identify the micronucleus chromosomal origin.

3- When the increased susceptibility to chromosome 21 malsegregation is proven, micronucleus assay (MN) combined with FISH for chromosome 21 may be used as biomarker and could be added to the list of investigations for premarital and preconceptional counseling.

1. DOWN SYNDROME

Down syndrome (DS) or trisomy 21 is an over expression syndrome, where genes on chromosome 21 are expressed in 3 copies instead of 2 copies (figure 1). Down syndrome is the most common noninherited, 'organic' cause of mental retardation and occurs in approximately one of every 600 live births (BC Vital Statistics). Early detection and improvements in health care have led to a significant increase in the life expectancy of individuals with DS.However, children with DS have an increased risk of certain congenital anomalies. (**Freeman et al., 2007**).

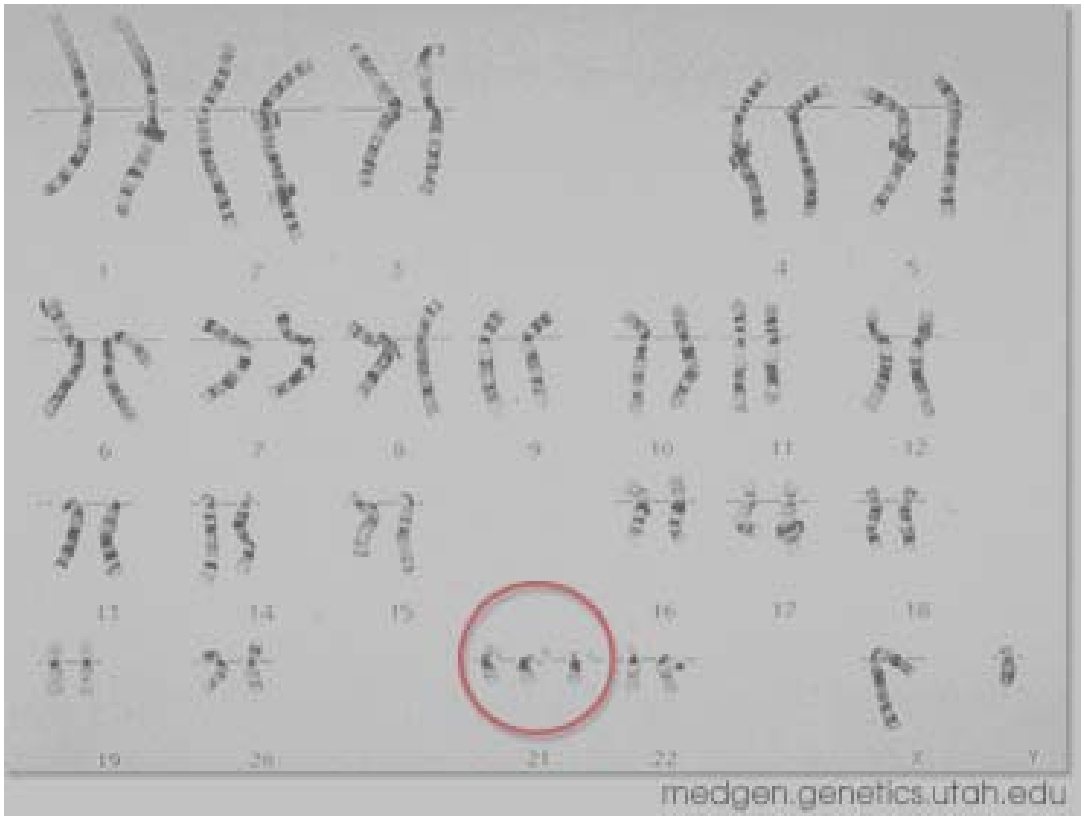

Figure 1: Female **Down Figure 1:** syndrome karyotype demonstrating non disjunction trisomy 21. (Karyotype prepared by Dave McDonald), **(Luthardt and Keitges, 2001).**

1.1.1 Down Syndrome: Historical Aspects

In 1866, the British physician John Langdon Down (1828-1896) published an article which described children with a common phenotype and with intellectual disability **(Kieser et al.,2003)** He accurately described the features of DS including hypotonia, mental retardation and facial features, and classical pattern of palmar creases of hands. The affected individuals have upward slanting of palpebral fissures which give the impression of mongolian people. He referred to the patients as "mongoloids". In 1959, the French geneticist Jerome Lejeune showed that DS is caused by a trisomy of chromosome 21 which subsequently confirmed by Jacobs and her co workers **(Jacobs et al., 1959).** In 1961, the WHO informally recommended to stop using the term “mongolism” and to describe people with DS as trisomy 21 anomaly **(Howard-Jones, 1979).**

More than 40 features that may be associated with DS (figure 2).However, not all features are observed in one individual with DS, but variable features occur to some degree in each individual with trisomy 21. Relationship between extra genes or gene products on chromosome 21 and craniofacial abnormalities, hypotonia, heart defects, duodenal atresia, mental retardation and dermatoglyphics has contributed to the construction of a phenotype map within the Down syndrome critical region (DSCR). The mechanism for phenotypic variability is not understood **(Castillo et al., 2013).**