

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

**Thesis**

Submitted for Fulfillment of Masters Degree in

**Pediatrics**

**By**

**Mohamed Badie Taher Kattaria**

M.B., B.Ch.

**Supervised By**

**Prof. Dr.**

**Sawsan Abd-Elhady Hassan**

*Professor of Pediatrics and Genetics*

*Faculty of Medicine, Cairo University*

**Prof. Dr.**

**Hanan Hosny Afifi**

*Professor of Human Genetics*

*Clinical Genetics Department*

*National Research Centre*

**Assoc. Prof. Dr. Eman Abdel Ghany Abdel Ghany**

*Associate Professor of Pediatrics, Faculty of Medicine, Cairo University*

**Faculty of Medicine**

**Cairo University**

**2014**

## **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, they 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 malsegregation of chromosome 21 using FISH probe revealed high susceptibility of malsegregation ( $p=0.0001$ ) in young mother of Down syndrome children compared to controls mothers.

### **Key words:**

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

## ACKNOWLEDGEMENT

First of all, I thank **Allah** to whom I relate any success I have reached and might reach in the future.

I have great pleasure in expressing my deep sense of gratitude and indebtedness to **Prof. Dr. Sawsan Abdel-Hady Hassan**, Professor of Pediatrics, Cairo University, for her expert supervision and constant source of encouragement to produce this work.

I am heartily thankful to my supervisor **Prof. Dr. Hanan Hosny Afifi**, Professor of Human Genetics, Clinical Genetics Department, National Research Centre, whose encouragement; guidance and support from the initial to the final level enabled me to develop a better understanding of the subject.

My gratitude and appreciation for **Dr. Eman Abdel Ghany**, Associate Professor of Pediatrics, Cairo University for her sincere help, enthusiastic encouragement and close supervision throughout the work.

I would like to express my deepest gratitude to **Dr. Maha Eid**, Assistant Professor of Human Cytogenetics, Human Cytogenetics Department, National Research Centre, for her relentless efforts and for providing the time to perform Cytokinesis-block micronucleus assay and FISH analysis for all cases and controls.

This dissertation would not have been possible without the guidance and help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

I would like to express my sincere thanks to all controls, patients with Down syndrome and their families for their cooperation during the study.

Lastly, I offer my regards and blessings to my family and all of those who supported me in any aspect during the completion of the study. Each of you can share in this accomplishment, for without your support it would not have been possible, thank you for everything.

***Mohamed Badie Taher***

# CONTENTS

<b>Table of Content</b>	<b>Page</b>
<b>Acknowledgement</b>	
<b>Abstract and Keywords</b>	
<b>Table of Contents</b>	
<b>List of Tables</b>	
<b>List of Figures</b>	
<b>List of Abbreviations</b>	
<b>Introduction and Aim of the Work</b>	<b>1</b>
<b>1.Review of Literature</b>	<b>4</b>
<b>1.1. Down Syndrome</b>	<b>4</b>
1.1.1. Down syndrome: Historical aspects	<b>5</b>
1.1.2. Features of Down syndrome	<b>7</b>
1.1.2.1. Newborn assessment	<b>8</b>
1.1.2.2. Cardiovascular defects	<b>10</b>
1.1.2.3. Orofacial defects	<b>10</b>
1.1.2.4. Hearing defects	<b>10</b>
1.1.2.5. Respiratory anomalies	<b>11</b>
1.1.2.6. Endocrinal defects	<b>12</b>
1.1.2.7. Hematological defects	<b>12</b>
1.1.2.8. Immunological defects	<b>12</b>
1.1.2.9. Ophthalmological defects	<b>13</b>
1.1.2.10. Skin features	<b>13</b>
1.1.2.11. Orthopedic features& Atlanto-axial Instability (AAI)	<b>13</b>
1.1.2.12. Gastrointestinal tract anomalies	<b>14</b>
1.1.2.13. Urogenital anomalies	<b>14</b>

1.1.2.14. Developmental defects	14
1.1.2.15. Neurological features	15
1.1.3. Age-related demographics	16
1.1.3.1. Biological aging hypothesis	17
1.1.3.2. Genetic aging hypothesis	18
1.1.4. Sex-related demographics	20
1.1.5. Race-related demographics	20
1.1.6. Exogenous risk factors	20
<b>1.2. Micronucleus</b>	<b>22</b>
1.2.1. History	24
1.2.2. Uses and applications	25
1.2.3. Micronucleus in healthy and diseased individuals	26
1.2.4. Method of assay	28
<b>1.3. Micronucleus and Down Syndrome</b>	<b>34</b>
1.3.1. Chromosomal abnormality	34
1.3.2. Risks of Down syndrome and the increased micronucleus	42
1.3.3. Buccal cytome assay	45
1.3.4. Cytokinesis-block micronucleus ( <i>CBMN</i> ) assay	48
<b>2- Subjects and Methods</b>	<b>51</b>
<b>3- Results</b>	<b>59</b>
<b>4- Discussion</b>	<b>74</b>
<b>5- Summary</b>	<b>87</b>
<b>6- Conclusion</b>	<b>89</b>
<b>7- Recommendations</b>	<b>90</b>
<b>8- References</b>	<b>91</b>
<b>9- Arabic Summary</b>	

## LIST OF TABLES

Table	Title	Page
<b>Table 1</b>	Features among Down syndrome	7
<b>Table 2</b>	Neonatal signs of Down syndrome	8
<b>Table 3</b>	Some genes located on the long arm of chromosomes 21	39-40
<b>Table 4</b>	Comparison of age between mothers of DS and the control mothers	60
<b>Table 5</b>	Age ranges of Down syndrome offspring	60
<b>Table 6</b>	Characteristic features of the studied Down syndrome cases in comparison to published data	62
<b>Table 7</b>	Comparison of MN % between mothers of Down syndrome and the control mothers	65
<b>Table 8</b>	Comparison of malsegregation between mothers of Down syndrome and the control mothers	68
<b>Table 9</b>	Sensitivity, specificity and predictive values for MN% in detection of YMDS	70
<b>Table 10</b>	Best cut-off level of MN% for detection of YMDS in cases and control mothers	70
<b>Table 11</b>	Correlation between malsegregation and other studied variables	72
<b>Table 12</b>	Sensitivity, specificity and predictive values of FISH in detection of DS mothers	73

## LIST OF FIGURES

Figure	Title	Page
<b>Figure 1</b>	Down syndrome karyotype demonstrating non disjunction trisomy 21	4
<b>Figure 2</b>	Some associated congenital anomalies with Down syndrome	7
<b>Figure 3</b>	Features of Down syndrome	9
<b>Figure 4</b>	Telomere length (Kbp) among control and meiotic outcome groups stratified by age categories	19
<b>Figure 5</b>	Micronucleus	22
<b>Figure 6</b>	Formation of a micronucleus during cell division	26
<b>Figure 7</b>	Micronucleus (MN) formation, showing MN formation during the metaphase/anaphase transition of mitosis (cell division)	30
<b>Figure 8</b>	The mechanism of micronucleus formation in vivo	31
<b>Figure 9</b>	Ideogram of chromosome 21	35
<b>Figure 10</b>	Various segments of chromosome 21 showing the critical region for Down syndrome	36
<b>Figure 11</b>	Segmental map of human chromosome 21	41
<b>Figure 12</b>	Risk factor model for Down syndrome birth showing with increasing maternal age	42
<b>Figure 13</b>	Buccal Cytome Model	47
<b>Figure 14</b>	Routine diagnostic workup for identification of trisomy 21	49
<b>Figure 15</b>	Cytokinesis blocked MN assay (CBMN) shows binucleated cell without MN (control).	55
<b>Figure 16</b>	CBMN shows binucleated cell with MN (mother of DS).	55



<b>Figure 17</b>	FISH using LSI 21 combined with CBMN show normal segregation of LSI 21 (control).	56
<b>Figure 18</b>	FISH using LSI 21 combined with CBMN show malsegregation of LSI 21 one cell with one signal and other cell with 3 signals (mother of DS).	56
<b>Figure 19</b>	Distribution of DS cases in relation to age	61
<b>Figure 20A</b>	Characteristic features of the studied Down syndrome cases in comparison to published data	63
<b>Figure 20 B</b>	Characteristic features of the studied Down Syndrome cases in comparison to published data	63
<b>Figure 20 C</b>	Characteristic features of the studied Down Syndrome cases in comparison to published data	64
<b>Figure 21</b>	Comparison of MN% between mothers of Down Syndrome and the control mothers	66
<b>Figure 22</b>	Distribution of mothers of DS and control group in relation to MN%	67
<b>Figure 23</b>	Comparison of malsegregation between mothers of Down syndrome and the control mothers.	69
<b>Figure 24</b>	ROC curve for determining the best cut-off level of MN % in detection of DS in relation to FISH	71
<b>Figure 25</b>	Correlation between mother age and FISH result	72

## LIST OF ABBREVIATIONS

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

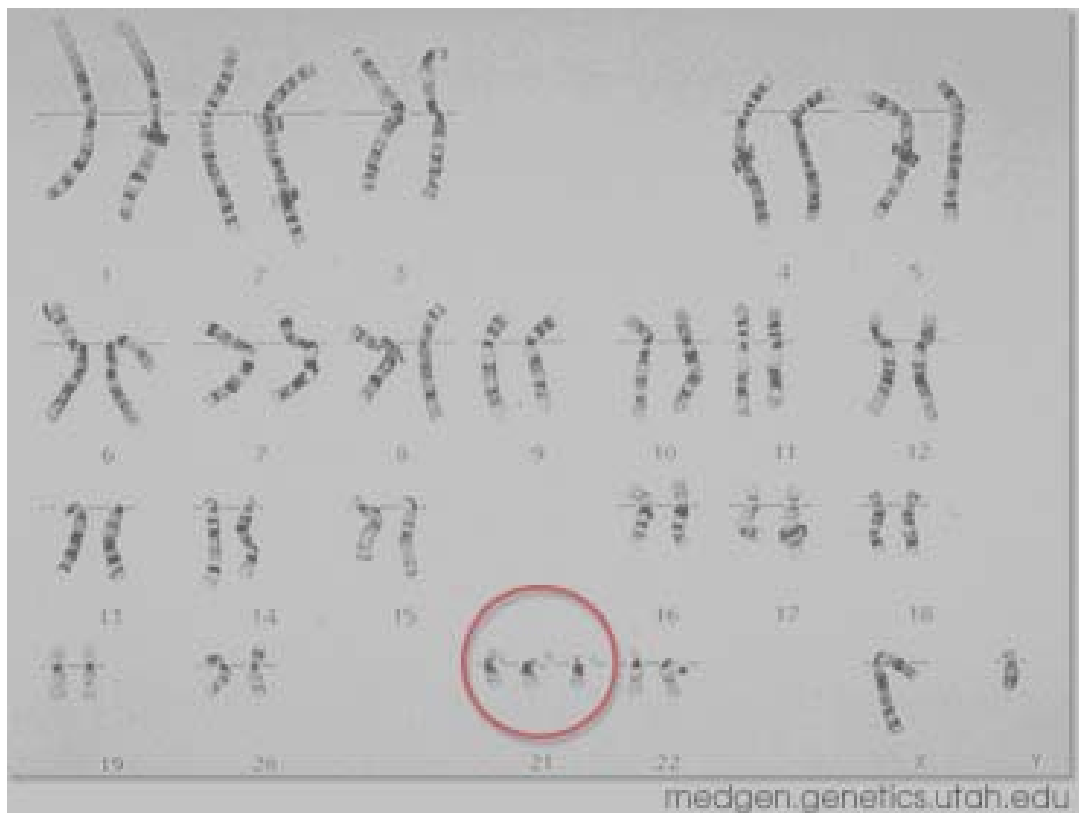
Migliore and her co-workers studied the susceptibility to chromosome 21 malsegregation in young mothers ( $< 35y$ ) with DS children (**Migliore et al., 2009**). They documented an increased frequency of MN which points to an increased aneuploidy in peripheral lymphocytes among young mothers of individuals 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 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**).