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Cytogenetic and Immunological analyses of Down Syndrome Children and their Parents

By

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B. Sc. Biotechnology, Al-Nahrain University, 2003

Ramdan ***1427***

October ***2006***

Acknowledgments

At the beginning, thanks to **Great Merciful God** who gave me health, faith, strength and patience, and facilitated the ways for me to accomplish this work.

I would like to express my deep gratitude, thanks and appreciations to my supervisors **Dr. Esmail K. Shubber** and **Dr. Mohammed Rafeeq** for their continuouses guidance, support and advices through whole period of research. This work would never have been done.

I would like to thank the Biotechnology staff in Al-Nahrain university Miss Oruba , Al-Yarmok Hospital Dr.Da'ad, Dr. Nariman, Mr. Taha , Hiba , Alla Teaching Center and Awat Teaching center in Al-Sulmaniya

My sincere gratitude goes to Dr. Hazim Al [illegible] Wahab for them assistance.

I want to thank to my colleagues and friends in the department of Biotechnology, college of science Mayassa Fadhil, Muntaha Abdulrazaq, Farah Haytham, Saefaldin R. Khalel , Ahmed Hassan, Bassam Sameir, Rafal Hussam, Worood Kamil, Hassan Abdulhadi and Sura Ali.

Special thank to Oday Adnan for his constant support throughout this work.

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Conclusions

1- Cytogenetic indices BI, MI, CAs, RI, MN and SCE are sensitive tools to ascertain the cytogenetic abnormalities in DS children and their parents which expressed their genomic unstability.

2- Analysis of the results of this study has revealed an increased frequency of CAs, MN and SCEs in lymphocytes of DS children and their parents compared with control, while a significant decreased observed MI, BI and RI.

3- The cell division reduction is a useful index for evaluating the sensitivity and resistance to the anticancer drugs MTX and 6-T-G.

4- Using chromosomal aberrations, sister chromatid exchanges and micronuclei as obvious immune dysfunction DS children.

Recommendations

1- Further investigation by molecular studies for evaluating the genomic instabilities in blood samples from DS children and their parents.
2- Further studies of mitochondrial DNA for mother of DS children and their children.
3- Further investigation of *HPRT* and *DFHR* enzymes activities in DS children and their parents to confirm the cytogenesis alterations at their gene loci combined by gene polymorphism.
4- Every mother should do prenatal test(diagnosis and prenatal screening) so as to make sure that the fetus is healthy .

1.1 Introduction

Down syndrome is a genetic condition resulting from the presence of all or part of an extra 21 chromosome. It is named after John Longdon Down; the British doctor who first described it in 1866.DS is characterized by a combination of major and minor abnormalities of body structure and function. Among features present in nearly all cases are impairment of learing and physical growth, and recognizable facial appearance usually identified at birth (Patton, 2003).

An interesting aspect of this syndrome is the increased incidence among children of older mothers, a fact known more than twenty-five years before the discovery of the cause of the syndrome (Schon, *et al*., 2000).

Study of the incidence of DS in Iraq showed a significant increase during post-war period (1991-1997) in comparison with pre-war period. The incidences [illegible] doubled in the old [illegible] period (Al-Taha, 1998).

Numerous studies have documented immune dysfunction in DS including decreased IgA, low white cell counts, and low levels of T-cells. These deficits probably lead to the increased incidence of upper respiratory, ear, and gastrointestinal infection rate in DS most evident in children but even present in adults (Chaushu, *et al*. 2002).

There are three types of DS, although it is generally thought that there is no clinical difference in the three genotypes.

(1) ***Trisomy 21*** (94%): The extra 21 chromosome (three instead of the usual two) produces a complement of 47 chromosomes.

(2) ***Translocation*** (5%): A segment of a 21 chromosome is found attached to other pairs of chromosomes (usually #14, thus referred to as a14/21 translocation). These individuals have the normal complement of 46 chromosomes.

(3) ***Mosaicism*** (1%): Nondisjunction occurs at a later stage of cell division, therefore, some cells have the normal complement of 46 chromosomes and other cells 47 chromosomes (with an extra 21 chromosome)(Patterson, 1987).

The aim of this study:

1- Study of cytogenetic analyses of DS children and their parents (Blast index, Mitotic Index, chromosomal aberration, SCE, RI and [illegible]).

2- Study some of immunological analyses of DS children and their parents (Blast index and phagocytosis activity).

3- Determine the sensitivity of human blood lymphocytes from DS children and their parents to anticancer drug 6-Thiogaunine (6-T-G) and Methotrexate (MTX).

Literature Review

1-2: Down syndrome (DS)

Down syndrome is a genetic disorder occurring in approximately 1 in 650 to 1000 live births (Hook, 1982). It is the most common genetic cause of mental retardation accounting for 25-30% worldwide. John Langdon Down (1866), an Englishman published the first clinical description of Down syndrome. Lejeune *et al*. (1959) confirmed the presence of trisomy 21 in Down syndrome (McLaren and Bryson 1987).

1.2.1: Physical Characteristic of DS

chromosomally normal children (Patton, 2003).

1.2.2: Prenatal Screening for DS

Obstetricians and Gynecologists are always searching for a noninvasive way to screen for genetic abnormalities of the fetus. Of particular interest is Down's syndrome. Between 1984 and 1988 several investigators began reporting on serum markers. Maternal serum alpha—fetoprotein (MSAFP) levels were found to be low in association with fetal aneuploidy, and elevated human chorionic gonadotropin (hCG) levels were found in many Down's

syndrome pregnancies, along with reduced maternal serum unconjugated estriols. The value of routine ultrasound studies as a fourth marker (Haddow, *et al*., 1992; Cheng, *et al*., 1993).

1.2.3: Diagnostic test of DS

The ways of DS diagnosis are by obtaining fetal tissue samples by amniocentesis, choroinic villus sampling, it would not be appropriate to examine every pregnancy this way. Besides greatly increasing the cost medical care, these methods do carry a slight amount of risk to the fetus. In some circumstances, it may be useful to obtain a sample of fetal blood. This technique, referred to as cordocentesis or percutaneous umbilical blood sampling (

The homologous gene quantitative polymerase chain reaction (HGQ-PCR) is practical and may be used for the prenatal diagnosis of Down's by [illegible] (Lee *et al*., 19

1.2.4: Genetic Form of DS

Three genetic variations can cause DS. In most cases, approximately 94% of the time, DS is cause by the presence of the extra chromosome 21 in all cells of the individual. In such cases, the extra chromosome originates in the development of the either the egg or the sperm. Consequently, when the egg and sperm unite to form the fertilized egg, three-- rather than two—chromosomes 21 are present. As the embryo develops, the extra chromosome is repeated in every cell. This condition, in which three copies of chromosome 21 are present in all cells of the individual, is called **trisomy 21**. This is form of DS that increases in incidence with increase maternal age.

In approximately 2%of cases, DS is due to **mosaic trisomy 21**. This situation is similar to simple trisomy 21, but, in this instance, the extra chromosome 21 is present in some, but not all, cells of the individual. For example, the fertilized egg may have the right number of the chromosomes, but due to an error in chromosome division in early embryonic development, some cells acquire an extra chromosome 21. Thus an individual with DS due to mosaic trisomy 21 will typically have 46 chromosomes in some cells, but will have 47 chromosomes (including an extra chromosome 21) in others. In this situation, the range of the physical problems may vary depending on the proportion of cells that carry the additional chromosome 21.

However, approximately 5 % of individuals with DS have cells containing 46 chromosomes, but still have the features associated with DS. In such cases, material from one chromosome 21 gets stuck or translocated onto another chromosome. Under prior to or at conception, a parent with DS has two normal chromosome 21 material on the translocated chromosome 21, resulting in the features associated with DS. In such situations, the individual with DS is said to have **translocation trisomy 21** (Hernandez and Fisher 1996).

1.2.5: Immune Dysfunction

Impairment of immunological defenses in patients with DS can be inferred from a number of clinical and epidemiological studies. A large body of research focusing on abnormalities in the systemic immune system of DS was performed, demonstrating selective cell-mediated immunodeficiencies, defective neutrophil polymorphonuclear leukocyte chemotaxis, impaired antibody response to specific pathogens, low T-cell lymphocyte counts, and

immature subsets of T-lymphocytes (Ugazio, *et al*., 1981; Reuland-Bosma and Van Dijk, 1986; Lockitch *et al*., 1987; Morinushi *et al*., 1997)

Young DS patients have high rates of infections, malignancies, and autoimmune diseases (Desai, 1997), which may be seen in non-DS subjects at a much older age.

Histological aberrations of the thymus as well as abnormal distribution of T-cell populations suggest that the majority of the immunological disabilities may be ascribed to an abnormal thymic physiology. The impairment in both B- and T-cell function is expressed in abnormal antibody and immunoglobulin production (Nespoli *et al*., 1993).

Acquired hypothyroidism in DS is at least partly attributable to autoimmune thyroiditis, because thyroid autoantibodies are found in 65% of individuals with DS and hypothyroidism (Tuysuz, and Beker, 2001).

Children with Down syndrome (DS) are at a 10- to 15-fold increased risk of developing acute leukemia, including both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) (Hasle *et al*., 2000).

1.2.6: Risk Factors Associated to the Down Syndrome Occurrence

i: Maternal Age

The main risk factor for DS is maternal age. Many studies having shown an increased incidence of Down's syndrome with increased maternal age. (Schimmel *et al.*, 1997) Among mothers younger than 30 of age, the risk is less than 1/1000. It increases to approximately 1/400 at age 35 years, 1/100 at age 40, and approximately 1/25 after age 45 as seen in figure 2-1. (Cash, 2004)

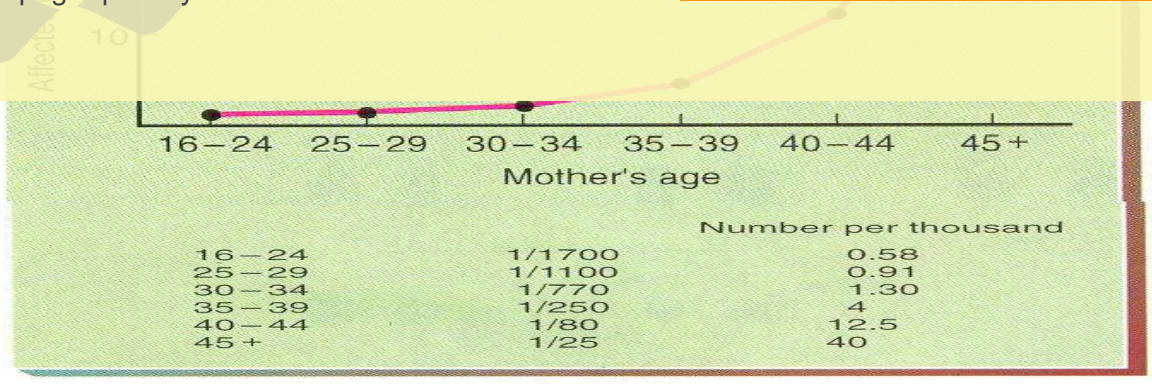

Figure 1-1 increased risk of trisomy 21 attributed to the age of the mother (Hooke, 1979).

Direct studies of the frequency of chromosomal abnormalities in sperm & egg cells indicate that the pattern is in fact due to an increase in non-disjunction among older mothers. Since all of a female's oocytes are formed during her embryonic development, an ovum of a 45-year-old woman is also 45 years old. It has been stated recently that in the aneuploid oocyte, a number of events occur, beginning with hormonal imbalance, sub-optimal micro-vasculature around the ovarian follicle, reduced blood flow, increased carbon dioxide and lactic acid inside the follicle, decreased pH in the oocyte, reduced mitotic spindle size, spindle displacement and non-disjunction (Aardeme *et al*, 1998).

It was hypothesized that the mutations in the mitochondrial DNA that increase between the 30 and 40 years in diverse tissues could also occur in the oocyte, determining defects in the phosphorylation which could affect the maturation and the meiotic process (Arbuzova, 1995; Schon *et al*, 2000)

Down syndrome with the use of cont [illegible] has been suggested an increase of trisomy 21(Tabor and Philip.1997).

ii: Paternal age

Paternal non-disjunction of chromosome 21 accounts for 5–10% of all trisomy 21 .The effect of paternal age is minor; this is because spermatocytes, unlike oocytes, are egenerated throughout the life of the male (Cash, 2004).

iii: ionising radiation

There is a strong association between the incidence of DS and a history of maternal abdominal radiation. Radiation effect may be age-dependent. (Zuftan, and Luxin, 1986).

Research compared population live at high background to control with low background radiation. The observed frequency was significantly higher than in controls. Higher frequency of cases of Down's Syndrome born to mothers aged 30-39.There was an association between low dose radiation exposure of older maternal age, suggesting that the damaging event accelerates oocyte aging and causes primary trisomy rather than translocation trisomy (Kochupilla *et al*,

to increase exposure of mothers to pollutions, which resulted from the war activities and enhanced by the adverse effect of embargo. (AL-Taha, 1996).

1.2.7: The 21st Chromosome and Down syndrome

DS disorders are based on having too many copies of the genes located on chromosome 21. In general, this leads to an overexpression of the genes. (Mao, et al., 2003).

The 21st chromosome may actually hold 200 to 250 genes (being the smallest chromosome in the body in terms of total number of genes); but it's estimated that only a small percentage of those may eventually be involved in producing the features of DS. Right now, the question of which genes do what is highly speculative. However, there are some suspects.

Genes that may have input into DS include:

- Superoxide Dismutase (SOD1)-- overexpression may cause premature aging and decreased function of the immune system; its role in Senile Dementia of the Alzheimer's type or decreased cognition is still speculative
- COL6A1 -- overexpression may be the cause of heart defects
- ETS2 -- overexpression may be the cause of skeletal abnormalities
- GART -- overexpression may b
- Cystathione Beta Synthase (CBS) -- overexpression may disrupt metabolism and DNA repair
- DYRK -- overexpression may be the cause of mental retardation
- CRYA1 -- overexpression may be the cause of cataracts
- GART -- overexpression may disrupt DNA synthesis and repair
- IFNAR -- the gene for expression of Interferon, overexpression may interfere with the immune system as well as other organ systems

Other genes that are also suspects include APP, GLUR5, S100B, TAM, PFKL, and a few others. It is important to note that no gene has yet been fully linked to any feature associated with DS (Rahmani *et al*., 2005).

One of the more notable aspects of DS is the wide variety of features and characteristics of people with trisomy 21. The first possible reason is the difference in the genes that are triplicated; genes can come in different alternate forms, called "alleles." The effect of overexpression of genes may depend on which allele is present in the person with trisomy 21. The second reason that might be involved is called "penetrance." If one allele causes a condition to be present in some people but not others that is called "variable penetrance," and that appears to be what happens with trisomy 21 (Hemandez and Dierssen, 1999).

1. 3: Drug Resistance

Development of drug resistance is a common problem in cancer chemotherapy (Wittes and Golden, 1986).Celullar drug resistance is mediated by different mechanism operating at different steps of the cytotoxic action of the drug. After several different mechanisms are switched on in the cells but usually one major mechanism is operating. The most investigated mechanisms with clinical significance are:

1. Altered transport of the cell.

A-decrease influx.

B-increased efflux.

2. Increase in total amount of target enzyme/protein (gene amplification).
3. Alteration of the target enzyme/protein (low-affinity enzyme).
4. Elevation of cellular glutathione.
5. Inhibition of drug induced apoptosis (Stavrovskaya, 2000)

1.3.1: Methotrexate

This drug has been in use since 1951, and has achieved the prominence of being the most widely used anticancer drug, as seen in figure 1-2 (Osborn *et al*., 1958).

Figure 1-2 Chemical structure of MTX (Kamen, 1997).

It is a folate antagonist, which kills the proliferating cells by inhibiting the enzyme dihydrofolate reductase (*DHFR*), thereby blocking the pathway of de novo DNA synthesis (Williams and Flintoff, 1995; Dunlap *et al*., 1971).

Today, MTX is still used extensively in the treatment of human leukemia, breast cancer, head and neck cancer, choriocarcinoma, and lymphoma. (Takimoto and Allegra, 1995).

The demonstration in 1985 that low – dose, intermittent MTX is potent and effective therpy for rheumatoid arthritis (RA) (Weiblatt *et al*., 1985).

MTX was found to have a clastogenic effect which appeared to be particularly severe when the cells were exposed continuously to high concentrations of the drug (Mondello *et al.*, 1984).

Cowan and Jolvet (1983) have shown that the resistance to MTX exhibited by a human breast cancer cell line was due to decreased formation of MTX polyglutamation in these cells.

A number of studies have revealed decreased influx of MTX due to low level or nonfunctioning of folate carrier protein (Wong *et al.*, 1995; William and flintoff, 1995).

Amplification of the *DHFR* gene is one of the most common forms of MTX resistance observed in experimental system (Allegra, 1996).

The first evidence of this phenomenon in mammalian cells was provided by Schimke and his team (19٧٨) who demonstrated significant increase in level of dihydrofolate reductase (*DHFR*) in an MTX-resistant cell line.

The sensitivity of the enzyme towards the drug remains the same; however there would be an excess of enzyme relative to the concentration of the drug inside the cell. Thus, the pathways or biochemical processes, which were to be inhibited by the drug, would continue and the cell would escape inhibition by the drug. (Schimke, 1980).

Finally, mutations in *DHFR* leading to a decreased binding of MTX have been reported in a number of tumors (Schweitzer *et al*., 1990; Melera, [illegible])

(Krynetskaia *et al*, 2001).

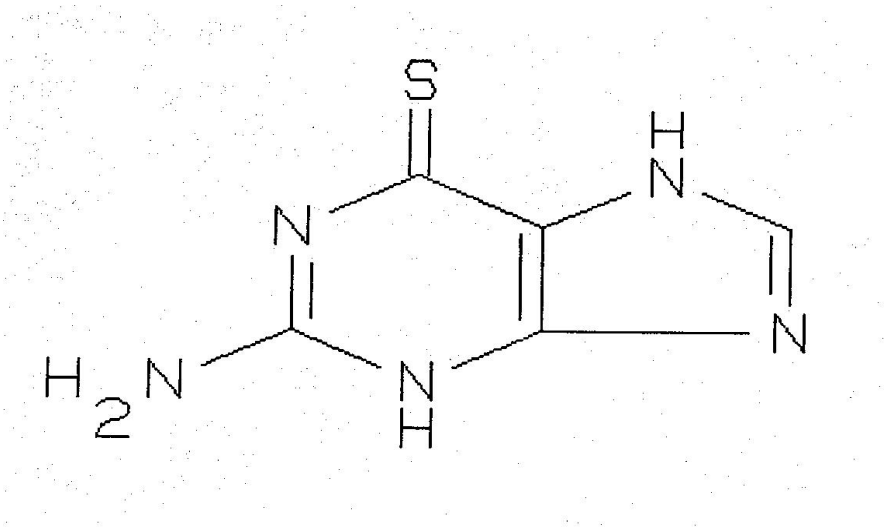

Figure (1-3) Chemical structure of 6-thioguanine (Murray *et al*., 1988)

6-Thioguanine inhibits de novo purine synthesis and purine interconversion reactions, and their nucleotide metabolites are incorporated into nucleic acids. It is converted to its nucleotide form by hypoxanthine guanine phosphoribosyl transferase (*Hprt*). This active metabolite interferes with de novo purine synthesis. (Hande and Garrow, 1996).

***Resistance to 6TG**

Several laboratory and clinical observations suggest that *Hprt* deficiency causes cellular resistance to 6TG.For example, cells from Lesch-Nyhan syndrome patients lack *Hprt* and are resistant to 6TG (Dempsey *et al*., 1983; Yamanaka *et. al*, 1985).

Most chemically induced mutant cells that are resistant to 6TG show significantly reduced *Hprt* activity (Sato *et al*., 1972).

Additional mechanisms of 6TG-resistance include lower affinity of *Hprt*, increased degradation of 6MP, decreased incorporation of the analog into polynucleotides and failure of the analog to enter the cell. (Brockman, 1974).

1.4: Cytogenetic analysis

Numerous diverse environmental and industerial chemicals are capable of causing cytogenetic damage in experimental animal's .The potential for similar effects in a man are obvious. Since cytogenetic damage in humans is generally associated with severe clinical disorders (Burns, 1972; Riccardi, 1977), it is imprevative to determine if chemicals to which man may be exposed are capable of inducing this type of genetic damage (Evans, 1976).

Cytogenetic methods have been used longest and most extensively in the biological monitoring of population exposed to physical and chemical carcinogens and mutagens. (UNEP, 1988)

1.4.1: Mitotic index

The MI is counted as a ratio of nuclei in the mitotic stage to interphase nuclei in a thousand cells. It is a useful and sensitive test for detection of cytotxic effects of chemical and physical agents as well as mutagenic and carcinogenic agents (King *et al*., 1982).

MI is also employed to assess the toxogenic and carcinogenic effect of some drugs and radiations (Shubber and salih, 1988).

It is affected by culture conditions in cultured lymphocytes, it was found to be increased by the increase of colchicine concentration, but was unaffected by bromodeoxyuridine (BrdUrd) concentration in the medium (Shubber and Al-Allak, 1986).

1.4.2: Chromosome Aberrations (CAs)

The basic principles of aberration formation were laid out in the early 1930 when even though the molecular structure of the eukaryotic chromosome was not known at that time. Techniques to prepare and stain chromosomes have improved gradually, parellel with increased ability to identify and quantify chromosome aberration, thus leading to a better understanding of their origin (Figueiredo *et al*., 2004). The CA assay in cultured cells has been widely used for many years, and it has proved to be useful and sensitive test for detection of genotoxic agents. The damaged is scored by microscopic examination of chromosomes in mitotic metaphase cells (Galloway, 2000).

only a single breakage event while complex aberrations (rearrangements within and between chromosomes)involve multiple breakage and misrepair events.(Savage,1975).

Numerical CAs (i.e aneuploidy, polyploidy) refers to changes in chromosome number that occur due to abnormal cell division The most common type of chromosome anomaly is trisomy, where there is an extra version of a single chromosome. Trisomy can arise for any of the chromosomes; Trisomy 21 alone has the ability to achieve adulthood (Oshimura and Barrett, 1986).

Numerical and structural aberrations are important both in congenital abnormalities and tumors (Obe and Natarajan, 2004).

1.4.3: Micronucleus (MN)

Micronuclei formation was first described by scientists such as Carlson,Sax, Koller in the 1930's and 1940's who noticed the formation of micronuclei after x-irradiation ; however the formation of micronuclei by chemical mutagens wasn't discovered until after Word War II.(Vian *et al.* ,1993).

Micronuclei are small, extranuclear bodies that arise from acentric chromosome fragments or from whole chromosome that are excluded from the nucleus during mitotic cellular division. They can be a consequence of DNA damage by agents that act on damaged DNA, as template for inhibition of DNA synthesis, failure of any of the mitotic apparatus, disorders and mechanical disruption. Micronucleus is a good indicator of mutagenicity. It is unclear however, whether MN formation has a specific role in carcinogenesis. (Albertini *et al.*, 2000)

Micronucleus analysis can be used for a number of the cells, both *in vitro* and *in vivo*,including lymphocytes (Bolognesi *et al.* , 2004) and buccal epithelial cells (Pastor *et al.* , 2002).

The first serious attempt to use micronuclei as a monitor of cytogenetic damage appear to be that reported by Evan and his team (1959); the used micronucleus frequency to measure the cytogenetic damage induced in root-tips by fast neutron and X-rays in the presence and absence of oxygen.It was found that all chromatid ,chromosome,and chromatid breaks ,as well as asymetrical

and incomplete symmetrical exchange ,will give rise acentric fragments at mitosis ,and that these fragments are freguently excluded from daughter nuclei and appear in the following interphase as micronuclei.

This assay is more easily scored than the chromosome aberration assay and utilize relatively small amounts of test article, thus requiring less time to make an assessment of mutagenic potential of a chemical, therefore, this assay has been widely used as an alternative means to screan mutagens. (Kirsch-Volders *et al.*, 2003).

1.4.4: Cell Cycle

undergoes growth and protein synthesis, it needs enough protein for two cells,priming it to be able to divide. Once this is complete, the cell finally enters the fourth and final phase of the cell cycle: the M (Mitosis) During M phase, the cell splits apart into two daughter cells. Following mitosis the daughter cells may re-enter the G1phase or proceed to a 5^{th} phase called “G0”, where growth and replication stops (Adams, 1980).

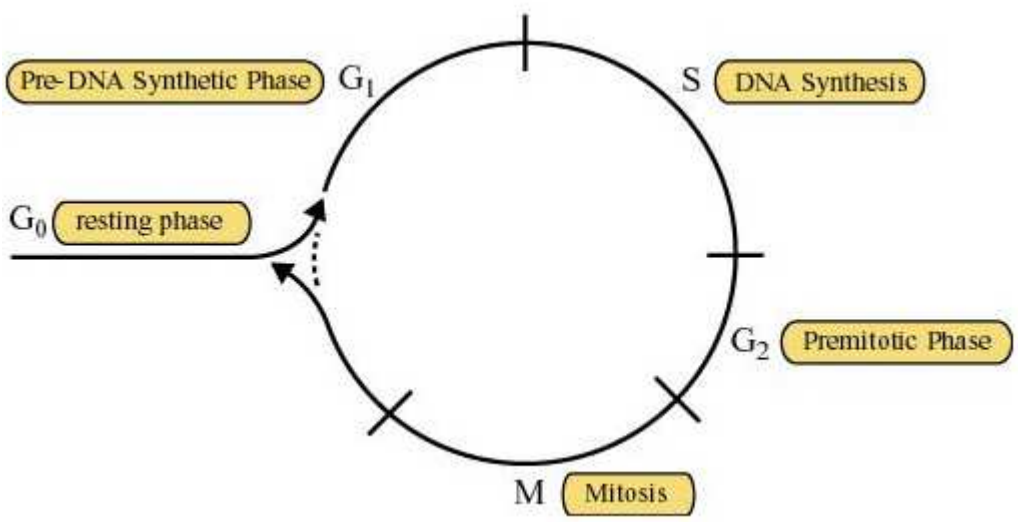

Figure 1-4 Cell Cycles (Adams, 1980).

1-4-5 Cell cycle progression (CCP)

Cell cycle progression is the ability of cells to pass through M1, M2 and M3.Cell cycle progression is used as an assay to know the ability of chemical and physical material in reduction cell replication kinetics and it is measured by decreasing the percentage of M3 and increasing the percentage of M1 (Tice et *al*., 1976; Lamberti *et al*., 1983).

In vitro CCP was found to be affected by the medium, BUdR levels, and colchicine (Shubber and Al-Allak, 1986).

The CCP consist of three types of division:

1- First cell division:

These groups of cells have no incorporated BUdR or in single DNA strand

During S phase. The chromosomes of this phase all appear bright under the light microscope.

2- Second cell division:

This group had the ability to corporate BUdR during two S phases and display a typical differential staining of sister chromatids one dull and one bright.

3-Third cell division:

These cells corporated BUdR during three S phases so they contained BUdR-substituted DNA in both sister chromatids, half number of the chromosomes will be [illegible] brightness because of BUdR (Lamberti *et al.*, 1983).

[illegible] considers, at the same time, the number of M1, M2 and M3 metaphase cells.

Many indicators were considered, but the most suitable was the RI. This index is formulated using the following equation:

RI= (1xM1%) + (2xM2%) + (3xM3%)/100(Lamberti *et al.*, 1983).

1.3.7: Sister chromatid Exchange (SCE)

Since the discovery of SCE by Taylor, Woods and Hughes (1957), many studies have been carried out on the mechanism as well as the biological role of SCE.

SCE arise from equal exchange of DNA replication products between two identical sisters' chromatids of duplicated chromosome. They are thought to arise as a consequence of "error free" homologous recombinational repair or bypass of DNA lesions during replication on a damaged DNA template, possibility at the replication fork. (Tucker *et al.*, 1993).

In the most commonly used method of SCE analysis, DNA replication is required for two consecutive cell cycle, hence bromodeoxyuridine is add to the culture medium and cells are scored in the second division metaphase. (DeFerrari *et al.*, 1991).

There is evidence that the measurement of SCE frequency is a very sensitive method for detecting physical and chemical mutagens (Latt, 1981).

Cytogenetic assessment of SCE levels [illegible] & an index of the mutagenic potential [illegible]. Importantly, ~10 SCE occur spontaneously in normally cycling human cells (Galloway, and Evan, 1975), suggesting a link between SCE and DNA replication.

Elevated spontaneous SCE levels are observed in cells from Bloom syndrome patients (German and Ellis, 1998), acute lymphoblastic leukemia (Otter *et al.*, 1979), chronic myeloid leukemia (Shirini and Sanberg, 1980), patient with Schistomiasis(Shubber,1987)and patients with Multiple Sclerosis(Karki *et al.* , 1986).

The effect of different chemotherapeutic drugs on the frequency of SCE of the peripheral lymphocyte from normal adults was

studied.Methotrexate(MTX),Mitomycin C(MMC) , adriamycin (ADM) , Cyclophsphamide (CP),and Actinomycin D(ActD) were tested. All of these agents induced significantly high frequency of SCE (Emere and Salkizli, 1994).

1.5: Immunological Analysis

The immune response is a complex series of the cellular interaction activated by the entry into the body of foreign (nonself) antigenic materials such as infectious agents and variety of macromolecules (Roitt *et al.*, 1998).

Two important tests used to study the activity of the immune response:

a: Lymphocyte Transformation (blast genesis)

The lymphocyte transformation test (LTT) is based on the observation in 1960 that incubation of lymphocytes with phytohemagglutinin, a mitogen, leads to cell activation and proliferation therefore, it is sometimes also called the “lymphocyte proliferation test”. (Klein, 2004)

The test has been applied by different research groups for the evaluation of various cell-mediated immune reactions (Von Baehr, et al, 2001). Initially, the LTT was used to investigate the immunological competence of cancer patients, Knight and Davidson (1975) found that the lymphocyte transformation, in response to PHA, is reduced in breast cancer even very early in the disease and certainly preoperatively.

However, more recently, the LTT used in association with recall antigens has became an important tool for investigating the functional competence of the specific immune system in patients suffering from HIV, cancer and recurrent viral or intracellular bacterial infections. Furthermore, the LTT seems to be a useful tool not only for screening cellular sensitization to several conventional allergens such as foods or antibiotics (Reekers et al., 1996; Ronnau et al., 1997), but also in applications to patients with suspected contact dermatitis, a disease which is mostly initiated by haptens (i.e. some drugs, heavy metals such as nickel or gold as well as different chemicals and solvents) and not by immunogenic allergens (Everness et al., 1990; Nyf- bler and Pichler, 1997).

Furthermore, the LTT has been used as a part of the diagnostic evaluation of patients suffering from an active infection with persisting [illegible]

[illegible] (1969) found that impairment in [illegible] blood lymphocytes to respond to phytohemagglutinin (PHA) in vitro in patients with Down's syndrome.

b: Phagocytosis:

The first line of innate cellular immune defence mechanism is phagocytosis by which living cells (phagocytes) ingest or engulf other cells or particles. The entire process of phagocytosis of microorganisms by phagocytes can be divided into three main steps:

The first step involves the initial **binding** of the target particle to receptors at the phagocyte surface, a recognition process mediated by a limited number of specific receptor–ligand interactions at the contact interface. The

second **activation** step involves the interaction of the cytoplasmic tails of clustered receptors with cytosolic molecules, resulting in transmission of a transmembrane signal from the ligated receptor to intracellular signaling pathways. In the third step, i.e. the process of **entry**, pseudopod extensions are formed around and closely attached to the target particle, or the particle sinks into the ingesting cell, leadingto its complete encapsulation by host cell plasma membrane. This is followed by membrane fusion events, allowing the formation of an intracellular vesicle around the particle (the **phagosome**) (Hazenbos and Brown, 2006).

The clinical significance of phagocytosis is augmented if we consider the consequences of phagocyte defects, which are observed in some inherited human diseases, for instances, cyclic neutropenia, Chediak-Higashi syndrome, myeloperoxidase deficiency and chronic granulomatous. These diseases are characterized by recurrent severe infections

List of abbreviations

6- TG	6- thioguanine
BI	Blastogenic Index
BudR	5-Bromodeoxyuridine
CAs	Chromosomal Aberrations
D.W.	Distilled water
CCP	Cell cycle progression
DDW	double Distilled water
DHFR	Dihydrofolate reductase
DS	Down syndrome
LTT	Lymphocyte transformation test
MI	Mitotic Index
PHA	Phytohaemagglutinin
KCl	Potassium Chloride
RI	Replecative Index
RPMI 1640	Rosewell Park Memorial Institute
MN	Micronucleus
MTX	Methotrexate
SCE	Sister chromatid exchange
UV	Ultraviolet

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Chapter Two: Materials and Methods

2.1. Materials:

2.1.1: Equipments and Apparatus:

The following equipments and apparatus were used in this study:

Apparatus	Company
Autoclave	Gallenkamp(England)
Centrifuge	Gallenkamp(England)
Water bath	Gallenkamp(England)
Cold incubator	Gallenkamp(England)
Electric balance	Metler (Switzerland)
Electric oven	Gallenkamp (England)
Microscope	Carl Zeiss (Germany)
pH-Meter	Orien Research (USA)
U.V. light lamp	CAMAGLL,1987
Laminar air flow	Metalab (France)
Reflux	Gallenkamp(England)
Magnetic stirrer	Retsch (Germany)
Electric shaker	Merk(Germany)

2.1.2: Chemical Materials:

The following chemical materials were used in this study and its company:

Material	Company
phytohaemoaglutinine	Radiobiology center of The Ministry of Science and Technology
Anhydrous sodium	BDH(England)
Methanol	Fluka(Switzerland)
Glacial acetic acid	Fluka(Switzerland)
Hydrochloric acid (HCl)	BDH
Potassium chloride(KCl)	Fluka
Heparin	Denemarca
RPMI(1640)	Flow laboratories (UK)
Hepes	Sigma chemical company (USA)
Sodium bicarbonate($NaHCO_3$)	
Penicillin, Streptomycin)	Samara drug factory (Iraq)
Giemsa stain	Fisher
Colchicin	Ibn-Hayan (Syria)
Bromodeoxyuridine	BDH
Fetal calf serum	Sigma
NaCl	Sigma
Na_2HPO_4	Sigma
KH_2PO_4	Sigma
HOCHEST	BDH
Methotrxate	Serva
6-thioguanine	OncoHexa

2.2: Chemicals Preparation:

1. Antibiotic:

Streptomycin was prepared by dissolving 1 g of streptomycin in 100ml D.W. Penicillin was prepared by dissolving 1000000 unite /100 ml D.W., both antibiotic were sterilize by filtration under aseptic conditions.

2. Normal Saline(0.85mg\ml):

Prepared by dissolving 8.5 gm of NaCl in 1 liter of D.W.

3. Bromodeoxyuridine (BUdR)(13.33mg\ml):

This solution was prepared by dissolving 50 mg of 5-bromo-deoxyuridine in 37.5 ml of D.W.The solution was sterilized by filtration through a (0.22µm) millipore sterile filter, distributed in sterile tubes with final concentration 1.33 mg/ml and stored at -20 ˚C until used (Al-Amiry, 1999).

4. Colchicines (0.5 mg/ml):

One tablet 0.5 mg of colchicines was dissolved in 10 ml of D.W. to make a stock solution. This solution was stored at -20 ˚C until used.

5. Fixative Solution:

This solution was freshly prepared by mixing 3 parts of absolute methanol with 1 part of glacial acetic acid and then kept in 4˚C until use (Allen *et al*., 1977)

6. Potassium Chloride (KCl) (Hypotonic Solution)(0.57mg\ml):

The hypotonic solution was prepared by dissolving 5.75 gm of KCl in one liter D.W. to get 0.075 M concentration of KCl. The solution was sterilized by autoclaving and stored at 4˚C.The solution was warmed up to 37˚C before use

While formicronucleus test was prepared by dissolving 7.4 gm of KCl in one liter D.W. to get 0.1 M concentration of KCl. The solution was sterilized by autoclaving and stored at 4˚C.The solution was warmed up to 37˚C before use (Allen *et al*., 1977).

7. Sodium Bicarbonate Solution(44mg\ml):

It was prepared by dissolving 4.4 gm of ($NaHCO_3$) in 100ml of sterile D.W. this solution kept at 4˚C until used.

8. Phoshosphate Buffered Slain (PBS)

The solution was prepared by dissolving of the following chemicals in 1000 ml of D.W. and the pH was adjusted to 7.2:-

Sodium chloride (NaCl)	8gm
Potassium chloride (KCl)	0.2gm
Sodium hydrogen phosphate (Na_2HPO_4)	1.15gm
Potassium dihydrogen phosphate (KH_2PO_4)	0.2gm

This solution was sterilized by autoclaving and stored at 4˚C.(Verma and Babu, 1989).

9. RPMI 1640 Medium:

This medium contained the following components :-

RPMI 1640 medium base	10 gm
Fetal calf serum (heat inactivated)	15% (v.v)
Hepes	1% (w.v)
Sodium bicarbonate	1% (w.v)
Penicillin	100 iu/ml (final)
Streptomycin	100 µg/ml (final)

BUdR	15µg/ml (final)

The volume was completed with sterile D.W. to 1000 ml, and the pH was adjusted to 7.2 and sterilized by filtration using 0.22 size filter under aseptic conditions, after that 2 ml of the medium was transferred into sterile test tubes and kept at 4˚C until used (Shubber *et al.*, 1991).

10. Preparatin of Methotrexate

MTX was obtained from vial at concentration (50 mg/ 2 ml) as stock solution and from this solution, the concentrations 0.4, 2 and 4 µg/ml werepreprepared, then sterilized by filtration and kept at 4° C.

11. Preperation of 6- Thioguanine

6-T-G was obtained by dissolving. 20 mg of 6-T-G in 10 ml of PBS to make stock solution .Three concentrations were prepared 0.8, 10 and 20 µg/ml from the stock solution, and then sterilized by filtration and kept at (4° C).

12. Stock suspension of bacteriophage test

Pure bacteria colonies obtained from (Health laboratory Center)

Cultured on nutrient agar media, incubated for 24 hours at (37 °C), the colonies were collected in normal saline or PBS. The bacterial cells were then washed 3 times and reconstituted in concentration of 1x10^{٦} cells / ml and stored at 4 °C until used.

13. Giemsa Stain:

a.Stock

The stock of Giemsa stain was prepared by dissolving 1g of Giemsa stain powder in 33ml of glycerine. The solution was transferred to brown bottle then placed in a 60 °C water bath for two hours with shaking from time to

time.Then the solution was cooled down, and after cooling 66ml of methanol was added gradually with continuous mixing.Giemsa stock solution was strored in the dark at room temperature.

b.Working Giemsa stain

It was prepared by mixing the following:

1ml of Giemsa stain stock.

40ml of distilled water.

0.5 ml of sodium bicarbonate solution.

1.25ml of methanol.

2.2 Population studied

Subjects whom selected for this study were children and their parents. Subjects were divided into two groups. The first group represented ten DS children from both gender, with ages less than 12 years as shown in figure 2-1, and their parents aged 20-40 years for mothers and 24-45 years. All those patients were selected from Genetic Counsilling Unit. AL-Yarmouk Teaching Hospital Baghdad and from Hibatto- Allah Teaching Center for Down syndrome in Baghdad.

The second group represented five normal children and their parents were considered as the controls.

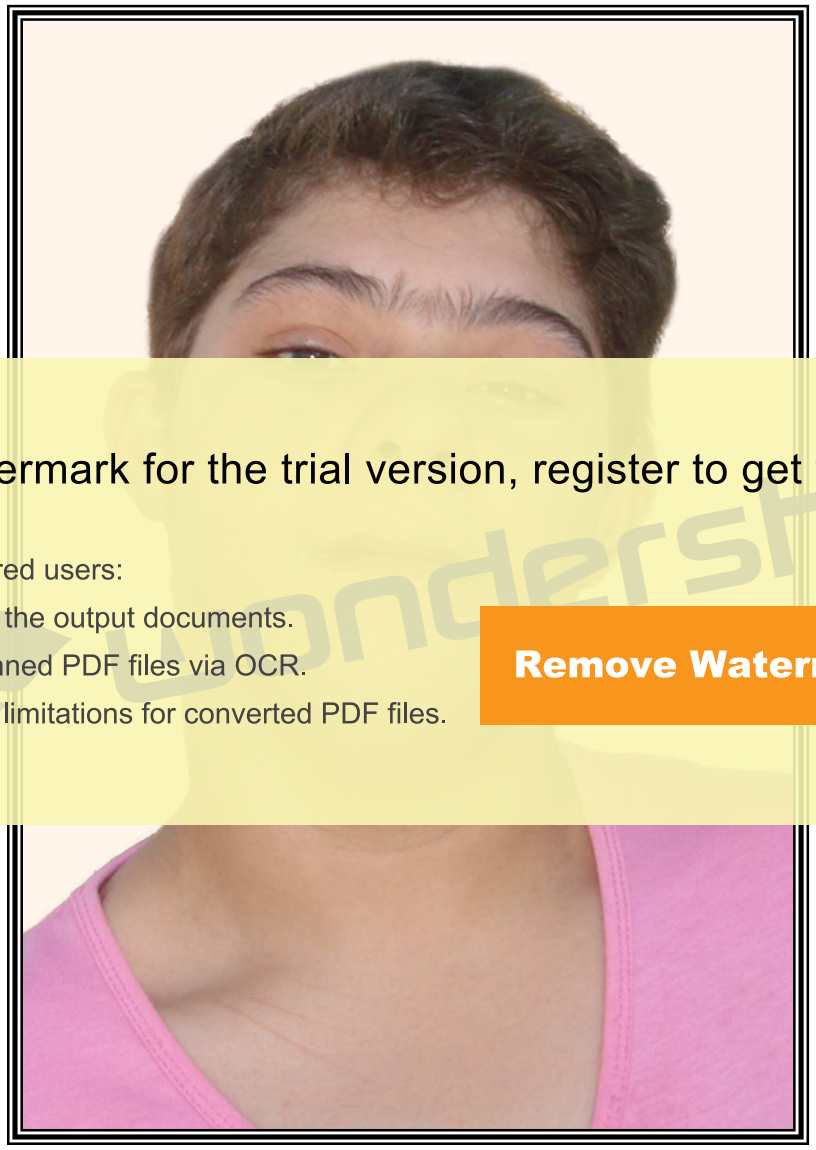


Figure 2-.1 Picture of DS child

2.4: Cytogenetic Analysis

2.4.1: Cytogenetic Analysis of Human Blood Lymphocytes

This was performed according to the method used by Shubber, (1987) which was adapted from Nowell (1960).

1. Human blood was collected into heparin-coated syringe.
2. Peripheral blood (0.25 ml) was added into test tube containing 2 ml of culture medium (RPMI 1640).
3. PHA (0.25 ml) was added, the components were mixed very well. (0.1 ml) of of control. the components was mixed very well and transferred to 37° C incubator.

4- the test tubes were gently shaken each (24 hours) one try at least .The incubation period was completed to 72 hours.

5. A portion of 0.1mg/ml of colchicine was added to each tube 1/2 hr. before harvesting the cells.
6. The test tube was centrifuged at 2000 rpm for 10 min.
7. The supernatant was removed and 5 ml of potassium chloride (KCl) as added as hypotonic solution at (0.075 M), then the test tubes were left for 30 min in the incubator at 37°C and shacked from time to time.

8. The tubes were centrifuged at 2000rpm for 10 min.

9. The supernatant was then removed and the fixative solution was added as drops on the inside wall of the test tube with continuous shaking and then, the volume was fixed to 5 ml and the contents were shacked well.

10. The tubes were kept at 4°c for 30 min to fix the cells.

11. The tubes were transferred in to the centrifuge at 2000 rpm for 10 min. The process was repeated for 3 times and after that, the cells were suspended in 2 ml of the fixative solution.

12. By a pasture pipette, few drops from the tube were dropped vertically on the chilled slide from a height of 3 feet at a rate of 4-5 drops to give the chance for the chromosomes to spread well. Later, the slides were kept to dry at room

13. The slides were stained with giemsa stain and left for 15 min, and then washed with D.W.

14. Three slides for each tube were prepared for cytogenetic assays.

Another slides for each concentration were stained with Hoechest stain (33258) for the analysis of cell cycle progression and sister chromatid exchange.

2.4.2: Hochest (33258) Staining

This was done according to the method described by Ian-Freshney, 2000

1. The slides were immersed in a coplin jar Hochest 33258 at a concentration of 20µg/ml for 10 min.

2. The slides were transferred to a slide rack, and 500 µl of 2x SSC were dropped.
3. The slides were covered with 22-mm x 50- mm cover slips, and the edges were sealed with a temporary seal, such as cow gum, to prevent evaporation.
4. The covered slides in the slide rack (cover slip facing downwards) were placed on a short-wave UV box. Maintain a distance of approximately 4 cm between the slides and the UV source. The longer the pale chromatid will become, expose the slides for about 24-60 min.
5. The covered slips were removed from the slides, and the slides were washed three times in ultra purified water (UPW), 5min. per wash. The holder was covered with aluminum foil.
6. The slides were air dried in the dark.
7. The slides were stained in a coplin jar containing 3.5% Giemsa solution in [illegible] for 3-5 min.
8. The slides were carefully rinsed in tap water, and drained using a paper tissue.
9. The slides were air-dried on the bench for 1 hour, and dipped into xylene, 4 drops of DPX mountant were dropped onto the slide and a 22-mmX50- mm cover slip was lowered, expressing any air bubbles with tissue.
10. The slides were air dried in a fume hood overnight.

2.4.3: Micronucleus test in human blood lymphocytes culture

This was applied to the method adopted by Al-Adami, 2000

1-Human blood was collected in heparinized syringe.

2- (o.25 ml) of peripheral blood was added into test tube containing (2 ml) of culture medium (RPMI-1640).

3- (0.25 ml) of PHA was added, mixed the components very well and transferred to (37° C) incubator.

4- the test tubes were gently shaken each (24 hours) one try at least .The incubation period was completed to (72 hours).

5-The test tubes were centrifuged at speed of 800 rpm for (5 min).

6- the supernatant was removed [illegible] (5 ml) [illegible] hypotonic solution (0.1M) and then the te[illegible] water bath at (37° C) and the tubes were shake from time to time.

7- the tubes were centrifuged at 800 rpm for (5 min.).

8- after that, the supernatant was removed and the fixative solution was added as drops on the inside wall of the test tube with continuous shaking, and then, the volume was fixed to (5 ml), and the contents were shaken well.

9- the tubes were kept at (4° C) for (30 min.) to fix the cells.

10- the tubes were transferred into the centrifuge at 800 rpm for (5 min.). The process was repeated 3 times and after that, the supernatant was discarded and one drop of the pellet was smeared on a clean slide and left to dry at room temperature.

11- the slides were stained with Giemsa stain and left for (15 min.), then washed with D.W.

2. 5: Cytogenetic parameters analysis:

1- Mitotic Index (MI) Assay:

This was performed according to the method used by Shubber and AL-Allak, 1986.

The slides were examined under high power (40 X) of compound light microscope and (1000) of divided and non divided cells were counted and the percentage rate was calculated for only the divided ones according to the following equation:

Mitotic index =no. Of the divided cells/ total no. Of the cells (1000) ×100

2-Blasto Index (BI) Assay:

This was done according to method described by Al-Shawk, 1999.

The slides were examined under high dry power (40X) of compound light microscope and (1000) cells were counted to calculate the percentage rate of the blast cells according to the following equation:

Blast index (BI) =no. of the blast cells/total no. of cells (1000) ×100.

3-Chromosomal Aberration (CA) Assay:

The prepared slides were examined under the oil immersion lens for 100 divided cells per each blood lymphocytes culture, and the cells should be at the

first metaphase stage of the mitotic division where the chromosomal aberrations are clear and the percentage of these aberrations was estimated.

4-Sister Chromatid Exchange (SCE) Assay:

Sister chromatid exchange were counted in 50 well spread second metaphases stained with Hochest and Giemsa.

5-Replicative index (RI) assay

This was applied to the method adopted by Lamberti, 1983.

The replicative index (RI) was determined by counting the number of cells at the first, second and the third metaphase in (100) cells at metaphase, the RI were calculated according to the following equation:

RI= (1xM1%) + (2xM2%) + (3xM3%)/100 .

2.6: Experimental Deign

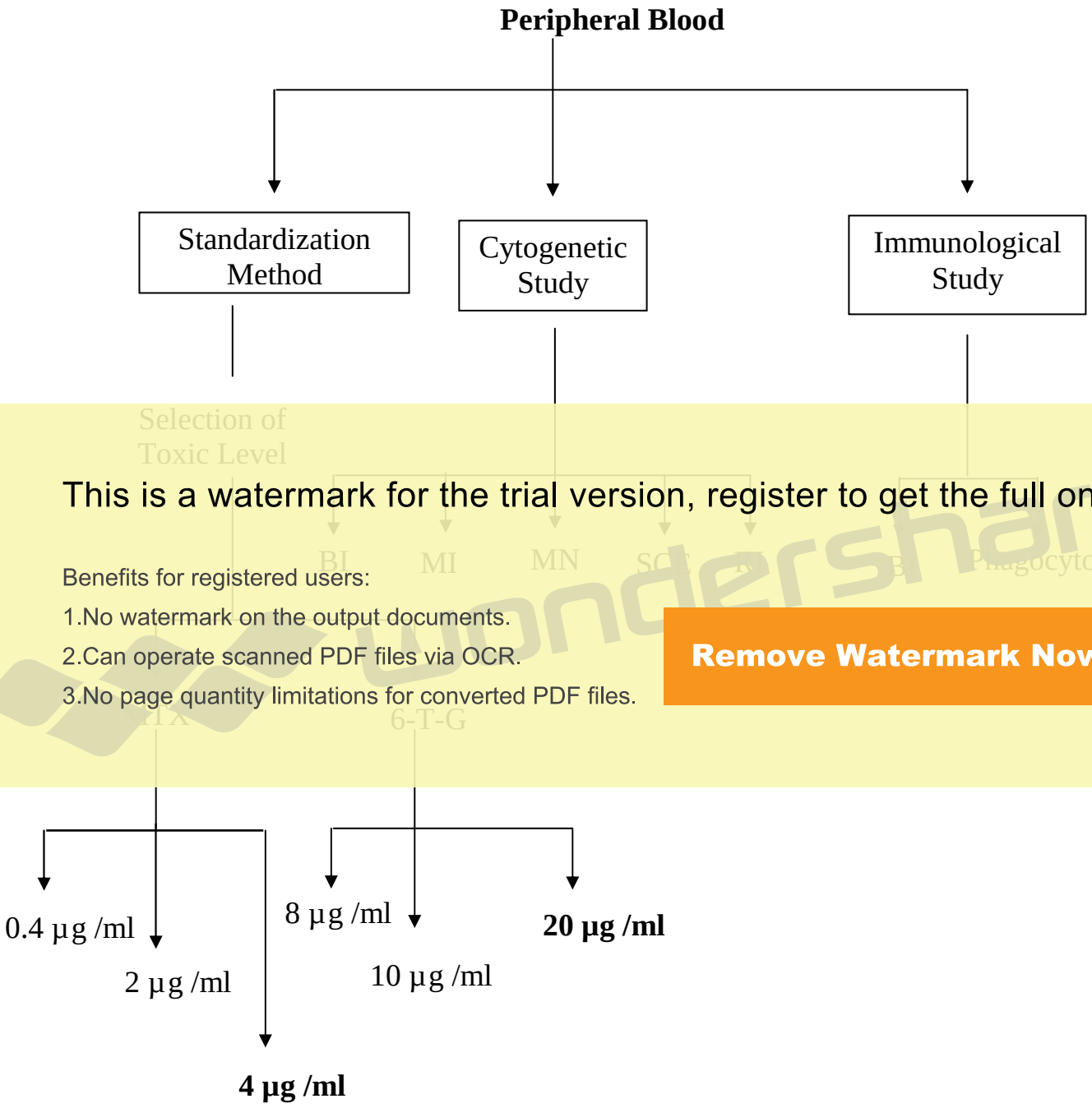
3.6.1: Standardization of Doses

[illegible] dose that can [illegible] and 4 µg/ml) MTX, and three concentrations of the (8, 10 and 20 µg/ml) 6-T-G were tested against the cell from healthy individuals. It was found that a dose of (4 µg/ml) MTX and (20 µg/ml) of 6-T-G could inhibit the mitosis. These two concentrations were tested against the cell of the families of DS (mother, father and DS).

3.6.2: Assessments of drug Effects

For each subject 4 cultures were set-up. The first was a drug free, while the second and third were treated with toxic level of MTX and 6-T-G. Finaly the fourth untreated culture for micronucleus assay.

To assess the drug effect, the BI, MI, cell division reduction, RI, SCE were evaluated. A schematic presentation of such procedure is outlined below:



Figure(2-2)
Outlines of the laboratory assessments

2.7: Immunological Analysis

2.7.1: Phagocytosis Assay

This assay was performed according to the method used by Furthe *et al*, 1985.

One ml of heparinized blood was mixed with 1 ml of bacterial suspension ($1x10^{٦}$ cell ml). The tube was then incubated at 37 °C for 30 minutes with slow movements.

A blood film prepared on dry clean slide, and the slide was covered with methanol for 5-10 minutes followed by staining with Giemsa stain for 5-10 minutes then washed with D.W. Three slides to each tube. The slides were studied under light microscope,

And the percentage of phagocytosis was calculated

Phagocytic % = phagocytosis cells / total (phagocytic + non phagocutic cells) X 100.

This was done according to methods described by AL-Mohammed *et al*, 1986 through Hazim Esmail.

One-way of analysis of variance was performed to test whether group variance was significant or not, the comparison between groups were used analyses of variance test Duncan.

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3.1: Cytogenetic analyses

3.1.1 Cytogenetic analyses of untreated cultures

The cytogenetic analyses were performed on peripheral blood lymphocytes obtained from DS children and their parents. Moreover, normal children and their parents were included and considered as control group.

The cytogenetic analyses (Blast index, Mitotic index) of DS children and normal children are provided in table 3–1. The blastogenetic transformation that was referred to as blast index (BI) was reduced increasingly in lymphocytes of DS children (24.94±0.95) whereas the BI value for control group was (34.02±1.5). The difference was significant ($p<0.05$).

The mean of mitotic index (MI) for DS children was (1.72±0.2). This is significantly lower than that of control (2.68±0.1, $p<0.05$).

This depression of BI and MI in-patient with DS reflects an impairment of cellular immune function in these patients, which may be one of the factors contributing to the vulnerability of these patients to repeat or persistent infections (Watkins *et al*., 1987).

These results in this study are in agreement with Agarwal *et al*. (1969) who indicated the abnormality of lymphocyte stimulation in patients with DS is not due to a non specific environmental factor prevalent in the situation, for these reasons the impaired responsiveness seems to be an innate characteristic of lymphocytes of patients with DS.

Table (3-1) Cytogenetic analyses of blood lymphocytes obtained from Down syndrome Children and normal Children

Groups	Blast Index% mean±SE	Mitotic Index% mean±SE
Normal Children	34.02±1.5 a	2.68±0.1 a
DS Children	24.94±0.95 b	1.72±0.2 b

Different letters in the same columns: significant difference (P<0.05)

Other cytogenetic analyses micronuclei (MN) and chromosomal aberrations (CA) of DS children and control are provided in table 3-2.

The micronuclei percentage for lymphocyte of DS children (1.25±0.13) significantly ($p<0.05$) higher than normal children (0.42±0.07)

The figure (3-1) showed Micronucleated blood lymphocyte from Down syndrome child.

Spontaneous frequencies of chromatid break, chromosome break, deletion, dicentric, acentric that were observed in lymphocyte from DS children, not observed in normal children, only the gap was found in two group.

A number of proteins play protective role against oxidative stress:superoxide dismutase(SOD),catalase(Cat) and glutathion peroxidase (GSHPX) are well-known antioxidant enzymes that scavengers Reactive Oxygen Species (ROS). In DS oxidative stress may result from excess of SOD activity: the ratio of SOD, which is gene located on chromosome 21, GSHPx is altered, meaning that more potentially damaging hydrogen peroxide is generated by SOD than Cat and GSHPx can neutralize, leading to severe oxidative imbalance. Oxidative damage to DNA can take in the cells many forms, ranging from oxidized bases to deoxyribose DNA damage, such as DNA strand breaks and the formation of micronuclei (MN) (Dettaan *et al.*, 1997).

Table (3-2) Chromosomal aberrations of blood lymphocytes obtained from Down syndrome children and normal children

	Percentage of Chromosomal aberrations (mean±SE)							Micro-nucleus% mean±SE
	Chromosome break	Chromatid break	Deletion	Gaps	Ring	Fragment	Total	
Normal Children	0 a	0 a	0 a	0 a	0 a	0 a	0.25 ±0.02	0.42 ±0.07 a
DS Children	0.05 ±0.01 B	0.19 ±0.01 b	0.03 ±0.01 b	0.25 ±0.02 b	0.21 ±0.02 b	0.03 ±0.01 b	0.79 ±0.03 b	1.25 ±0.13 b

Different letters in the same columns: significant difference (P<0.05)

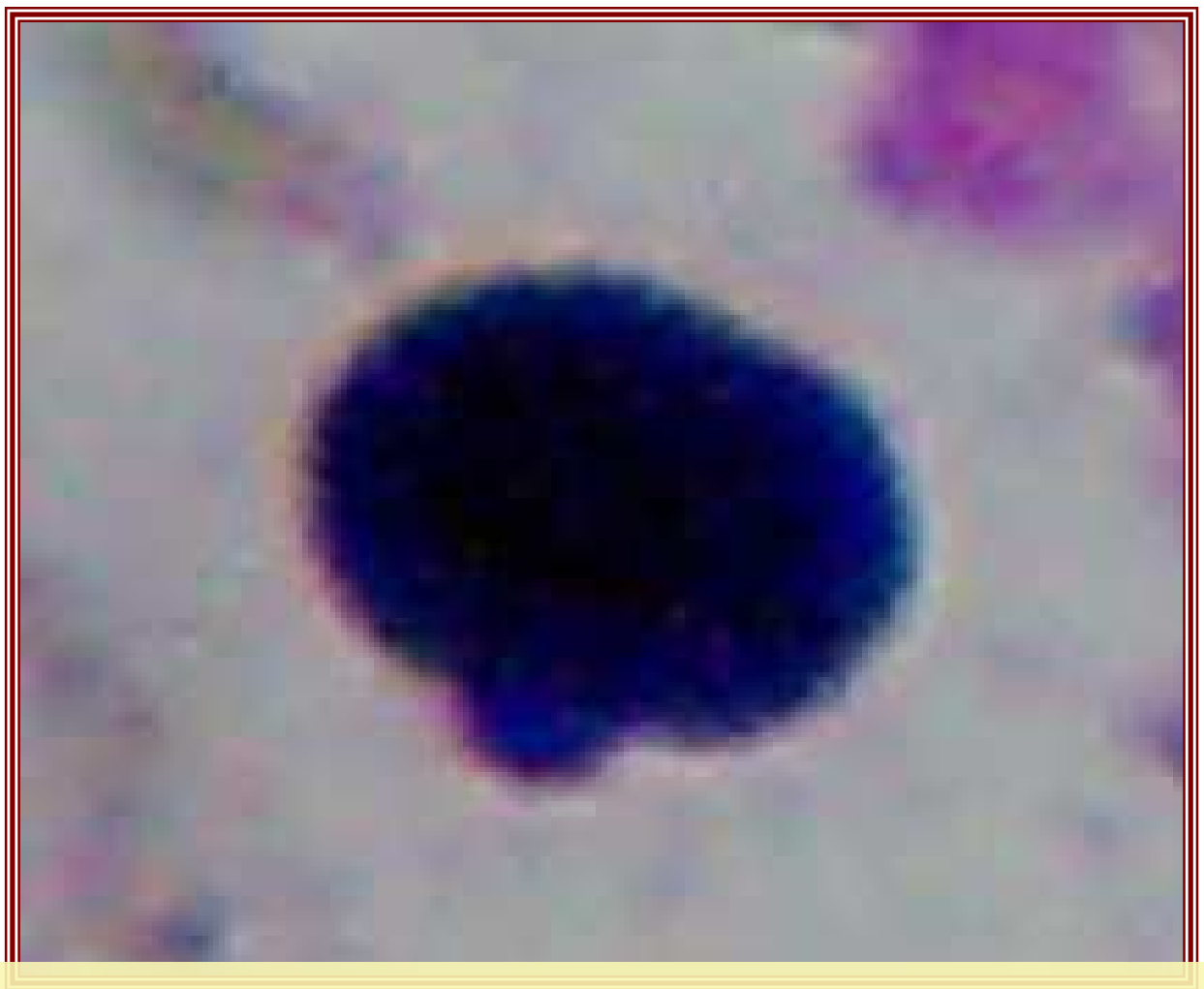


Figure 3-1 Micronucleated blood lymphocyte from Down syndrome child.

The incidence of SCEs and RI in blood lymphocytes of normal and DS children are presented in table (3-3).

The mean frequency of SCEs for normal children was 5.8±0.2 while for DS children 8.37±0.16 SCE\cell.

DS children immunologically distrubted and have a defective cellular immune function (Watkins *et al.*, 1987). These conditions may lead to a change in the relative proportion of T-lymphocyte subsets, and when SCEs frequencies have been determined they have often been found to be higher than in healthy donors (Parkes *et al.*, 1985; Shubber, 1987).

It is also suggested that defective DNA-repair mechanisms may lead to abnormal SCE frequencies .Individuals with DS were reported to have defective DNA-repair processes (Swift, 1977).

The RI, for the DS and normal children were 1.44±0.09 and 2.35±0.08, respectively. This may explain the defective cellular immune function (Sigh *et al.*, 1986).

Table (3-3) Cytogenetic analyses of blood lymphocytes obtained from Down syndrome Children and normal Children

	Replicative Index mean±SE	Cell cycle progression% mean			Sister Chromatid Exchange /cell mean±SE
		M1	M2	M3	
Normal Children	1.75 ±0.04	a			a 5.8 ±0.2
DS	b				b
Children	**1.44 ±0.03**	**65.75**	**21.62**	**12.62**	**8.37 ±0.16**

Different letters in the same columns: significant difference (P<0.05)

Table (3-4) describes the blast and mitotic index of lymphocytes for parent of children with DS and normal families, there was no difference between two groups.

A significant increase ($P<0.05$) in the percentage of CAs and MN observed in parent of DS children, as shown in table (3-5).

The MN for father and mother of DS children 1.8±0.06 and 2.1±0.05, respectively, as shown in figure (3-2).

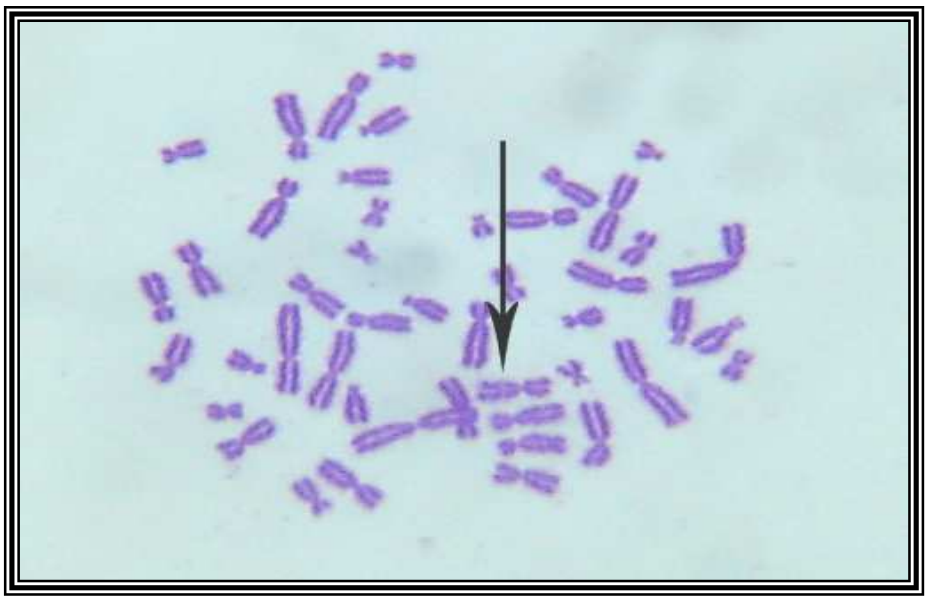
The spontaneous frequencies of chromosome break, chromatid break, dicentric and acentric that observed in mother of children with DS compared with mother of normal children, as shown in figure 3-2.

The mean of MN and CAs frequencies for mother higher than father, this is probably because their lymphocytes have more chromosomal material (3%) than males and their cell are more sensitive to DNA-damaging agents than male cells (Soper *et al.*, 1984; Margolin and Shelby, 1985; Wulf *et al.*, 1986).

Table (3-4) Cytogenetic analyses of blood lymphocytes obtained from parent of children with Down syndrome and normal parent

		Blast Index% mean±SE ±SE	Mitotic Index% mean±SE ±SE	
Normal parent	Father of Normal children	a 33.94±1.5	2.6±0.05	a
	Mother of Normal children	a 29.7±1.7	2.5±0.06	a
Parent of children with DS	Father of DS children	a 32.6±1.6	2.92±0.2	a
	Mother of DS children	a 31.5±1.4	2.72±0.2	a

Different letters in the same columns: significant difference (P<0.05)



(A)

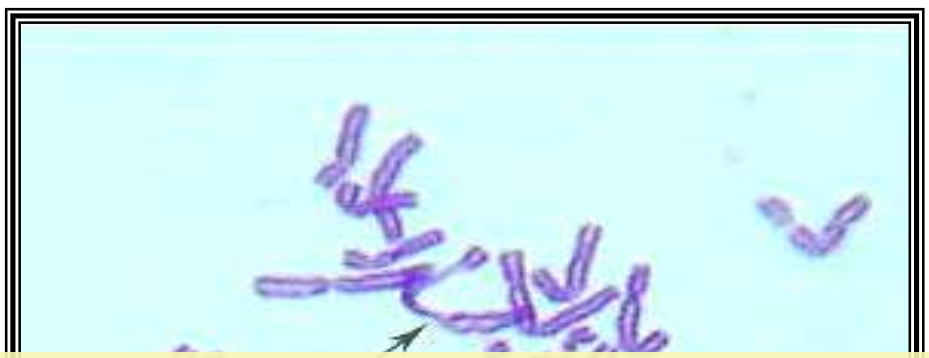


Figure (3-3): Metaphase for mothers of DS blood
Chromosome breakage; (B) 1- chromosomal damage, 2- gap.

Table (3-5) Chromosomal aberrations and Micronucleus of blood lymphocytes obtained from parents of children with Down syndrome and normal parents

Groups	Percentage of Chromosomal aberrations (mean±SE)							Micro-nucleus% mean±SE
	Chromosome break	Chromatid break	Deletion	dicentric	Gap	acentric	Total	
Mother of Normal children	0	0.02 ±0.004	0	0	0.07 ±0.01	0	0.09 ±0.01	a 1.02 ±0.09
Father of Normal children	0	a	a	a	a	a	a 0.26 ±0.04	a 0.96 ±0.1
Mother of DS children	0.29 ±0.03	0.46 ±0.03	0.02 ±0.007	0.13 ±0.02	0.27 ±0.04	0.06 ±0.01	b 1.27 ±0.06	b 2.1 ±0.05
Father of DS children	0.29 ±0.02	b 0.36 ±0.03	b 0.07 ±0.03	a 0.03 ±0.02	a 0.21 ±0.03	b 0.03 ±0.008	b 0.92 ±0.08	b 1.8 ±0.06

Different letters in the same columns: significant difference (P<0.05)

Other cytogenetic analyes, SCE, RI of parents' children with DS and normal parents are provided in table (3-6).

The mean SCE frequencies for mother and father of DS children were 8.35±0.7 and 9.06±0.02, respectively.

These values were significantly higher ($P<0.05$) than the mean frequency of the normal mother and father (6.0±0.2) and (6.6±. 0.14) respectively.

This result suggests that the high level of SCE is a trait that might be transmitted from parents to children.

No significant difference in the RI was seen between the parents of the two groups.

Table (3-6) Cytogenetic analyses of blood lymphocytes obtained from parent of children with Down syndrome and normal parent

Groups	Replicative Index mean±SE	Cell cycle progression% mean			Sister Chromatid Exchange \cell mean±SE
		M1	M2	M3	
Mother of Normal Children	a 1.87 ±0.03	40	37	23	a 6.6±0.14
Mother of DS	b 1.77 ±0.09	37	44	19.87	b 9.06 ±0.02
Father of Normal children	a 1.81 ±0.09	38	48	14	a 6.0 ±0.2
Father of DS children	**a 1.77 ±0.09**	**37**	**49**	**14**	**b 8.35 ±0.7**

Different letters in the same columns: significant difference (P<0.05)

3.1.2: Cytogenetic analyses of drug (6-T-G) and (MTX) treated culture.

3.1.2.1: Optimization of Doses:

In order to evaluate the response of blood lymphocytes obtained from DS children and their parents, the genotoxic effect of the two anticancer drug (6-T-G and MTX), were tested against cells of healthy individuals and methods are presented in section (**2.6.1**).

It is clearly showed that a significant reduction in BI and MI were seen in normal blood lymphocytes as a function of drug concentration (i.e. concentration dependent effect), The data were presented in table (3-7).

Accordingly the following concentrations 8, 10, 20 µg/ml of 6-T-G were tested. It was found that a concentration 20µg/ml was toxic level that reduced increasingly the BI compared with control (31.06±1.79, 27.18±1.9) respectively and inhibited mitosis.

The lymphocytes of healthy individual treated with 8µg/ml of 6-T-G showed no difference in blastogensis, to those of nontreated cells the BI was (32.4±1.14), while at concentration 10µ/ml there was a reduction in BI (29.53±1.83). This reduction, however, was not significant.

The reduction was more pronounced when lymphocytes of healthy individual were treated with 20µg/ml of 6-T-G; for 72h the mean of BI value was (27.18±1.9).

Marked decrease in MI of lymphocytes treated with 8µg/ml the first concentration of 6-T-G (1.78±0.2) and (0.8 [illegible] completely inhibition of MI was observed at con [illegible]

The reduction in MI can be explained as the effect of 6-T-G that induce chromatid damage and arrest the cells at S-phase (Wotring and Roti Roti, 1980).

Toxic level of MTX, the other cytotoxic agent was examined on lymphocyte from healthy individual treated with three concentration of MTX 0.4, 2, and 4µg/ml, *in vitro* for 72h.

There was a marked reduction in both parameters. The mean BI for lymphocytes treated with 0.4, 2, and 4µg/ml were (32.35±1.42, 31.06±1.79 and 27.78±1.14) respectively, while the BI for control was (35.26±1.64). Significant reduction ($P<0.05$) in MI was observed for human lymphocytes treated with 0.4, 2, and 4µg/ml of this antifolate drug.

This can be explained by the effect of MTX that stop the cells from proliferating through inhibiting the synthesis of DNA at the S (76%) and G2 (24%) phases (Duran, 2000).

These results are in agreement with the result of AL-Amiry (1999) who found that MTX cause mitotic index inhibition in breast cancer patients *in vivo*.

Table (3-7) Blastogenic and Mitotic Index of lymphocyte obtained from controls after treatment with different concentrations of drugs MTX and 6-T-G.

	Test Control	MTX 0.4 µg/ml	MTX 2 µg/ml	MTX [illegible] µg/ml	6-T-G [illegible] µg/ml	6-T-G 10 µg/ml	20 µg/ml
Mitotic Index% mean±SE	a 35.26 ±1.64	b	b	b	a	a 29.53 ±1.83	b 27.18 ±1.9
Blast Index% mean±SE	a 2.93 ±0.25	b 1.38 ±0.25	c 0.35 ±0.11	c 0	b 1.78 ±0.2	c 0.83 ±0.25	d 0

Different letters in the same columns: significant difference (P<0.05)

3.1.2.2: Cytogenetic analyses of lymphocyte grown for 72h *in vitro* in presence of 6-T-G and MTX (toxic level) treated cultures.

Blood lymphocytes taken from DS children and their parents were cultured *in vitro,* in the presence of toxic level of 6-T-G and MTX to determine the resistant cells.

All DS children and their mother showed resistance to toxic level of 6-T-G, while all fathers of DS children were sensitive to toxic level of 6-T-G.

No significant difference in BI and MI was seen between the R group of DS children lymphocytes treated with 6-T-G and untreated DS children lymphocyte as shown in table (4-8), while in mothers of DS children R group, their was a significant reduction in BI and MI compared with control (without 6-T-G). The BI and MI value of R group of mother of DS children were (32.0±1.02, 2.72±0.2) and (26.25±0.78, 1.3±0.13) respectively, as shown in table (3-9).

To determine the incidence of such thioguanine resistant cells TG^r we calculated the cell division reduction and blast index reduction of these cells. The cell division reduction of DS children was (20.72±5.4), while cell division reduction of mothers of DS children was (41.38±6.1). The BI reduction of DS children was (11.95±0.3) and for their mothers (17.97±0.95).

The basis for thioguanine resistance is loss of HPRT activity as a consequence of point mutation or loss of HPRT locus as a result of deletion or aberration involving the long arm of the X-chromosome where the HPRT locus is located (Vigaud-Daum and Evans, 1984). Cytogenetically detectable x-chromosome aberrations (deletion and

translocation) in about 40 percent of thioguanine mutant human fibroblast induced by various types of ionizing radiation. HPRT deficiency renders the cells to be hypersensitive to chemical and/or physical carcinogens (Shubber *et al.*, 1991). The result of this study, suggesting that the resistance could be maternally inherited, these result are in agreement with the results of Shubber *et al.* (2000) who found that the HPRT enzyme deficiency could maternally inherited.

Similar observations were reported on genetic analysis of HPRT mutation in somatic cells from patients with partial or complete deficiency of HPRT enzyme activity (patients with Lesh Nyhan syndrome, gout and hyperuricemia) and their parents (Keoch *et al.*, 1988; Ogusawara *et al.*, 1989, Skopeck *et al.*, 1989) and revealed that such mutation is likely to be maternally inherited.

Table (3-8) Cytogenetic effects of toxic level 6-T-G (20µg/ml) on human blood lymphocytes from Down syndrome children

Groups	Blast Index% mean±SE		Blast index reduction%	Mitotic Index% mean±SE		Cell Division Reduction% mean±SE
	DS Children Without 6-T-G	DS Children With 6-T-G		DS Children Without 6-T-G	DS Children With 6-T-G	
Sensitive	–	–	–	–	–	–
Resistance	24.94±0.9 a	22.05±0.95 a	11.59±0.3	1.7±0.1 a	1.3±0.09 a	20.72±5.4

Different letters in the same columns: significant difference (P<0.05)

Table (3-9) Cytogenetic effects of toxic level 6-T-G (20µg/ml) on human blood lymphocytes from mother of children with Down syndrome

Groups	Blast Index% mean±SE		Blast index reduction%	Mitotic Index% mean±SE		Cell Division Reduction% mean±SE
	Mother of DS Children Without 6-T-G	Mother of DS Children With 6-T-G		Mother of DS Children Without 6-T-G	Mother of DS Children With 6-T-G	
Sensitive	–	–	–	–	–	–
Resistance	2.0 ±0.1	a 26.13 ±1.2	b 17.97	2.72 ±0.1	a 1.38 ±0.13	b **47.38 ±6.1**
	Father of DS Children Without 6-T-G	Father of DS Children With 6-T-G		Father of DS Children Without 6-T-G	Father of DS Children With 6-T-G	
Sensitive	**29.7 ±1.7**	**a 21.52 ±1.3**	**b 27.55 ±1.4**	**2.5 ±0.06**	**a 0**	**b 100**
Resistance	–	–	–	–	–	–

Different letters in the same columns: significant difference (P<0.05)

No difference was observed in chromosomal aberration frequencies between 6-T-G treated and untreated DS children and their mothers, as shown in table (3-10) and (3-11) respectively.

A significant ($P<0.05$) increase in SCEs was observed in DS children and their mothers treated with 6-T-G compared with untreated groups, as shown in table (3-12) and (3-13).

No significant difference in RI in DS children with and without 6-T-G, while there was a significant decrease in RI of mothers of DS children treated with 6-T-G and untreated group.

Table (3-10) Chromosomal aberrations of blood lymphocytes obtained from Down syndrome childrens treatedwith6-T-G (20µg/ml)

Groups	Percentage of Chromosomal aberrations (mean±SE)						Total
	Chromatid break	Isochromatid break	Chromosome break	Dicentric	Ring	Acentric	
DS Children	0.05 a	0.19 a	0.03 a	0.25 a	0.21± a	0.03 a	**0.79 ±0.03 a**
DS Children With 6-T-G	0.24 ±0.01	0.42 ±0.02	0.02 ±0.01	0.20 ±0.09	0.32 ±0.02 b	0.02 ±0.009 a	**0.87 ±0.03 a**

Different letters in the same columns: significant difference (P<0.05)

Table (3-11) Chromosomal aberrations of blood lymphocytes obtained from mothers of childrens with6-T-G (20µg/ml)

Gaoups	Percentage of Chromosomal aberrations (mean±SE)							Total
	Chromosome break	Chromatid break	Deletion	Dicentric	Gap	Acentric		
Mother of Wild 6-T-G	a 0.29±0.01	a	a	a	a	a 0.06±0.01		a 1.2±0.06
Mother of DS Children With 6-T-G	a 0.24±0.01	a 0.41±0.02	a 0.02±0.01	a 0.13±0.01	a 0.32±0.02	a 0.05±0.01		a 1.18±0.02

Different letters in the same columns: significant difference (P<0.05)

Table (3-12) Cytogenetic effects of toxic level 6-T-G (20µg/ml) on blood lymphocytes from Down syndrome

Groups	Replicative Index mean±SE	Cell Cycle Progression% mean			±Sister Chromatid Exchange % mean±SE
		M1	M2	M3	
DS children Without 6-T-G	1.7 a ±0.03	65.75	21.62	12.62	8.37 a ±0.16
DS children With 6-T-G	1.35 b ±0.03	76	12.8	11.2	16.66 b ±0.68

Different letters in the same columns: significant difference (P<0.05)

Table (3-13) Cytogenetic effects of toxic level 6-T-G (20µg/ml) on blood lymphocytes from mother of children with Down syndrome

Groups	Replicative Index mean±SE	Cell Cycle Progression% mean			Sister Chromatid Exchange \cell mean±SE
		M1	M2	M3	
Mother of DS Children Without 6-T-G	[illegible]	[illegible]	[illegible]	[illegible]	8.35 ±0.7 a
Mother of DS Children With 6-T-G	1.5 ±0.01 b	61.75	26.5	11.75	11.44 ±0.14 b

Different letters in the same columns: significant difference (P<0.05)

In the case of MTX, lymphocyte from DS children and their parents were cultured *in vitro* in the presence of toxic level of MTX to determine the resistant cells. All mothers of DS children, 30% of DS children and 40% of father of DS showed marked resistance to MTX so they did represent the resistance group (R), while 70% of DS children and 60% of father of DS children represented the sensitive group (S).

Toxic level of MTX reduced BI and MI in the sensitive group of DS children and their parents as shown in table (3-14) and (3-15) respectively.

The BI value significantly decrease to (18.97±1.1) in S group of DS children compared with control (24.94±0.9).

The BI for R group of DS children decreases to (22.03±1.9) compare with control (24.94±0.9), but this reduction not significant. The reduction of BI was clear in the R group of mother of DS children; the BI value was (23.4±1.4) while for control was (32.0±0.95).

The lymphocyte blastogenic transformation after stimulation with PHA is normally occurred, because PHA (mitogen) is able to cross-link the lymphocyte receptors, and induce their transformation (Roitt *et al*., 1998).

McCowage *et al*., (1996) have demonstrated that the anticancer drugs may cause abnormalities in lymphocytes receptors involved in mitogen recognition such effect may result in a reduction of BI.

MI for R group of DS children (0.4±0.1) decreases significantly (P<0.05) when compared with controls (1.7±0.1).

The marked reduction was observed in MI of R group for mother and father of DS children (0.4±0.1, 0.3±0.04) respectively.

As we mentioned before this result can be explain by the cytotoxic effect of MTX that stop the cells from proliferating by inhibiting DNA synthesis (Duran, 2000).

Another Effects of MTX were significant decrease in the cell division reduction and BI reduction in R group compared with S group of DS children and their parents as shown in table (3-14) and (3-15).

The cell division reduction percentage for S group was 100%, while for R group (77.5±5.2), (86.4±4.1) and (98.01±3.2) for DS children and their mothers and fathers respectively.

The BI reduction for S group of DS children and their fathers were (23.94±1.2) and (19.53±1.2), while for R group were (11.66±1.6), (26.88±1.1) and (17.35±1.4) of DS children and their mothers and fathers.

The mechanism of resistance to MTX has been shown to be due to the amplification of the gene coding for DFHR (Alt *et al*., 1978).

Mutation in DFHR that leading to a decreased binding of MTX has been reported in a number of tumors (Schweitzer *et al*., 1990; Maley, 1991).

Impaired ability to transport MTX into cells carrier also can cause resistance (Guo *et al*., 199

Cowan and Jolvet (1983) have shown that the resistance to MTX exhibited by a human breast cancer cell line was due to decreased formation of MTX polyglutamation in these cells.

Table (3-14) Cytogenetic effects of toxic level 6 MTX (4µg/ml) on blood lymphocytes from Down syndrome children

Groups	Blast Index% mean±SE		Blast index reduction%	Mitotic Index% mean±SE		Cell Division Reduction% mean±SE
	DS Children Without MTX	DS Children With MTX		DS Children Without MTX	DS Children With MTX	
Control	24.94 ±0.9 a	22.03 ±1.9 a	11.66 ±1.2 b	1.7 ±0.1 a	0.4 ±0.1 a	77.5 ±5.2 b
Resistance	24.94 ±0.9	22.03 ±1.9	11.66 ±1.2	1.7 ±0.1	0.4 ±0.1	77.5 ±5.2

Different letters in the same columns: significant difference (P<0.05)

Table (3-15) Cytogenetic effects of toxic level MTX (4µg/ml) on blood lymphocytes from parent of children with Down syndrome

Groups	Blast Index% mean±SE		Blast index reduction%	Mitotic Index% mean±SE		Cell Division Reduction% mean±SE
	Mother of DS Children Without MTX	Mother of DS Children With MTX		Mother of DS Children Without MTX	Mother of DS Children With MTX	
Sensitive	–	–		–	–	–
Resistance	32.0±0.9				0.4±0.1 b	86.4±4.1
	Father of DS Children Without MTX	Father of DS Children With MTX		Father of DS Children Without MTX	Father of DS Children With MTX	
Sensitive	**29.7±1.7 a**	**23.9±1.4 a**	**19.53±1.2**	**2.58±0.06 a**	**0 b**	**100**
Resistance	**29.7±1.7 a**	**24.55±1.4 a**	**17.35±1.4**	**2.58±0.06 a**	**0.3±0.09** b	**89.01±3.2**

Different letters in the same columns: significant difference (P<0.05)

Another cytogenetic effect of MTX in human blood cells in vitro from DS children and their parent was the increase in the spontaneous frequencies of CAs, as shown in table (3-16) and (3-17).

Those increases in CAs were significant ($P<0.05$) in R group of DS children and their parents as compared with control as shown in figure (3.2).

The effects of MTX on the induction of CAs may be explained by the intracellular accumulation of MTX resulting in an enhancement of enzyme inhibition that might cause an imbalance in the deoxyribonucleotide triphosphate (dNtp) pool due to storage of thymidylate and purine nucleotides and as a consequence lead to DNA lesions (Kasahara *et al.*, 1993; Jackson *et al.*, 1996 and Keshava *et al.*, 1998).

These results are in agreement with the results of Branda *et al.* (1988) who found MTX treatment markedly increases the number of single-strand breaks DNA, and also produce single and double strand breaks in a variant NIH3T3 cells (Lorico *et al.* 1988).

Table (3-16) Chromosomal aberrations of blood lymphocytes obtained from Down syndrome children treated with toxic level MTX (4µg/ml)

Groups	Percentage of Chromosomal aberrations (mean±SE)						
	Centromeric break	Acentric break				Acentric	Total
DS Children Without MTX	0.03 ±0.01	a	a	a	a	a	a 0.79 ±0.03
DS Children With MTX	b 0.22 ±0.04	b 0.43 ±0.05	a 0.02 ±0.02	b 0.44 ±0.02	a 0.31 ±0.02	a 0.03 ±0.02	b 1.46 ±0.06

Different letters in the same columns: significant difference (P<0.05)

Table (3-17) Chromosomal aberrations of blood lymphocytes obtained from parents of children with Down syndrome treated with toxic level MTX (4µg/ml)

Groups	Percentage of Chromosomal aberrations (mean±SE)`						
	Chromosome break	Chromatid break	Deletion	Dicentric	Gap	Acentric	Total
Mother of DS Children Without MTX	0.29 ±0.03 a	0.46 ±0.03 a	[illegible] a	[illegible] ±0.02 a	[illegible] a	0.06 ±0.01 a	1.23 ±0.06 a
Mother of DS Children With MTX	[illegible] a	[illegible] a	[illegible] a	[illegible] b	[illegible] a	0.07 ±0.02 a	1.57 ±0.05 b
Father of DS Children Without MTX	0.29 ±0.02 a	0.36 ±0.03 a	0.07 ±0.03 a	0.03 ±0.02 a	0.21 ±0.03 a	0.03 ±0.008 a	0.99 ±0.08 a
Father of DS Children With MTX	0.31 ±0.004 a	0.43 ±0.004 a	0.11 ±0.009 a	0.14 ±0.01 a	0.40 ±0.03 a	0.14 ±0.01 b	1.53 ±0.04 b

Different letters in the same columns: significant difference (P<0.05)

Table (3-18) Cytogenetic effects of toxic level MTX (4µg/ml) on human blood lymphocytes from Down syndrome children

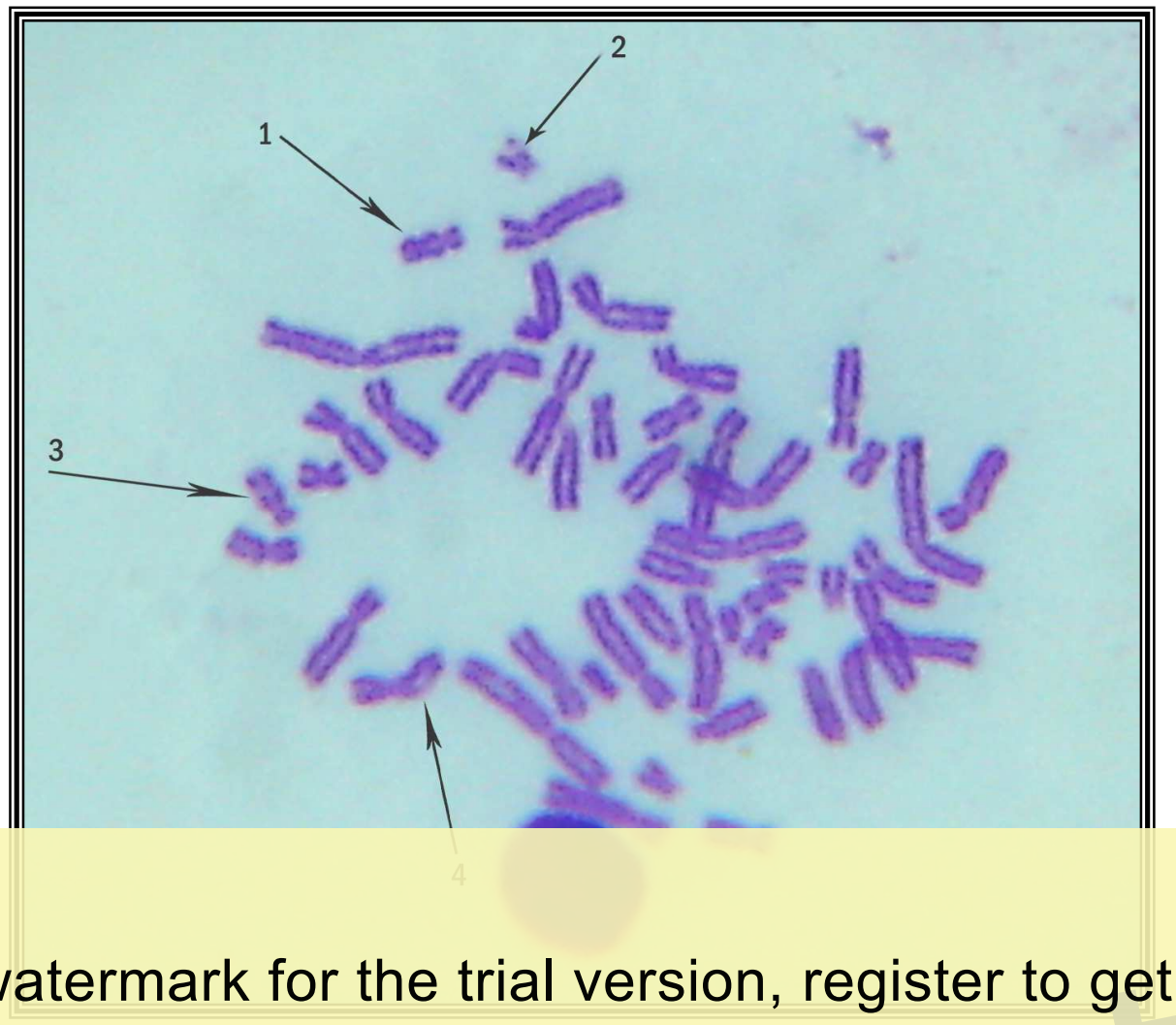
Groups	Replicative Index mean±SE	Cell cycle progression% mean			Sister Chromatid Exchange \cell mean±SE
		M1	M2	M3	
DS [illegible] Without [illegible]	[illegible] ±0.03	[illegible]	[illegible]	12.62	a 8.37 ±0.16
DS children With MTX	b 1.25 ±0.04	79.33	15.66	5	b 20.83 ±0.04

Different letters in the same columns: significant difference (P<0.05)

Table (3-19) Cytogenetic effects of toxic level MTX (4µg/ml) on human blood lymphocytes from parent of children with Down syndrome

Groups	Replicative Index mean±SE	Cell cycle progression% mean			Sister Chromatid Exchange \cell mean±SE
		M1	M2	M3	
Mother of DS Children Without MTX	a 1.8 ±0.02	59.5	25.5	15.0	a 8.35 ±0.7
Father of DS Children With MTX	b 1.17±0.49	64.5	16.5	7	b 13.86 ±0.16
Mother of DS Children With MTX	b 1.77 ±0.09	37	49	14	a 9.06 ±0.02
Father of DS Children With MTX	b 1.17±0.49	64.5	16.5	7	b 13.9 ±0.01

Different letters in the same columns: significant difference (P<0.05)



Our previous explanations about cytogenetic analyses of untreated and treated cultures with MTX and 6-TG (toxic level) of DS children and their parents are summarized in the three tables below:

Table (3-20) Cytogenetic analyses of blood lymphocytes obtained from Down syndrome Children and

Parameters	Children		Parent			
	Normal children	DS children	Parent of normal children		Parent of DS children	
			Mother	Father	Mother	Father
Blast Index	a 34.02 ±1.5	b 24.94 ±0.95	a 36.00 ±1.6	a 33.94 ±1.5	a 32.0 ±0.8	a 29.7 ±1.7
Mitotic Index	a 2.68 ±0.1	b 1.72 ±0.1	a 2.9 ±0.1	a 2.6 ±0.05	a 2.72 ±0.2	a 2.5 ±0.06
Total CA	a 0.25 ±0.02	b 0.79 ±0.03	a 0.29 ±0.01	a 0.26 ±0.04	b 1.27 ±0.06	b 0.92 ±0.08
Replicative Index	**a 2.35 ±0.04**	**b 1.44 ±0.03**	**a 1.87 ±0.15**	**a 1.81 ±0.09**	**a 1.8 ±0.02**	**a 1.77 ±0.09**
SCE\cell	**a 5.8 ±0.2**	**b 8.37 ±0.16**	**a 6.6 ±0.14**	**a 6.0 ±0.2**	**b 9.06 ±0.02**	**b 8.35 ±0.7**

Different letters in the same column: significant difference (P<0.05)

Table (3-21) cytogenetic analyses of lymphocytes grown for 72h in vitro in the presence of 6-TG and MTX from Down syndrome children and

Groups	Parameters	Toxic level of 6-TG		Toxic level of MTX	
		Sensitive	Resistance	Sensitive	Resistance
DS children	BI%	-	22.04±0.09	18.97±1.1	22.03±1.9
	MI%	-	1.3±0.09	0	0.4±0.1
	Total CA	-	0.87±0.03	-	1.46±0.06
	RI%	-	1.53±0.03	-	1.25±0.04
	SCE\cell	-	16.66±0.68	-	20.83±0.04
Mothers of DS children	BI%	-	26.25±0.78	23.4±1.4	23.4±1.4
	MI%	-	1.38±0.13	0	0.40±0.1
	Total CA	-	1.18±0.02	-	1.5±0.05
	RI%	-	1.5±0.01	-	1.38±0.02
	SCE\cell	-	11.44±0.14	-	13.86±0.16
Fathers of DS children	BI%	12.52±1.01		23.9±1.4	24.55±1.4
	MI%	0	-	0	0.3±0.0
	Total CA	-			1.53±0.04
		-			

Table (3-22) the Blast Index and cell division reduction of lymphocytes grownfor 72 h in vitro in the presence of 6-TG and MTX (toxic level) for Down syndrome children and their parents

Groups	Toxic level 6-T-G (20µg/ml)				Toxic level 6 MTX (4µg/ml)			
	Sensitive		Resistance		Sensitive		Resistance	
	BI Redu-ction	Cell Division Redu-ction	BI Redu-ction	Cell Division Redu-ction	BI Redu-ction	Cell Division Redu-ction	BI Redu-ction	Cell Division Reduc. ction
DS children	–	–	17.97 ±0.92	47.38 ±6.1	–	–	11.66 ±1.2 26.88 ±1.6	77.5 ±5.2 86.4 ±4.1
Father of DS children	**27.55 ±1.4**	**100**	**–**	**–**	**19.53 ±1.2**	**100**	**17.35 ±1.4**	**89.01 ±3.2**

Different letters in the same columns: significant difference (P<0.05)

3.2: Immunological analyses

To evalute immunological response in DS children and their parents, two main types of immunological analyses performed

-Transformation analysis, which its results discussed before in section (31.1).

-Phagocytocytosis: The analyses of the mean values for the percentage of phagocytic activity of DS children and their parents are presented in table (3-14).

The phagocytic activity was decreased significantly in DS children compared with normal children. The mean percent of phagocytosis of DS children was (50.5%±1.7) while for the control (61.4%±1.4).

These results agreed with the results of Ugazio *et al.* (1989) that indicated that phagocytosis activity in DS children display some characteristics functional impairment, with low chemotactic ability and reduced production of oxygen radicals.

No significant difference observed in phagocytosis activity in parent of DS children compared with parent of normal children.

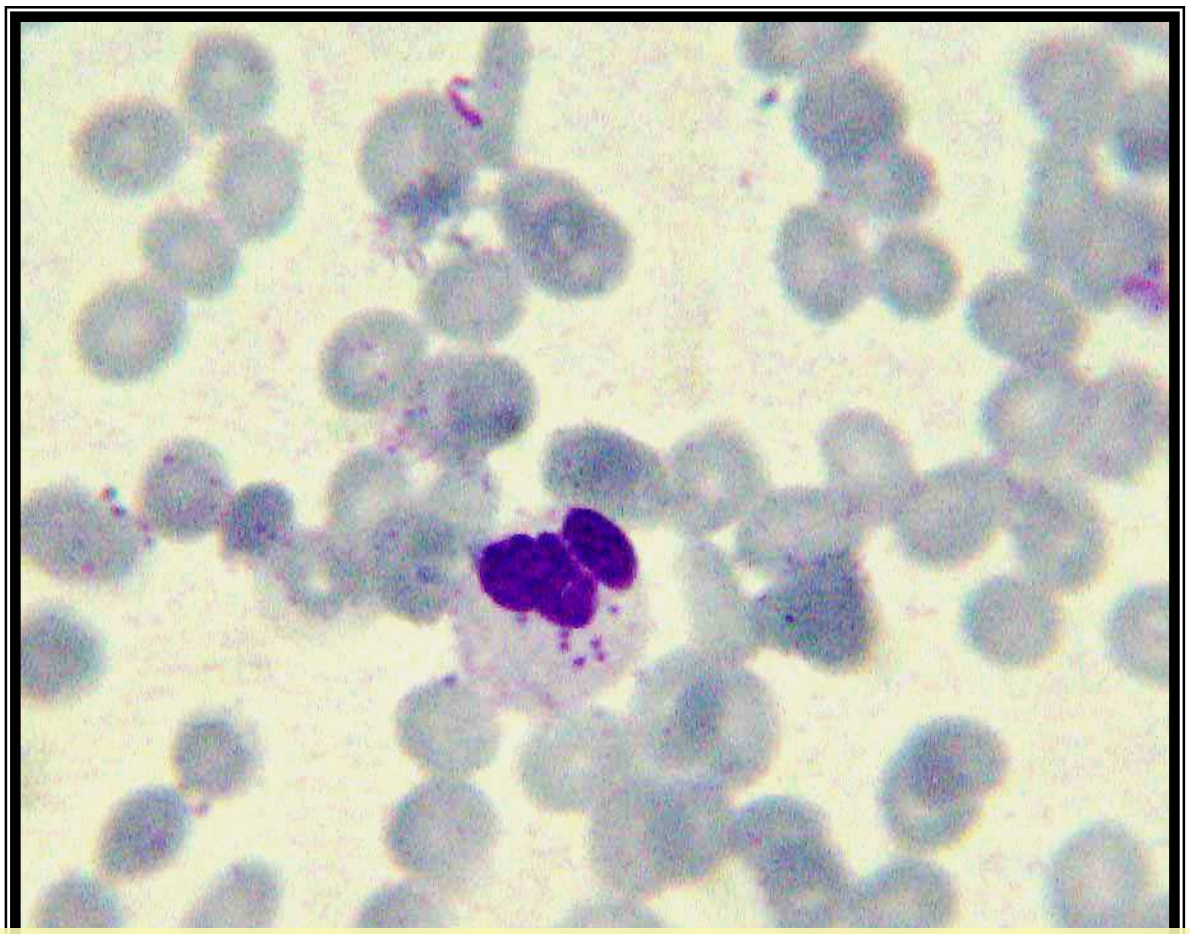


Table (3-23) Percentage of phagocytosis of Down syndrome families and normal families

	Normal Children	DS children	Normal parents		Parents of DS children	
			Mother	Father	Mother	Father
Percentage phagocytosis	61.4±1.4				a 1.09	59.4±1.5 a

Differences a, a, are significant (P<0.05) to compression rows

Summary

The present study was conducted to evaluate some cytogenetic and immunological parameters in Down syndrome (DS) children and their parents. Ten families with children of DS were selected; in addition five families with normal children were included as a control group.

The cytogenetic parameters; blastogenic index (BI), mitotic index (MI), chromosomal aberrations (CA), micronucleus (MN), sister chromatid exchange (SCE) and replicative index (RI) were assessed in lymphocytes of cultured peripheral blood.

Blast index, MI and RI were decreased in DS children while their parents were in normal range when compared with control group.

A significant increase was observed in SCE, CA and MN for DS children and their parents.

The cell division used to determine the sensitivity of DS children and their parents by the application of anticancer

response to the drugs, as a sensitive (S) and resistant (R) groups.

All DS children and their mothers were resistant to 6-TG at a concentration 20µg/ml, while their fathers were sensitive.

At the concentration 4µg/ml of MTX all mothers was resistant, while the 30% of DS children and 40% of their fathers were resistant. In the case of control groups all of them are sensitive. ``

The DS children and their parents' blood were also tested for the lymphocyte transformation test and phagocytosis against *Staphylococcus aureaus.*

Summary

The lymphocyte transformation and phagocytosis percentage were lower in DS children compared with normal children and their parents with normal range.

DEDICATION

SOLAF

بسم الله الرحمن الرحيم

صدق الله العظيم

السورة: الروم

الاية: 22

In the name of Allah, the Compassionate, the Merciful.

And of His signs is the creation of the heavens and the earth, and the difference of your languages and colors. Lo! Herein indeed are portents for men of knowledge.

CHAPTER*: 30 ALRUM,(ROMANS)*
NUMBER*: 22*

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانه الاحيائيه

التحليلات وراثيه- خلويه و مناعيه لمرضى متلازمة الداون و ذويهم

من قبل

سولاف جوهر علي

بكلوريوس تقانه احيائيه جامعة النهرين ٢٠٠٣

رمضان ١٤٢٧
تشرين الاول ٢٠٠٦

الخلاصه

صممت هذه الدراسه لتقيم بعض المعايير الخلويه الوراثيه و المناعيه لاطفال مصابين بمتلازمة الداون و ذويهم . اختيار عشرة عوائل لديهم اطفال مصابين بمتلازمة الداون و كذلك خمسة عوائل لديهم اطفال طبيعين اعتبروا كمجموعة سيطره.

درست المقايس الوراثيه الخلويه التاليه: المعامل الارومي،معامل الانقسام،التغيرات الكروموسوميه، معامل التضاعف، والتبادل الكروماتيدي الشقيقي و معدل تكون النوى الصغيره في خلايا الدم المحيطي للانسان.

بينت النتائج المعامل الارومي و معامل الانقسام و معامل التضاعف هنالك انخفاض معنوي لاطفال متلازمة الداون بالمقارنه مع مجموعة السيطره بينما كانت ضمن المعدل الطبيعي لذويهم

جميع اطفال متلازمة الداون و امهاتهم اظهروا مقاومه واضحه لدواء 6-T-G بتركيز ٢٠ ميكروغرام\مل بينما الاباء كانوا جميعا مقاومين.

اما بالنسبه لدواء MTX وبتركيز ٤ميكروغرام\مل فأن كل الامهات و ٣٠% من اطفال متلازمة الداون و ٤٠% من الاباء اظهروا مقاومه واضحه.

ومن الجانب المناعي فوجد انخفاض واضح في عملية البلعمه ضد بكتريا *Staphylococcus aureus* لاطفال متلازمة الداون مقارنة بالاطفال الطبيعين، بينما لذويهم فلم يكن هناك اختلاف واضح بين المجموعتين.