# A cytogenetic Study of Down's Syndrome in Iraqi Population

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**ABSTRACT**

Down's syndrome is the most common type of chromosomal abnormality found in neonates. It is associated with characteristic abnormal facial features and mental retardation. However, clinical diagnosis is not sufficient to confirm the disease. Classical karyotyping has long been used as a diagnostic tool for confirming Down's syndrome. The present study was conducted to confirm the cytogenetic composition of individuals suspected with Down's syndrome after the preliminary clinical a diagnosis in Basra, Iraq. Karyotype analysis was carried out for 249 suspected cases of Down's syndrome. 249 patients were confirmed to have Down's syndrome after cytogenetic tests. Nearly 90% of these patients had pure trisomy 21 and about 5% of cases had translocations. Less than 4% of cases were reported having mosaic trisomy and only 1.4% patients carried additional chromosomal abnormalities. In our study, the disease showed significant gender bias, with an excess of males over females (sex ratio was 1.39). The identification of specific chromosomal abnormalities in different variants of Down's syndrome patients work as first step towards improving their quality of life. The study also provides the basic information required to forecast the various complications associated with Down's syndrome.

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## 1. Introduction

Down's syndrome is a genetic disorder caused by trisomy of chromosome 21, leading to developmental disorders arising during embryogenesis [1]. Earlier known as 'Mongolism', it is the most common chromosomal anomaly seen in humans. In 1866, a British physician, John Langdon Down, described the disease and later, it was named after him as Down's syndrome [2]. Both intellectual and dysmorphologies are developed due to this genetic disorder [3]. The morbidities is well correlated with the age category and associated intellectual disorders, autoimmune disease and the cardiovascular diseases [4]. Globally, 1 in every 1000 babies born have trisomy 21[2]. Genetically, Down's syndrome can be classified into three forms: Pure Trisomy, Translocation and Mosaic trisomy [3]. In rare occasions, trisomy 21 is accompanied by some other kind of chromosomal aberrations [4,5]. In case of pure trisomy, the cells have an extra 21$^{st}$ chromosome and 47 chromosomes totally. This trisomy is the result from abnormal chromosomal segregation at either the first or the second meiotic division during spermatogenesis or oogenesis. Studies have indicated that in approximately 95% of cases, the trisomic chromosome comes from the oocyte [6]. The pure trisomy (karyotype: 47,XX,+21 or 47,XY,+21) is the most common type of Down's syndrome, with an incidence rate of 90-95 % of all reported Down's syndrome cases. In case of translocation Down's syndrome, a fragment of one chromosome gets transferred to another (chromosome 21). Translocation trisomy [karyotype: 46, XX,t(21;21) or 46, XX,t(14;21) for girls and 46, XY,t(21;21), or 46, XY,t(14;21), for boys] is identified in 5-6% of all Down syndrome patients[7]. In case of mosaic trisomy, some of the cells have an extra copy of chromosome 21 while others don't, i.e., some of the cells are normal while others are trisomic. It is reported in 2-3% of all Down's syndrome cases [8]. The patients of Down's syndrome exhibit structural abnormalities coupled to functional disorders of the Central Nervous System, heart, musculo-skeleton system and digestive system. Patients also suffer from metabolic disorders, nutritional deficiencies, immune defects, defective endocrine system, intellectual disabilities and leukaemia [9,10]. Patients often showcase late development with impaired speech, memory, response, perception and social integration [11,12]. Morphological features (face, eyes, ears, nose and limbs) of patients suffering from Down's syndrome play a significant role in primary screening and diagnosis, however further confirmation is done using classical karyotype and fluorescent *in-situ* hybridization assays. Down's syndrome patients are also at increased risk of early onset of Alzheimer's disease because of the presence of three copies of APP gene, a gene known to play a

causative role in the Alzheimer's disease [13,14]. Chromosomal aberrations in Down's syndrome vary. Approximately, 90-95% carry trisomy 21, 5-7% carry translocations, 3-4% carry mosaic trisomy and very few have additional chromosomal abnormalities [3]. Very little information is available regarding the epidemiology of Down's syndrome in Iraq [14]. The aim of the study was to carry out a cytogenetic evaluation of suspected cases of Down's syndrome using karyotype and to report the incidence of different variants of Down's syndrome in both genders in Iraqi population.

## 2. Methods

A total of 249 individuals, who were referred to Albayan Diagnostic Laboratory in Basra, Iraq, were evaluated prospectively for the cytogenetic analyses of suspected cases of Down's syndrome from 2012 to 2017. All children were subjected to go through thorough clinical examination. Karyotyping was done for cytogenetic analysis of all these patients. Aseptically 2 mL of whole blood were collected from each patient in heparin lithium tube, 0.7 ml of the blood sample was added to 15 ml falcon tubes containing .10% FBS- RPMI 1640 medium and incubated for 69 hours at 37. The culture was provided with 100 µl of PHA as a mitogen and gently inverted few times, then the culture tubes were layed at 45° angle at 37C. The incubation was terminated at 69$^{th}$ hour by adding 100 µl of colcemide at a concentration of 10ug/mL for 30 minutes in 37C. Finally, the mixture was treated with 0.75M KCl for 35 min in 37 C. The cells were harvested by Carnoy's fixative (3:1) of methanol to glacial acetic acid for four washes, and the clear pellet with suspension of 2 ml volume was stored at 4°C overnight. A drop of the suspension was placed on pre- chilled clean slides and left 24 hours for aging. The drop was banned using trypsin – Giemsa and karyotyping was conducted using cytovision system.

## 3. Results

A total of 249 children with clinical suspicion of Down's syndrome were transferred to Albayan Private Diagnostic Laboratory. The cytogenetic findings of these Down's syndrome patients were distributed into four categories: Pure trisomy, translocation, mosaicism and additional chromosomal aberrations Table 1.

Table 1: Distributions of the karyotypes in Down's syndrome children

| No. | Cytogenetic characteristics | Karyotypes | No. of cases | | |
|---|---|---|---|---|---|
| | | | Total | Male | Female |
| 1. | Pure trisomy | XX+21 or XY+21 | 249 | 145 | 104 |
| 2. | Translocation | 46,XX,t(14;21) or 46,XY,t(14;21) | 9 | 4 | 5 |
| | | 46,XX,t(21;21) or 46,XY,t(21;21) | 6 | 5 | 1 |
| 3. | Mosaicism | 46,XX/47,XX+21 or 46,XY/47,XX+21 | 8 | 6 | 2 |
| 4. | Additional chromosomal abnormalities | ✓47,XYqh-,+21<br>✓47,XX,inv(9q),+21<br>✓45,XY,der(11)t(11;21)(p15.3;q11.2) | 3 | 2 | 1 |

Pure trisomy was the most common genetic composition found in the Down's syndrome patients reported in our study. Of all the patients with simple trisomy of chromosome 21, 145 were males (Karyotype: XY+21) and 104 were females (karyotype: XX+21) with a sex ratio of 1.39. Mosaic Down's syndrome for trisomy of chromosome 21, with karyotype 46,XX/47,XX+21 or 46,XY/47,XX+21, was also recorded in 8 children, 6 males and 2 females. Children with Down's syndrome were also detected with translocation. The translocation in these cases were created by merging of chromosome 21 with one of the other acrocentric chromosome (either chromosome 12 or chromosome 14). Seven children (3 males and 4 females) were reported to carry translocation formed between chromosomes 14 and 21 [karyotype: 46,XX,t(14;21) or 46,XY,t(14;21)]. Additional 4 children (3 males and 1 female) were diagnosed with translocation between chromosomes 21 and 21 with cytogenetic composition as 46,XX,t(21;21) or 46,XY,t(21;21).

In 3 cases, trisomy 21 was associated with additional chromosomal aberrations. One male patient carried an aberration which revealed size variation of chromosome Y (karyotype: 47,XYqh-,+21). Another female with trisomy 21 had an additional chromosomal abnormality and this aberration was structural with inversion of chromosome 9 at the long arm (karyotype: 47,XX,inv(9q),+21). One was a complicated case of mosaicism where some cells of the male patient were genetically showing a derived chromosome 11 with translocation between chromosomes 11 and 21 with breaks in 11p15.3 and 21q11.2 [karyotype: 45,XY,der (11)t(11;21)

(p15.3;q11.2)]. While other cells showed an isochromosome for chromosome 21 at 21q10 [karyotype: 46,XY,i(21)(q10)].

## 4. Discussion

The incidence of Down's syndrome is around 1 every 1000 live births, globally [2]. Various abnormalities like learning disability, morphological differences, heart ailments and Alzheimer's disease can be seen in Down's syndrome patients. Studies have pointed towards excessive or deficient nutrient uptake in Down's syndrome patients [3]. Deficiency of vitamin B group coupled with abnormal blood homocysteine levels leads to retarded intellectual development in such patients[15] . Zinc deficiency results in short height and improper functioning of thyroid [16]. The neurological disorders are associated with reduced numbers of neurons, abnormal neuronal differentiation, degeneration and apoptosis of neurons, which can reportedly be prevented by treating with free radical scavengers [17,18]. However, it should be noted that the Down's syndrome patients, having similar genetic composition, display different symptoms. For example, some Down's syndrome patients develop leukaemia, while others do not, nearly 70% of patients develop Alzheimer's disease and congenital heart defects occurs in about 50% of Down's syndrome patients[19-23]. The present study is the first report of Down's syndrome in Iraqi population during the period 2012-2017. This six years duration study focuses on documenting the various types of cytogenetic abnormalities in Down's syndrome children. The cytogenetic study conducted on 249 Down's syndrome patients revealed that the simple trisomy 21 is more frequent (~90%) than the other types of trisomy 21 observed. Less than 4% of children showed mosaicism and even fewer patients showed translocation and other additional chromosomal aberrations. These findings are in concordance with a recent study at Duhok province in Iraq[24]. To analyse the steps that could be taken to prevent the incidence of this chromosomal abnormality and manage this disease, it is essential to understand the factors that might lead to the non-disjunction of chromosome 21. The maternal age is one of the most critical factors leading to incidence of Down's syndrome. Studies have shown that women of age more than 35 years are more prone to give birth to babies with Down's syndrome. Other factors include birth order of affected children, number of maternal miscarriages, physical, biological and chemical mutagens [25].

The maternal age is a critical factor in incidence of Down's syndrome. In various Western countries, the mean maternal age was 35 years [25] while in Iraq, the average maternal age of

Down's syndrome individuals was 32 years, slightly lower than the reports of other countries [24]. This might be attributed to many environmental in combination with genetic factors such as tobacco smoking and contraceptive pills which displayed a strong correlation with the mother age [26]. In fact, in order to identify the associated risk factors, the nondisjunction which is an interaction of many genetic aberration should be dissected to its individual parts [27]. Another explanation could be the difference in dietary habits which reduce the level folic acid and vitamin B12, hence affect the segregation of chromosomes leading eventually to non-disjunction. Studies have shown that the women with birth order of third or higher order were more prone to giving birth to children with Down's syndrome [28]. However, Chan *et al*., suggested that the increased parity and incidence of Down's syndrome are not related[29]. Consanguineous marriages have been associated with the higher rate of Down's syndrome in Arab countries, including Iraq which exceeds 40% of total marriages[30] . Consanguineous marriages are common practices in Middle East countries[31]. In earlier studies conducted in Iraq and Oman, it was shown that the increased consanguineous marriages increased the risk of Down's syndrome [24-32]. The non-disjunction of chromosome might be associated with a recessive alleles or recessive gene(s) and the consanguineous marriages increases the probability of carrying this rare recessive allele and might lead to increased aneuploidy rates in their progeny [33,34].

Mothers of Down's syndrome children had higher incidence of prior miscarriage than mothers of healthy children and this could have been attributed to defence mechanism, where the inherited risk of chromosomal aneuploidy is combatted by early and natural termination of pregnancy [35]. In fact, there are a few markers that could be identified in the sonography of the women pregnant with Down's syndrome babies, such as nuchal fold thickness, cardiac abnormalities, duodenal atresia, femur length and pyelectasis [36]. In the present study, males are predominant with the sex ratio of (M:F = 1.39:1) and this is in concordance with other studies done in Iran (M:F = 1.5:1), Egypt (M:F = 1.14:1) and India (M:F = 2.3:1), Algeria (M:F = 1.75:1) [37-40]. However, there are reports where females were found to be predominant, like in Jordan (M:F = 1:1.2) and some regions of Duhok, Iraq (M:F = 1:1.3) [24, 41]. The variable sex distribution in Down's syndrome individuals is still unclear. However, various theories were suggested to explain the phenomenon including timing of insemination with respect to ovulation, the joint segregation of chromosome 21 and chromosome Y during spermatogenesis and non-disjunction of chromosome 21 at the time of meiotic division during oogenesis [42-44]. In addition, the sexual habit of the DS parents creates

the difference in the sex ratio, since the males have more frequent intercourse which might have an impact on the chromosome segregation in addition to other habits and environmental factors [45-51]. Scientific interventions in research and diagnosis of the Down's syndrome might result in prevention or better management of the associated conditions. This leads to significant increase in life expectancy of individuals with this genetic abnormality. Dietary intervention during early phase of life benefits the quality of the life by delaying some of the conditions associated with Down's syndrome.

## Conclusions

In our study we carried out a comparative cytogenetic analysis of suspected cases of Down's syndrome in children of both sexes in Basra, Iraq using classical karyotyping. We report the incidence of four different cytogenetic variants of Down's syndrome. Our study clearly concludes that simple trisomy 21 is the most abundant cytogenetic variant followed by translocation and mosaic trisomy. Chromosomal aberrations in addition to trisomy 21 is a very rare event and was least common in all the subjects registered in the study. This cytogenetic analysis provides basis for the genetic counselling of families of the patients and also emphasis on the importance of managing the disease to improve quality of life.

## List of Abbreviations:

| | |
|---|---|
| APP | Amyloid Precursor Protein |
| RPMI 1640 | Roswell Park Memorial Medium 1640 |
| PHA | Phytohemagglutinin |

## Declarations

### Ethics approval and consent to participate

This manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. Bas J Sci. My data have no images or videos related to any patient in my study neither adults nor children.

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## دراسة خلوية جينية لمتلازمة داون في المجتمع العراقي

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**المستخلص**

تعد متلازمة داون هي النوع الأكثر شيوعاً من الشذوذ الكروموسومي الذي يعبر عنه في حديثي الولادة. تتميز هذه المتلازمة بملامح وجه غير طبيعية وتخلف عقلي. ومع ذلك، فإن التشخيص السريري لا يكفي لتأكيد الإصابة بالمرض لذا يستخدم تحليل الكاريوتايبنك كأداة تشخيصية لتأكيد متلازمة داون. تم إجراء الدراسة الحالية لتأكيد التركيب الكروموسومي للأطفال المشتبه بهم بعد التشخيص السريري الأولي في البصرة، العراق. تم إجراء تحليل الكاريوتايبنك لـ 249 حالة مشتبه بها. تم تأكيد إصابة 249 مريضًا بمتلازمة داون . ظهرما يقرب من 90٪ من هؤلاء المرضى ذوو طراز جيني صافي للكروموسوم 21، ونسبة 5٪ تقريباً من الحالات كانت لديها انتقالات مواقعية. كما ظهر أقل من 4٪ من الحالات ذوو طراز موزاييكي، و 1.4٪ فقط من المرضى تم تشخيصهم بأعتلالات إضافية للكروموسومات. كما ظهر المرض انحيازًا جنسيًا ملحوظًا، حيث كان هناك زيادة في عدد الذكور مقارنة بالإناث (نسبة الجنس كانت 1.39). يعتبر تحديد للشذوذ الكروموسومي النوعي في مرضى متلازمة داون خطوة أولى نحو تحسين جودة حياتهم. كما توفر الدراسة المعلومات الأساسية اللازمة لتوقع التعقيدات المختلفة المرتبطة بمتلازمة داون.