Egyptian Journal of Medical Human Genetics

RESEARCH Open Access

The demographic data and the high frequency of chromosome/chromatid breaks as biomarkers for genome integrity have a role in predicting the susceptibility to have Down syndrome in a cohort of Egyptian young-aged mothers

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Abstract

Background: Down syndrome (DS) is a common numerical chromosome disorder that has its burden on both family and community. The well-known risk factor for chromosome 21 nondisjunction is advanced maternal age which failed to explain the occurrence of Down syndrome born to mothers less than 35 years. This study aimed to assess the effect of demographic data (consanguinity, residency area, and socioeconomic state) and chromosome/chromatid breaks as biomarkers for genome integrity on the susceptibility of young mothers to have a child with Down syndrome.

Results: Fifty mothers with a history of at least one DS pregnancy before the age of 35 were compared to 50 control mothers. There was a significant increase in DS births in consanguineous parents (46%) compared to 20% in non-consanguineous ones (OR = 3.40; 95% CI = 1.4–8.20, $P = 0.006$). Young mothers with DS children were more likely to be from rural areas (60%) than urban areas (40%) (OR = 2.66; 95%, CI = 1.18–5.98, $P = 0.017$) and of a low socioeconomic status (62%) rather than a high socioeconomic status (38%) (OR = 3.80; 95%, CI = 1.65–8.74, $P = 0.001$). Chromosome/chromatid breaks were detected in 76% of DS young mothers and 32% of control mothers ($P < 0.001$). There was an odds ratio of chromatid breaks of 8.50 (3.411–21.17) and chromosome breaks of 3.93 (1.40–11.05) with significant difference between the studied groups ($P < 0.001$ and $P = 0.009$ respectively).

Conclusion: In addition to advanced maternal age, consanguinity, residency in rural areas, and low socioeconomic status could be considered as possible risk factors for Down syndrome. The high frequency of chromosome/chromatid breaks in young mothers with a previous history of DS children highlights the impact of genome integrity on the tendency to chromosome 21 nondisjunction. These findings are valuable in predicting having a Down syndrome baby and providing proper genetic counseling for high-risk families.

Keywords: Young mothers, Down syndrome, Risk factors, Chromosome breaks, Consanguinity, Malsegregation

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Background

Down syndrome or trisomy 21 (OMIM #190685) was first described by Down in 1866 [1]. Clinically, affected patients had a combination of mental retardation and characteristic faces. Down syndrome (DS) is one of the commonest chromosomal abnormalities seen in neonates, and it is considered one of the commonest causes of mild to moderate mental retardation in children. The worldwide prevalence of DS is estimated to be 1 in 650 to 1,000 live births [2].

Patients with DS have higher mortality and morbidity rates than normal populations. Recently, a decrease in mortality rates among affected children was recorded and attributed to improved early diagnostic and management health services [3, 4].

The gold standard test for the diagnosis of DS is chromosome analysis, which has revealed that 94% of individuals with DS have three copies of chromosome 21 caused by chromosome nondisjunction. In almost 90% of such cases, nondisjunction occurs during maternal meiotic division, most frequently in maternal meiosis I. Only 5% of patients have translocation of chromosome 21 to one of the acrocentric chromosomes, usually chromosome 14 or 21. In very few cases of DS, there is detectable mosaicism for a trisomic and a normal cell line [5, 6].

Advanced maternal age is the only well-known risk factor for chromosome aneuploidy. In 1933, Penrose recognized that older mothers are more susceptible to having a child with DS [7]. Later studies confirmed these observations. It is now clear that advanced maternal age is significantly associated with chromosome aneuploidy for most, if not all, human chromosomes [8, 9].

In spite of this, many DS individuals are born to mothers aged 35 years or younger, suggesting a genetic susceptibility to early nondisjunction for chromosome 21 in blood lymphocytes as well as germ cells, which increases the tendency to chromosome aneuploidy in such women [10, 11].

The correlation between DS and risk factors other than maternal age has been poorly studied. The aim of the study was to investigate the possible correlation between demographic data (consanguinity, residency area, and socioeconomic state) and chromosome/chromatid breaks as cytogenetic biomarkers of DNA integrity on the susceptibility of young mothers to have children with Down syndrome.

Methods

The present case-control study recruited 50 mothers aged less than 35 years at the time of conception of a child with DS that was confirmed by cytogenetic analysis and a control group of 50 healthy mothers with matched age who had no history of a child affected by DS or any other genetic disease. All were selected from the Genetic Clinic, Department of Human Genetics, Medical Research Institute, Egypt, between January 2015 and May 2016. Residency in urban and rural areas for studied cases is determined according to the data of Central Agency for Public Mobilization and Statistics (CAPMAS, 2015) [12] and The Egypt Demographic Health Survey (EDHS) [13].

Mothers aged more than 35 years or had a history of exposure to irradiation, chemotherapy, smoking, or oral contraceptive pills were excluded from the study.

The study was approved by the ethics committee, and a written informed consent was obtained from 50 DS mothers and 50 control mothers before participation in the study, according to the Declaration of Helsinki.

Detailed history and pedigree analysis were assessed. A comprehensive questionnaire was completed and included full data regarding the age, consanguinity, education, occupation, and income of the mother and her husband, place of living, reproductive history of abortion or stillbirth, and the presence of a previous child with DS or any other chromosome anomaly in the family or among other relatives.

Updated Fahmy scale [14] was used to assess the socioeconomic status of the included mothers. High socioeconomic status was indicated at 70% or more of the total score while low socioeconomic status is less than 40%.

Cytogenetics study

Peripheral venous blood samples were taken from all mothers in the study. Under complete aseptic conditions and with sterile disposable syringes, 4 ml of blood were collected in heparinized vacutainer tubes for peripheral blood lymphocyte culture. Two tubes for each mother were prepared, and blood lymphocyte cultures were set up within 24 h of sampling according to the conventional method [15].

Whole blood cultures were established by placing 0.5 ml of blood with an RPMI medium supplemented with 20% fetal calf serum and 1.5% phytohaemagglutinin.

Cultures were incubated at 37 °C for 48 h in one tube for the detection of chromosome breaks and 72 h in the other tube for the diagnosis of any numerical or structural chromosome anomalies.

The harvesting process was started by the addition of colchicine (0.1 mg/ml) for the last 2 h of incubation to arrest the cells at metaphases. Cells were incubated with hypotonic KCI (0.075 M) at 37 °C for 10 min and fixed in four changes of cold 3:1 methanol/acetic acid.

Slides were prepared by the heat drying technique and were stained with aqueous Giemsa solution for the tubes incubated for 48 h. Trypsin pretreatment was used before Giemsa staining for tubes incubated for 72 h.

For chromosome numerical and structural abnormalities, 30 metaphases were counted; 5 metaphases were

analyzed and 2 were photographed for each sample prepared after the 72 h culture procedure.

For the samples prepared after the 48 h incubation, a total of 100 metaphase cells per sample were scored at random and analyzed for chromosome and chromatid aberrations according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016 [16].

Achromatic areas less than a chromatid width (gaps) were excluded in the calculation of chromosomal breakage frequencies. Achromatic areas taking a chromatid width were scored as a single chromatid break, and chromatid breaks involving both chromatid widths were considered as chromosomal breaks and were scored as two breaks each [16].

Statistical analysis

Data obtained were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp.), where a statistically significant difference between DS mothers and control mothers is set when $P < 0.05$. Qualitative data were described using number and percent and the association between studied factors (consanguinity, residency, social status, and chromosome breaks), and the risk of having a child with DS was calculated by chi-square test, odds ratio (OR), and 95% confidence interval (CI). Further analysis of the results was calculated in a 4 × 4 table to assess the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPP), and accuracy [17].

Results

The study recruited 100 mothers divided into two groups: group 1 included 50 mothers with a mean age of 27.52 ± 3.9 years who had a history of at least one DS child, and group 2 included 50 control mothers with a mean age of 26.96 ± 4.1 years.

As shown in Table 1 and Fig. 1, there was a significant difference between DS mothers and control mothers regarding consanguinity: the frequency of newborns with DS was higher among consanguineous mothers (46%) compared to 20% in the control mothers (OR = 3.40; 95% CI = 1.4–8.20, $P = 0.006$).

Table 1 Demographic data of young mothers with DS in comparison to control mothers

Variable		DS mothers	Control mothers	OR (95% CI)	*P* value
Consanguinity	Present	23 (46%)	10 (20%)	3.40 (1.4–8.20)	0.006*
	Absent	27 (54%)	40 (80%)		
Socioeconomic status	High	19 (38%)	35 (70%)	3.80 (1.65–8.74)	0.001*
	Low	31 (62%)	15 (30%)		
Residency area	Rural	30 (60%)	18 (36%)	2.66 (1.18–5.98)	0.017*
	Urban	20 (40%)	32 (64%)		

*Statistically significant at $P \leq 0.05$

Again, the frequency of DS was significantly higher among young mothers from rural areas as compared to urban areas (OR = 2.66; 95%, CI = 1.18–5.98, $P = 0.017$) (Table 1, Fig. 1).

There was a significant difference between young mothers of low socioeconomic status and mothers of high socioeconomic status regarding the birth of children with DS (OR = 3.80; 95%, CI = 1.65–8.74, $P = 0.001$) (Table 1, Fig. 1).

The frequency of chromosome/chromatid breaks was significantly higher in DS mothers than in the control group (76% versus 32%; $P < 0.001$) (Table 2).

There were no numerical or structural chromosome anomalies in blood samples of DS mothers and control mothers after72 h blood culture.

We found a significant increase of the frequency and mean of chromatid breaks in mothers with DS children compared to the control group (OR = 8.50; 95%, CI = 3.411–21.17, $P < 0.001$).

Regarding the chromosome breaks, Table 3 and Fig. 2 show that the frequency and mean of chromosome breaks in DS mothers was higher than that in control mothers (OR = 3.93; 95% CI = 1.40–11.05, $P = 0.009$).

Although the odds ratio was 3.033, Table 4 shows that there was no statistically significant correlation between the presence of chromosome/chromatid breaks in young mothers and low socioeconomic status ($P = 0.104$).

The sensitivity of the chromosome/chromatid breaks test was 69.2%, specificity was 73.9%, P.P.V was 72.0%, N.P.V was 68.0%, and the accuracy was 70% (Table 5).

Discussion

Down syndrome is a well-known chromosomal disorder that is caused in the majority of cases by chromosomal nondisjunction and the presence of an extra copy of chromosome 21 (trisomy21), which commonly occurs during maternal meiosis [6]. The association between advanced maternal age (above 35 years) and trisomy 21 was observed by Penrose in 1933 [7].

However, the frequency of DS in newborns is high among young mothers less than 35 years old at the time of conception, suggesting that there are other factors than advanced maternal age that may play a role in the predisposition to chromosome aneuploidy.

When analyzing the demographic data of young mothers enrolled in the present study, we found a significant increase in DS births among consanguineous parents (46%) compared to 20% in non-consanguineous ones ($P = 0.006$). In spite of the small sample size, our findings are supported by Ray et al. 2018, who reported an increased risk of chromosome 21 nondisjunction during meiosis II in young mothers with consanguineous mating [18]. Increased frequency of chromosome

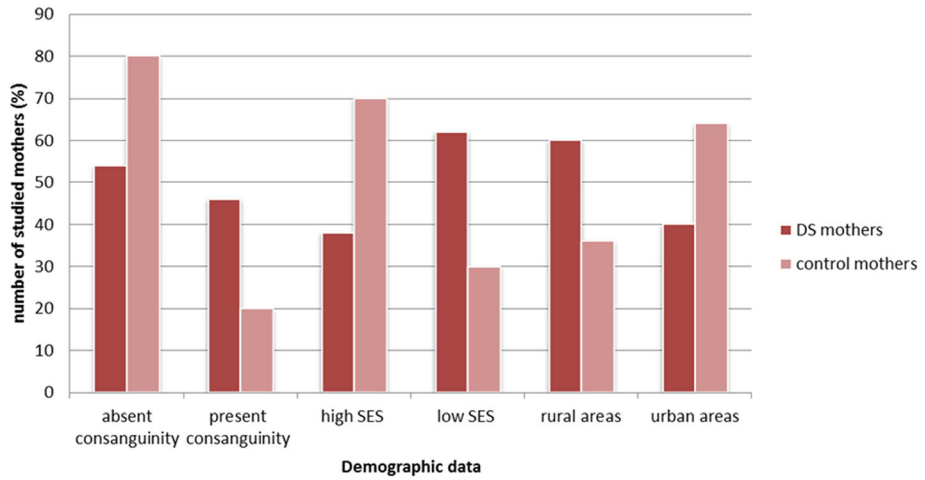

Fig. 1 The distribution of consanguinity, socioeconomic status (SES), and residency among young mothers of DS children in comparison to control mothers

nondisjunction in consanguineous parents was first postulated by Penrose in 1961 [19]. In 2013, Shawky et al. [20] found consanguineous marriage in 29.1% of patients with chromosome disorders and in 28.8% of DS patients. Conversely, El Mouzan et al. found no association between consanguinity and Down syndrome [21]. Consanguinity rates in Egypt range between 29 and 39% with some differences according to residency areas [22, 23]. Higher rates of consanguinity were found in rural areas compared to urban areas (46.0% versus 27.3%) and in upper Egypt compared to lower Egypt (46.5% versus 31%) [24]. In Egypt, like other Middle Eastern countries, consanguinity rates fluctuate depending mainly on religious as well as cultural morals, especially in rural areas [22, 24].

In the current study, by comparison to control mothers, young mothers with DS children were more likely to be from rural areas (60%) than urban areas (40%) and of a low socioeconomic status (62%) rather than a high socioeconomic status (38%), and these data match with a previous report from India [25]. These findings may be related to environmental and/or nutritional factors such as folic acid deficiency or pollution by pesticides [26, 27]. The poverty of proper prenatal diagnosis and lack of well-established health care services coupled with low education appears to be another factor that may cause higher DS birth rate in rural rather than urban areas [13, 28].

In 2013, Hunter [29] used data from the National Down Syndrome Project (NDSP), a large population-based case-control study, and found significant association between low socioeconomic status and nondisjunction during maternal meiosis II with no difference between young or old mothers.

From these observations, multifactorial etiology of nondisjunction might be assumed and further national studies are needed to evaluate the association between maternal socioeconomic status and chromosomal aneuploidy.

Failure of chromosome segregation during parental meiosis I, meiosis II, or post-zygotic mitosis results in an extra copy of chromosome 21 and a newborn with DS. Additional genetic and/or environmental factors other than advanced maternal age are still unclear. Recently altered patterns of recombination such as no exchange,

Table 2 Association between chromosome/chromatid breaks and young mothers with DS in comparison to control mothers

Studied group	Chromosome/ chromatid breaks		Total	χ^2	*P* value
	Present	Absent			
Young mothers with DS	38 (76%)	12 (24%)	50 (100%)	19.485*	< 0.001*
Control mothers	16 (32%)	34 (68%)	50 (100%)		

χ^2 chi-square test
*Statistically significant at $P \leq 0.05$

Table 3 The frequency of chromatid and chromosome breaks in young mothers with DS children and control mothers

Cytogenetic defect	DS mothers	Control mothers	OR (95% CI)	*P* value
Chromatid breaks				
Frequency	34/50 (68%)	10/50 (20%)	8.50 (3.411–21.17)	< 0.001*
Mean ± SD	3.68 ± 2.54	1.29 ± 0.84		
Chromosome breaks				
Frequency	18/50 (36%)	6/50 (12%)	3.93 (1.40–11.05)	0.009*
Mean ± SD	2.45 ± 3.32	0.86 ± 0.69		

*Statistically significant at $P \leq 0.05$

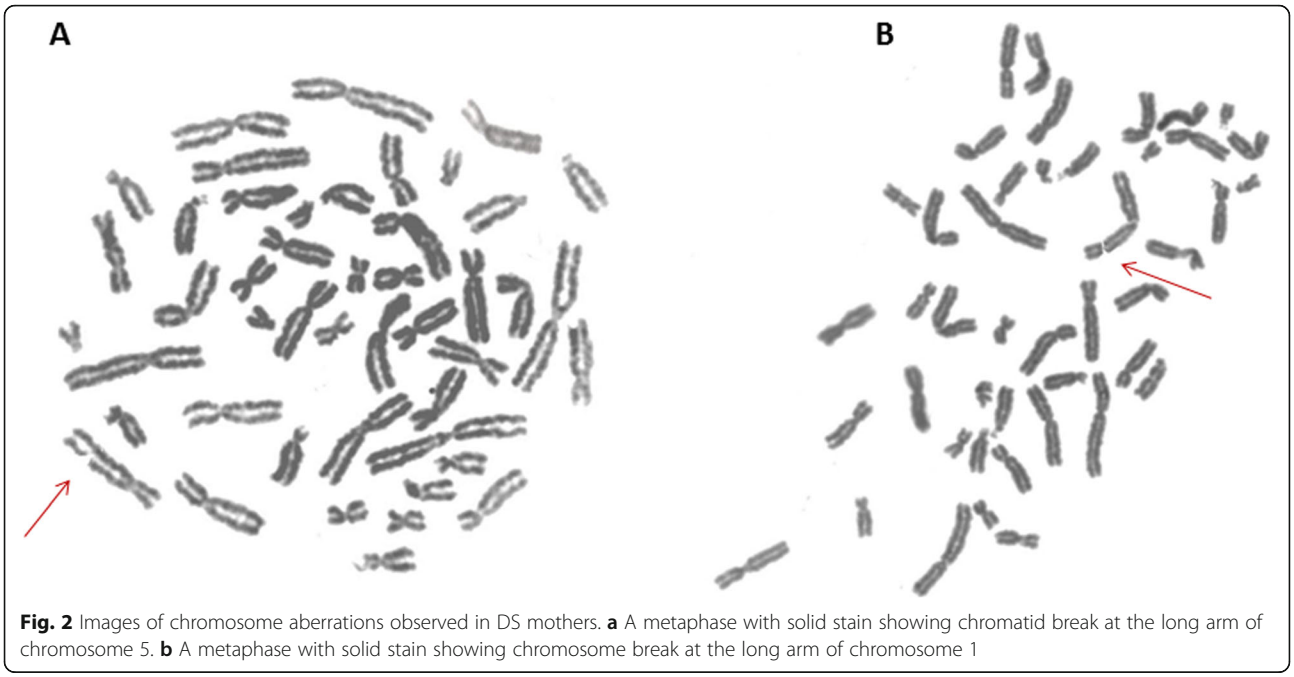

Fig. 2 Images of chromosome aberrations observed in DS mothers. **a** A metaphase with solid stain showing chromatid break at the long arm of chromosome 5. **b** A metaphase with solid stain showing chromosome break at the long arm of chromosome 1

telomeric, or pericentromeric exchanges have been found to have a role [30].

In reality, there is a paucity of published data regarding DS frequency in mothers aged less than 35 years. Studies have found an association between genome instability and young mothers who had at least one DS child. The genome instability is being indicated by biomarkers such as increased frequency of micronuclei [31], shorter telomeres [32], and premature centromere separation [33], in addition to impaired DNA methylation as a result of folate pathway genetic polymorphism, particularly the methylenetetrahydrofolate reductase (*MTHFR*) gene [26]. These studies suggested that young mothers of DS children are considered "biologically older" than mothers of the same age with normal babies.

In the present study of mothers less than 35 years old, there was a significant increase in the frequency of chromosome/chromatid breaks among mothers of DS babies in comparison to the control mothers (76% versus 32%; $P < 0.001$). To our knowledge, it is the first time in Egypt to use the frequency of chromosome/chromatid breaks as cytogenetic biomarkers to assess genome integrity and the risk of having a child with DS in mothers aged less than 35 years. In the current study, young mothers were at risk to have chromatid breaks by 8.5-folds and chromosome breaks by 3.9-folds ($P < 0.001$and $P = 0.009$ respectively) with 70% accuracy.

The frequency of chromosome/chromatid breaks in DS mothers of low socioeconomic status was 68.4%, and in DS mothers of high socioeconomic status, it was 31.6% ($P = 0.104$), while 57.9% of DS mothers from rural areas had chromosome/chromatid breaks in comparison to 42% of DS mothers from urban areas ($P = 0.589$). Neither socioeconomic status nor residency in rural or urban areas were found to have an impact on chromosome/chromatid breaks in our studied groups. Using a literature review, we did not find a relation between such factors and chromosome integrity.

Overall, the DNA damage seen under the light microscope in the form of chromatid/chromosome breaks is a result of failed DNA repair mechanisms during

Table 4 The association of socioeconomic status and residency area with chromosome/chromatid breaks in young mothers with history of a DS child

Studied variable		Chromosome/chromatid breaks		OR (95% CI)	*P* value
		Present (no. 38)	Absent (no. 12)		
Socioeconomic status	High	12 (31.6%)	7 (58.3%)	0.330 (0.087–1.254)	0.104
	Low	26 (68.4%)	5 (41.7%)	3.033 (0.797–11.539)	
Residency area	Rural	22 (57.9%)	8 (66.7%)	0.688 (0.176–2.684)	0.589
	Urban	16 (42.1%)	4 (33.3%)	1.454 (0.372–5.679)	

Table 5 Accuracy measures of chromosome/chromatid breaks in predicting DS pregnancy in young mothers

Accuracy measures	Value (%)
Sensitivity	69.2
Specificity	73.9
P.P.V	72.0
N.P.P	68.0
Accuracy	70.0

PPV positive predictive value, *NPV* negative predictive value

transcription and replication processes owing to replication errors, oxidative stress, impaired winding and unwinding of DNA strands due to topoisomerases defect, or damage by environmental triggers such as irradiation or chemical pollution.

Conclusion

Our findings suggest that maternal age should not be considered the only risk factor for Down syndrome. Consanguinity, residency in rural areas, and low socioeconomic status need to be considered in the disposition of having a child with DS.

Increased frequency of chromosome/chromatid breaks in peripheral blood lymphocytes of mothers less than 35 years of age and with a previous history of DS suggests that genome instability increases the tendency to chromosomal nondisjunction.

Further research is recommended to understand the biochemical and molecular mechanisms controlling DNA repair and its effect on chromosome 21 malsegregation in young mothers with a history of Down syndrome children.

Abbreviations

CI: 95% confidence interval; DS: Down syndrome; ISCN: International System for Human Cytogenetic Nomenclature; MTHFR: Methylenetetrahydrofolate reductase gene; NDSP: National Down Syndrome Project; NPP: Negative predictive value; OR: Odds ratio; PPV: Positive predictive value

Acknowledgements

We are thankful to all mothers who participated in this study.

Authors' contributions

LME conceptualized and designed the study, shared in the lab work, and participated in the analysis and interpretation of data, manuscript preparation, and submission. NMI provided the study patients and control, participated in the collection and analysis of primary data, shared in the lab work, and participated in the revision of the initial manuscript. HSM shared in the study design and revision of the initial manuscript. All authors approved the final version of the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The study was approved by the ethics committee of the Medical Research Institute (10 GR 0008812).
An informed written consent was obtained from all mothers before participation in the study according to the Declaration of Helsinki.

Consent for publication

A written consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

Received: 29 May 2019 Accepted: 30 August 2019
Published online: 04 October 2019

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