Research Article

Older paternal age and positive consanguinity increase the burden of β thalassemia disease

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Abstract

Background: β thalassemia has been considered one of the most common genetic diseases. It represents public health concern especially in Middle East and Mediterranean regions.

Objective: To determine the influential effect of paternal and maternal age son β thalassemia inheritance.

Materials and Methods: This case–control study was conducted at EL Fayoum University Hospital in the period from June 2013 to June 2014. The study included 94 children with β thalassemia. Full medical history was taken. The state of parental consanguinity and paternal and maternal ages at time of the child's birth were recorded (as given by history and confirmed by data from birth record of the child and identity record of the parents). Results were interpreted using Statistical Package for Social Science program.

Result: Fathers aged ≥40 years revealed increased risk to possess children with β thalassemia ($P = 0.000$, 95% CI: –0.038, 0.015). Children of relative parents had significantly increased chance to possess β thalassemia ($P = 0.000$, 95%CI: 0.219, 0.491). Maternal age did not have any influential effect on β thalassemia inheritance. There were no significant differences between thalassemia major and thalassemia intermedia with respect to father's age and consanguinity.

Conclusion: Parents with positive consanguinity and fathers aged ≥40 years showed increased risk to possess children with β thalassemia disease. These results must be considered on applying the rules of preconception screening and genetic counseling to thalassemia. Complementary genetic studies about this issue are recommended.

KEY WORDS: Paternal age, positive consanguinity, thalassemia risk, Middle East and Mediterranean regions

Introduction

Beta thalassemia has been considered one of the most common hereditary disorders. In Middle East and Mediterranean areas, thalassemia represents a major public health problem owing to the wide prevalence of the disease in these regions.[1] β thalassemia is inherited as an autosomal recessive disorder, which results in reduction or absence in β globin chain.[2] Point mutation in the β-globin gene is the cause of β thalassemia inheritance in majority of cases, while short deletion in the same gene may occasionally be the cause. More than 200 mutations that result in β-thalassemia are present all over the world.[3] Mutation type varies among different populations; in Egypt, there are about 19 mutations, the most common are IVS-I-110 (G-->A), IVS-I-1 (G-->A), and IVS-I-6 (T-->C).[4] If the mutation results in complete absence of β chain, then the phenotype will be βo or β thalassemia major. If the mutation permits some degree of β chain production, then the phenotype will be thalassemia intermedia.

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A higher percentage of spontaneous mutations in some genetic disorders are paternal origin,[5] and this should be deduced in an association between paternal age and inherited diseases. Fathers are more expected to transmit new mutations to their offspring with passing time.[6] Previous studies had been implemented to investigate the effect of older paternal age on some genetic diseases of various mode of inheritance. The effect of paternal age is established in a wide range of diseases (spontaneous dominant disorders as achondroplasia,[7] X-linked disorders as Lesch–Nyhan syndrome and ornithine transcarbamylase,[8,9] multifactorial inheritance, congenital anomalies,[10] childhood cancers,[11] autism,[12] schizophrenia,[13] bipolar disorder,[14] reduced neurocognitive abilities,[15] and increased telomere length[16]). However, the effect of maternal age is restricted to a narrow spectrum of disorders. The strongest example of these disorders is Down syndrome.[17]

However, the effects of parental ages on recessive disorders were not previously studied. Paternal age effect is more prominent with point mutation than with deletions as point mutations are almost entirely from sperm, whereas the deletions are derived from both parents.[18] The presence of point mutation as the main mode of genetic inheritance in thalassemia highlights the possibility of association between paternal age and thalassemia diagnosis. Presence of novel spontaneous mutation in thalassemia that is paternal in origin supports this assumption.[19] Appearance of β thalassemia in families with negative family history of the disease pays our attention to the occurrence of spontaneous mutation. This case–control study aimed to investigate the effect of paternal age as a risk factor for thalassemia disease, by using survival analysis and taking maternal age and consanguinity into account. The paternal and maternal ages-related risk to get β thalassemia in an epidemiological point of view are described.

Materials and Methods

A case control study was conducted in El Fayoum University hospital from June 2013 to June 2014. The parents gave written informed consent.

Participants

The study included Egyptian children diagnosed as β thalassemia major and intermedia. Diagnosis of thalassemia was based on the results of electrophoresis. The patients were born in El Fayoum city from 2001 to 2013. They attended the hospital for follow-up and for receiving appropriate treatment. Patients with associated genetic, congenital, or chronic diseases were excluded from the study. Control group comprised equal number of healthy children younger than 12 years chosen randomly. They attended the clinic for minor acute insult or for regular health checkup.

Sample Size

The sample size of the study was determined according to the following equation:

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2(Z_\beta + Z_{\alpha/2})}{\text{difference}}$$

n = sample size in each group;

r = the ratio of sample sizes in case and control groups;

σ = the common standard deviation of the outcome variable;

Z_β = standard normal variant for power (typically 0.84 for 80% power);

$Z_{\alpha/2}$ = standard normal variance for level of significance (typically 1.96 for 0.05 significance);

difference = the expected mean difference between cases and control (the effect size required to be detected).

The desired power of this study was 80%, and the desired level of significance was 0.05. The effect size was 5 years and the standard deviation 10 years.

Procedure

Cases were referred to study participation from pediatric clinic and inpatient units. They attended the hospital for receiving treatment or for regular follow-up. We classified the patients as thalassemia major if they required transfusion more than eight times till the age of 8 years, while thalassemia intermedia patients are those with no or occasional transfusion before the age of 4 years.[20,21]The parents who accepted to participate were included in the study. Parents were asked about their ages at the time of birth of the child. Paternal ages were confirmed by calculation of the parent ages at birth from the data of identity records of the parents and birth record of the child. State of the parent's consanguinity was also investigated.

The measured variants were paternal and maternal ages at birth of the child and state of parents' consanguinity.

Compliance with Ethics Guidelines

The Ethical committee of Faculty of Medicine, Fayoum University, approved the protocol for the research project. The work had been carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki 1975, as revised in 2000, for experiments involving humans). The parents gave written informed consent.

Data Analysis

Collected data were computerized and analyzed using Statistical Package for Social Science (SPSS), version 20. Demographic data of the patients were presented by using descriptive statistics. Frequencies and percentages were used categorical data. Continuous data were presented in the form of means and standard deviations. Independent sample T test was used to compare between patients and control and between patients of thalassemia major and thalassemia intermedia with respect to father's age, mother's age, and state of consanguinity. Univariate general linear model was used to study simple the effect of each factor on occurrence of the disease. Comparison with the effect of various paternal age groups was done using one-way ANOVA test. Pairwise comparison of the effect of different groups was performed to detect the exact effect of each group. Linear regression

analysis was used to detect independent effect of each factor. *P*-values less than 0.05 were considered statistically significant.

Results

The study enrolled 94 patients (50 boys and 44 girls) and 94 control children (49 boys and 45 girls). The age of the patients ranged from 1 to 12 years, and in control group, it ranged from 0.5 to 12 years. The mean values of fathers' age at the birth of the child were 36.42 years for cases and 32.6 years for control group. The mean values of mothers' age at the birth of the child were 29.48 years for patients and 29.17 years for control group. The rates of consanguinity were 53.2% of cases and 18% of control group [Table 1].

Fathers aged 40 years or older represented 44.7% of patients versus 19.1% of control group ($P = 0.001$). No significant differences were detected between the patients and control groups with respect to ages of mothers. Positive parental consanguinity is more frequent in patients than that in control groups [$P = 0.000$, OR (95%CI): –0.34 (–0.47, –0.21)] [Table 2].

The simple univariate test showed increased paternal age to be a significant risk factor for β thalassemia diagnosis [$P = 0.028$, OR (95%CI): 1.48 (1.39,1.58)]. Positive consanguinity was associated with increased risk of β thalassemia [$P = 0.000$, OR (95% CI): 1.44 (1.37, 1.52)] [Table 3].

On comparison of the effect of various paternal age groups on β thalassemia diagnosis, fathers aged 40 years or older showed significantly higher risk to possess children with thalassemia than those with younger age groups [Table 4].

No significant differences were detected between thalassemia major and intermedia concerning the impact of paternal age and positive consanguinity [Table 5].

The independent effects of paternal age and consanguinity on diagnosis of β thalassemia were studied using regression analysis test. Increasing paternal age and positive consanguinity were considered as independent risk factors for β thalassemia occurrence ($P = 0.000$) [Table 6].

Table 1: The characteristics of the patients and control groups

	Patients, *N* = 94	Control, *N* = 94
Child age (years), range	1–12	0.5–12
Sex, *n* (%)		
Male subjects	50 (53.2)	49 (52.1)
Female subjects	44 (46.8)	45 (47.9)
Diagnosis, *n* (%)		
TI	30 (31.9)	
TM	64 (68.1)	
Family history of other affected sibling, *n* (%)		
Positive	48 (51.1)	
Negative	46 (48.9)	
Father's age at birth of the child (years), mean±SD, range	36.42 ± 6.13, 22–50	32.68 ± 6.01, 22–45
Mother's age at the birth of the child (years), mean±SD, range	29.48 ± 5.36, 18–44	29.17 ± 4.45, 18–38
Consanguinity, *n* (%)		
Positive	50 (53.2)	18 (19.1)
Negative	44 (46.8)	76 (80.9)

TI, thalassemia intermedia; TM, thalassemia major; SD, standard deviation.

Table 2: Comparison between patients and control with respect to father's age, mother's age, and consanguinity

Tested factor	Group	Patients, *N* = 94		Control, *N* = 94		*P*	OR (95%CI)
		n	%	*n*	%		
Father's age groups (years)	<30	16	17	34	36.2	0.001***	0.69 (0.36, 1.02)
	≥30–35	19	20.2	24	25.5		
	≥35–40	17	18.1	18	19.1		
	≥40	42	44.7	18	19.1		
Mother's age groups (years)	<25	16	17	14	14.9	0.710	–0.26 (–1.67, 1.14)
	≥25–30	35	37.2	39	41.5		
	≥30–35	26	27.7	29	30.9		
	≥35	17	8.1	12	12.7		
Consanguinity	Positive	50	53.2	18	19.1	0.000****	–0.34 (–0.47, –0.21)
	Negative	44	46.8	76	80.9		

CI, confidence interval; OR, odd ratio.

Table 3: Univariate simple effect of paternal age, maternal age, and consanguinity using general linear model

	Patients	Control	*F* (effect)	*P*	OR (95%CI)
Father's age (years), mean	36.42	32.68	1.68	0.028*	1.48 (1.39, 1.58)
Mother's age (years), mean	29.48	29.17	1.45	0.103	1.47 (1.377, 1.56)
Consanguinity	Positive in 53.2%	Positive in 19.1%	26.69	0.000****	1.44 (1.37, 1.52)

CI, confidence interval; *F*, factor effect; OR, odd ratio.

Table 4: Pairwise comparison with the effect of paternal age groups to detect maximum group at risk

Age group (years)	Age group in comparison (years)	*P*	OR (95% CI)
<30	≥30–35	0.23	0.12 (0.07, 0.32)
	≥35–40	0.12	0.16 (–0.04, 0.37)
	≥40	0.000	0.38 (0.19, 0.56)
≥30–35	<30	0.226	–0.12 (–0.32, –0.07)
	≥35–40	0.69	0.04 (–0.17, 0.26)
	≥40	0.008	0.26 (0.07, 0.44)
≥35–40	<30	0.12	–0.16 (–0.37, 0.04)
	≥30–35	0.690	–0.04 (–0.26, 0.173)
	≥40	0.038	0.21 (0.01, 0.42)
≥40	<30	0.000****	–0.38 (–0.56, –0.19)
	≥30–35	0.008***	–0.26 (–0.44, –0.07)
	≥35–40	0.038*	–0.214 (–0.42, –0.01)

CI, confidence interval; OR, odd ratio.

Table 5: Differences in the impact of paternal age and consanguinity between thalassemia major and thalassemia intermedia using independent *T* test

	Thalassemia intermedia	Thalassemia major	*P*	OR(95%CI)
Father's age (years), mean	35.80	36.7	0.813	–0.92 (–3.62, 1.77)
Consanguinity	Positive in 46.6%	Positive in 56.25%	0.39	–0.09 (–0.12, 0.31)

CI, confidence interval; OR, odd ratio.

Table 6: Regression analysis to detect independent effect of father's age and consanguinity

	Standardized coefficients	*P*	OR	95%CI	*R*
Father's age	–0.33	0.000****	–0.026	–0.038, –0.015	0.465
Mother's age	0.07	0.305	0.008	–0.007, 0.023	
Consanguinity	0.34	0.000****	0.355	0.219, 0.491	

CI, confidence interval; OR, odd ratio.

Discussion

Premarital and preconception screening along with genetic counseling are strongly recommended as important lines of management in thalassemia, especially with absence of actual effective treatment up to the present time. Recognition of risk factors of the disease will help in qualifying the disease management strategy. This case–control epidemiological study aimed to investigate the effect of paternal and maternal ages at the time of birth on β thalassemia diagnosis. It is the first study that explores the effect of parental age on one of the autosomal recessive disorders.

The most important observation in this study is the impact of paternal age on β thalassemia disease. Fathers aged 40 years or older showed higher risk to get children with β thalassemia than those of younger age groups.

These results are in consistent with several results that associated older-aged fathers to several genetic diseases, especially when the main mode of inheritance is point mutation (autosomal dominant diseases such as achondroplasia, myositis ossificans, Marfan syndrome and Apert syndrome,[22] and X-linked recessive such as Lesch–Nyhan disease[8]).

In males, spermatogenia divides continuously throughout the reproductive life.[23] Continuous circles of DNA replications lead to occurrence of random copy-error mutational events in the male germ line.[24] Frequent exposures to mutagens with passing time may contribute to the accumulation of copy error. In addition, decreased level antioxidants enzymes with age[25] and absence of DNA repair mechanism in late spermatids and immature and mature spermatozoa[26] result in weakening the proficiency of editing DNA repair mechanism during spermatogenesis in older men.[27]Increase in the epigenetic mechanisms such as hypermethylation with age may add to the increased mutation rate.[28,29]

With respect to maternal age, no differences were detected between the patients and control groups. The study revealed absence of the maternal age effect on thalassemia diagnosis. In contrast to men, the oocyte in women remains in prophase arrest for most of its lifetime, therefore less-repeated rounds of DNA replications, and, so, random copy-error mutational events are seldom to be ensued. However, there are evidences that associate increased maternal age to chromosomal rearrangement such as Down syndrome, as all the postnatal phases of germ cell development in women are meiotic, and, so, maternal mutagenic events involve mostly recombination-related mechanisms.[30]

In this study, positive parental consanguinity was considered as a risk factor for β thalassemia disease. This is in consistent with a study by Hussein et al., in Sues Canal area, where consanguinity rate among patients with thalassemia was 60%. They considered that positive parental consanguinity could increase the risk of β thalassemia eight times.[31] Rates of consanguinity varied among different countries; highest rates are in the Middle East, North Africa, and South Asia,[32] which may contribute to high prevalence of thalassemia in these regions. As any recessive inherited disease, increased prevalence of consanguineous marriage among societies with high-disease mutations rates augments the burden of the disease as it leads to increase the possibility of homogenous mutation.

Conclusions

Fathers of older ages (≥40 years) are at increased risk to possess children with β thalassemia. These results can offer indirect evidence of developing spontaneous mutation in the gene of β globin. Positive consanguinity is significantly related to an increased risk of thalassemia inheritance. However, the mother age was not considered as an influential factor for thalassemia. These results highlight the importance of premarital and preconception counseling and screening as preventive measures in β thalassemia.

Recommendation

The findings of this study must be taken in consideration during applying the protocol of preconception screening and genetic counseling of thalassemia. They also must be concerned during safeguard measures for sperm donation; it is healthier to restrict the donor's age so as not to exceeding 40 years. In addition, performing more genetic studies to complement and confirm these epidemiological findings to detect the precise mutation level within the genome are recommended.

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