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Original Article

Genetic Investigation of 261 Cases of Turner Syndrome Patients Referred to the Genetic Clinic

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Abstract

Background: Turner syndrome (TS), also known as 45,X, is a genetic disorder caused by the partial or complete lack of an X chromosome. TS can cause a variety of medical and developmental conditions. We aimed to investigate TS mosaicism and variants pattern and research the presence of a correlation between the different variant's factors and TS occurrence.

Methods: From 1984-2018, 100,234 patients referred to the Farhud Genetic Clinic, Tehran, Iran, for karyotyping were studied. TS was determined by the chromosomal assay, and the patients' karyotype was obtained from amniotic fluid and blood samples. Different variants of the TS diagnosed patients were investigated, including maternal and paternal age at pregnancy, parental consanguinity, and the presence/absence of a family history of the disease.

Results: Overall, 261/100,234 (0.26%) were diagnosed with TS. These, 150 cases were identified to have the classical 45,X karyotype and 111 cases were identified to have either TS mosaicism or other less common variations of TS karyotyping. Higher parental age at pregnancy and TS data suggested that the occurrence of TS is significantly higher.

Conclusion: Data suggest parental age at pregnancy is an important factor for TS occurrence. Hence, prenatal screening in these groups of parents recommended. This study also implicates early medical diagnostic testing before the onset of puberty or as soon as symptoms arise is essential for early treatment.

Keywords: Turner syndrome; Chromosomal disorders; X chromosome; Primary amenorrhea

Introduction

Turner syndrome (TS) is one of the most common congenital chromosomal disorders worldwide with a prevalence of 1 in 2,500 female births. This syndrome is also well known under various names including monosomy X, 45,X, and Ullrich-TS (1, 2). The majority of TS individuals are phenotypically female, however, a few phenotypically male cases have been reported (3). The incidence of TS is more common in stillbirth and a known cause of spontaneous miscarriage (4).

TS can present with a variety of different karyotypes, leading to a range of presenting clinical features (5). The monosomy X TS karyotype means an X chromosome in the pair is completely missing. In comparison, mosaicism TS means there is an X chromosome or part of an X chromosome that is missing in a portion of the cells, but not all cells have been affected (6). The majority of TS patients suffer from the monosomy X karyotype (7). Sexual development and adult height are weaker in the monosomy X karyotype (3). Other symptoms of TS include secondary amenorrhea, webbed neck, low hairline, swollen hands and feet, lymphedema, skeletal abnormalities, renal, heart, and endocrine complications, broad chest with widely spaced nipples, skin pigmentation, and short IV metacarpals (8). Currently, researchers have not been able to identify specific genes in the X chromosome that cause the TS characteristics. But, a specific gene called the SHOX gene has been identified and proven to be essential for the growth of bone and the lack of a copy of the SHOX gene, in TS females, causes short stature and skeletal disabilities (1).

TS-related disabilities are not known to be hereditary, the cause of the chromosomal abnormalities has been found to be due to a defect in the reproductive cells, oocyte and sperm. This disorder occurs as a result of a meiotic error, leading to an abnormal number of chromosomes in the reproductive cell and cell division failure in the early embryonic stages (6). TS can be diagnosed in early pregnancy by soft markers present during the second-trimester sonography screening and with echocardiography (9). There are several prenatal diagnosis methods for TS, including ultrasound imaging, amniocentesis, or chorionic villus sampling. In addition, karyotype analyzing, especially in people with traces of 45, X, can help with the early-stage diagnosis of this syndrome. Furthermore, a variety of tests, such as molecular and cytogenetic tests, can be used to aid with the diagnosis (10, 11).

We aimed to investigate mosaicism TS and variant's pattern and highlight possible correlations between the different variant's factors with TS, including parental age at the time of birth; patient's age at the time of referral; and presence/absence of parental consanguinity.

Materials and Methods

This retrospective study was carried out on 100,234 patients referred to the Farhud Genetic Clinic, Tehran, Iran for karyotyping between 1984 and 2018. Both blood and amniotic fluid samples were collected for karyotyping in the study.

Ethical approval was obtained for the study from the Tehran University of Medical Sciences (IR.IAU.PS.REC.1397.297) and every patient signed the consent letter for the investigation.

Patients attending the clinic under the suspicion of genetic diseases or for genetic counseling were karyotyped using blood samples. About 3 ml blood was collected from each of these patients under sterile conditions by skilled technicians. Karyotyping was performed on the blood samples in a conventional and high-resolution format, as follows: cultivation, harvesting, lameness or chromosomal coverage, and chromosome staining. After 72 h, cultured chromosomes were extracted. To obtain chromosomes, fresh blood was added to the coagulant (sodium heparin), which allows cells to grow over the course of two weeks. The chromosomes were stained so that the observer could view them under a light microscope with 250x magnification. The observer examined the samples and reported on the probability of the disorder being present in the related chromosomes using specific metasystem software equipment.

Karyotyping of the amniotic fluid was used to screen pregnant women. Amniotic fluid samples

were collected under sterile conditions by specialists and prepared in order to examine the fetal chromosomes. Amniotic fluid was centrifuged at 1500 rpm for 15 minutes. Following this period of centrifugation, the supernatant was removed. 1cc amniomax was then added and the sample was examined under a microscope. The technique of amniotic fluid harvesting was revised to laminate the sample in order to obtain adherent cells and ensure chromosomes remain visible under the microscope, on 7 days of cells culture.

Data analysis was performed using PAST (ver. 2.17 software) and SPSS (ver. 17), Chi-squared tests were also performed.

Results

Out of 100,234 patients who underwent karyotyping, 261 patients tested positive for TS, which, 150/261 patients had the classic 45,X karyotype and 111 patients had either mosaicism TS or another less common karyotype. The components of the various karyotypes identified in these patients have been displayed in Table 1.

Table 1: Turner mosaicism and variants

Karyotype	*Number of pts**	*Percentage*
45,X / 46,XX	50	45.04
45,X / 46,XY	12	10.81
45,X / 46,XX / 47, XXX	11	9.90
45,X / 46, X (r)X	6	5.40
46, X, i (Xq)	6	5.40
45,X / 47, XXX	5	4.50
45,X / 46, X, i (Xq)	5	4.50
45, X / 46, XX / 47, XXX / 48, XXXX	3	2.70
45, X / 46, X (r)Y	2	1.80
45, X / 46, X, del (Xq)	1	0.90
45, X / 46, XX / 48, XXXX	1	0.90
45, X / 46, X, del (Xp)	1	0.90
45, X / 46, X, r, i (Xq)	1	0.90
45, XX $\frac{D}{G}$, t (14q;21q)	1	0.90
45, X, inv (16) (p12.2q11.1) / 46, XX, inv (16) (p12.2q11.1)	1	0.90
45, X, inv (9) (p11q13)	1	0.90
45, X / 46, X, tas (X:X) / (p22.3; p22.3)	1	0.90
46, X, iX	1	0.90
46, XX, del (Xq)	1	0.90
Unspecific karyotype	1	0.90
Total	111	

* *Data obtained from the patients' documents that underwent karyotyping*

* *Pts: Patients*

The reasons for referral were identified and reported in this study. A high proportion of patients presented with primary amenorrhoea, accounting for 45.45% of cases of TS; short-stature (9.09%); pre-marriage counseling (3.8%); pre-pregnancy counseling (3.3%); and all other reasons for presenting, each with a proportion of <3%, included: recurrent miscarriage; ambiguous genitalia; webbed neck; infertility; irregular menstruation; chromosomal abnormalities; lymphoedema; post-marriage counselling; lack of sexual characteristics; premature ovarian failure; intellectual disability; and hypothyroidism (Table 2).

Table 2: The reason of referring to the physician (n=209)

The reason for referring to the doctor	*Primary amenorrhea*	*Turner syndrome*	*Short stature*	*Pre-marriage counseling*	*Pre-pregnancy counseling*	*Recurrent miscarriage*	*ambiguous genitalia*	*Webbed neck*	*Infertile*	*irregular menstruation*	*chromosomal abnormalities*	*Lymphedema*	*Post-marriage counseling*	*Lack of sexual characteristics*	*Premature ovarian failure*	*intellectual disability*	*hypothyroidism*
Number of pts*	95	52	19	8	7	6	6	3	2	2	2	2	1	1	1	1	1
Percentage	45.45	24.8	9.09	3.8	3.3	2.8	2.8	1.43	0.95	0.95	0.95	0.95	0.47	0.47	0.47	0.47	0.47

* *Pts: Patients*

Other factors also extracted from the patients' documents, relating to the study, included: order of children (Table 3); patient's age when referred to the physician (Table 4); maternal age at conception and its correlation with TS (Table 5); and paternal age at conception and its correlation with TS (Table 6). Our data suggested that maternal age (59.26%) and paternal age (78.89%) of over 30 yr old at conception increased the risk of conceiving a child with TS (Table 5 and 6).

Table 3: The order of children (n=140)

The order of Children	*First*	*Second*	*Third*	*Fourth*
Number of pts*	61	51	20	8
Percentage	43.57	36.43	14.29	5.71

* *Data obtained from the patients' documents that underwent karyotyping*

* *Pts: Patients*

Table 4: Patient's age by referring to physician (n=36)

Patients' age (Years)	*0-4*	*5-9*	*10-14*	*15-19*	*20-24*	*25-29*	*30-34*	*35-39*	*40-44*	*45-49*	*50>*
Number of pts*	5	0	2	4	3	7	5	7	2	1	0
Percentage	13.8	0	5.5	11.11	16.6	19.4	13.8	19.4	5.5	2.7	0

* *Pts: Patients*

Table 5: Maternal age and its correlation with TS (n=108)

Age	*<20*	*20-30*	*>30*
Number of Patients with TS	9	35	64
Percentage	8.33	32.41	59.26

Table 6: Paternal age and its correlation with TS (n=90)

Age	*<20*	*20-30*	*>30*
Number of pts with TS*	1	18	71
Percentage	1.11	20	78.89

**Pts: Patients*

The UPGMA dendrogram was constructed based on the sex of the studied samples, female and male individuals intermixed in the different clusters. Investigated karyotypes have no association with the sex of the studied samples, and marriage type, identifying the presence/absence of consanguinity and its correlation (Table 7), have all been documented. Overall, 55 patients out of 144 had parents in consanguineous marriages (Table 7). Sixty-one individuals out of 140 were identified to be the first-born child, accounting for 43.57% of all the patients in the study (Table 3). However, no significant association was found between the order of the child and TS occurrence.

Table 7: Marriage (n=144)

Marriage Type	*Consanguineous*	*Non-consanguineous*
Number of pts*	55	88
Percentage	38.46	61.54
Percentage of Iranian Data (49)*	38.60	61.40

* *Type (consanguineous and non-consanguineous in patients compared to previous study done on Iranian population (49)*

* *Pts: Patients*

Moreover, in this study, an association between the patient's gender and TS karyotype (45,X or 46,XY) incidence was studied. However, no association was seen between the sex and the 45,X or 46,XY TS karyotype with the Chi-squared test. Cluster analysis of the sex and 45,X or 46,XY TS karyotype using average methods and the dendrogram showed no separate cluster for males or females with 45,X or 46,XY TS karyotype, which confirms that there is no correlation between gender and 45,X or 46,XY TS karyotype incidence (Fig. 1).

Discussion

This study is valuable due to the high-volume number of patients recruited (n=261) and the extended time period over which the study was carried out, from 1984 to 2018, running for over 35 years. These factors make this study the first of its kind to be carried out in on Iranian. Furthermore, the clinic in which the study was carried out is a reputable and well-known specialist clinic for genetic disorders and TS.

Chromosomal abnormality is the main cause of lack of development of secondary sexual characteristics, delayed puberty, miscarriage, and infertility in TS patients. The abnormalities identified include chromosomal deletions, duplications and translocations (12). Most patients with TS are not aware of their chromosomal abnormalities until primary symptoms arise. Nearly half of the patients referred to the genetic clinic with TS presented with complaints of primary amenorrhea. Another study conducted on 103 TS cases showed that TS patients with monosomy karyotype have delayed first menstrual bleeding and, on average, a higher BMI compare to other types of TS karyotypes (13). In this study, 38.46% of the patients with TS were born to parents in consanguineous marriages.

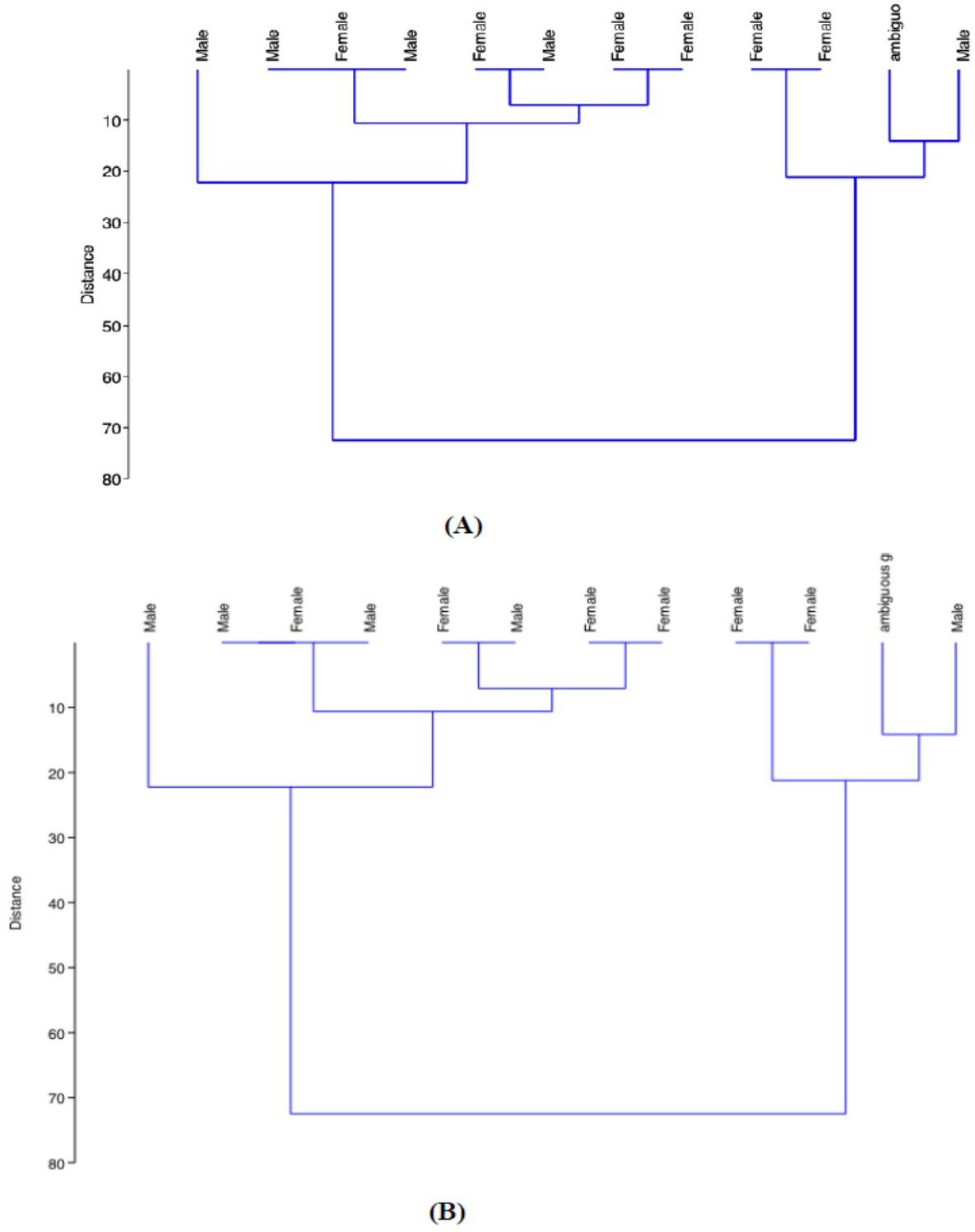

Fig. 1: Turner 45,X and 46,XY karyotype UPGMA dendrogram based on gender. (A) 45,X turner karyotype UPGMA dendrogram; (B) 46,XY turner karyotype UPGMA dendrogram

According to this study, 150 patients (57.47%) had the 45,X classic karyotype, compared to 57.1% and 74.3% identified in studies in Kuwait and Singapore, respectively (14, 15). 111 patients (42.53%) in the study had either mosaicism or another less common form of TS karyotyping, compared to studies reporting 36%, 45%, 32%, 42%, 28.6%, and 24% in America, Denmark, Tunisia, Minnesota, Brazil, and Czechoslovakia, respectively (16-21). Seven patients out of 19 (37%) were a single cell 45,X cell line and 63% of the patients were mosaic for TS. This study also commented on complementary and alternative investigations, consistent high-quality investigative methods and specialist input should not be overlooked when dealing with patient referrals to the cytogenetic clinic (12).

The results of Table 8 (22-32) from studies done in Metabolism and Endocrine Research Centers in Isfahan Medical University, Genetic enter of Urmia University, Medical Genetic group of Mashhad University, and Genetic group of Iran Cancer Research Center at Tehran Medical University on TS mosaic were 37.5%, 41.7%, 19%, and 25.81%, respectively (15, 33-35). A relevant study carried out at the endocrinology unit in Italy showed that the risk of diabetes mellitus in TS patients is significantly higher compared to the normal female population (36). In Iran looking at the overall types of marriages in the country reported rates of 38.60% consanguineous marriages and 61.40% non-consanguineous marriages among the general population in Iran (37, 38). Previous studies in Iran have shown that the risk of TS in consanguineous marriage is 36.52% (39).

In this study, of the fetuses identified to have TS, a maternal age range of 15-25 yr old carried 30.77% of the cases and that the maternal age range of 26-35 yr old carried 69.23% of the cases. From this data, there exists a relationship between increased maternal age and the likelihood of TS (38). A study conducted (37) on maternal age and its association with TS showed a significant relationship between maternal age and isochromosomal TS. Moreover, in mothers over 40, the probability of giving birth to TS girls is higher (3.2%) than the general population (1.8%) (39).

This study found a correlation between the increasing age of the father at the time of conception and the increasing number of females born with TS. Overall, 30 cases (20%) of the females with TS were born to fathers of 15-25 yr of age, and 120 cases (80%) of the females with TS were born to fathers of 26-35 yr of age. The direct correlation between paternal age and incidence of TS was analyzed and determined using the Pearson correlation coefficient (Table 6). However, no association was found between the patient's gender and TS. An interesting research was carried out on 35 females with TS, highlighted the differences in the prevalence of various dysmorphic features and a strong association between monosomy 45, X and phenotypic patterns including the more severe phenotype, lymphatic phenotype, and skeletal phenotype. The study also identified associations between the lymphatic phenotype and the presence of aortic coarctation in patients with TS.

In contrast, with recent reports, the study found that there was no association between patient karyotype and cardio-aortic malformations (40). In this study, 12 cases of TS Mosaicism were associated with the 45,X /46,XY karyotype (Table 9), which, with other supplementary tests, were determined according to each cell category. This study confirmed the associations between factors including maternal and Paternal age at pregnancy, familial history of the disease, consanguinity in marriage and incidence of TS.

Table 8: Present the internal and external studies

Type	*City/Country*	*Patterns found*	*SP*	*NOS*	*Year*
National Stidies	Mashhad	50% of cases were 45X, 22.5% mosaic and 15% iso-chromosome (34)	7	40	2003
	Tehran	The karyotype 45 X (full monosomal X) was the most commonly observed karyotype (111 cases, 45.7%), then karyotypes 46XX / 45X (33 cases,13.6%) isoXq, XX46 / 45X (25 cases, 3.10%) and isoXq / 45X (23 cases,9.5%). 12 patients (4.9%) with cell line, 46XY. (23)	30	243	2004
	Tehran	These patients were referred to the Endocrinology and Metabolism Institute (24)	1	35	2005
	Iran	Of the patients studied, 37.5% had a classical karyo-type of 45% and 62.5%of the remaining mosaic or structural disorders in the second sex chromosome (25)	5	40	2006
External Studies	Sweden	44%exhibited a 45,X karyotype and56% a second-cell line, while 27% of all hada45,X/46,XX mosai-cism. Five 45,X cases with a conventional karyotype were 45,X/46,XX mosaic according to FISH (26)	-	126	2007
	Denmark	45,X (n=64); 45,X/46,XX (n=8); karyotypes with isochromosomes (IsoXq) (n=23) and deletions (n=2); karyotypes with Y chromosomal material (n=5); and karyotypes with marker or ring chromo-somes (n = 5) (27)	-	107	2009
	Brazil	25.6% of patients presented CV abnormalities which 21.4%, 19% and 19% of them suffered from mitral regurgitation, bicuspid aortic valve and aortic coarc-tation, respectively. (28)	17	130	2010
	Sweden	Women who gave birth to girls with Turner syn-drome: maternal and neonatal characteristics (40)	39	-	2010
	Portugal	Karyotype: X monosomy-37/2%, mosaicism-37/2%, X structural changes-25/6%. Median FSH of 59/05 mIU/ mL (29)	-	79	2013
	Italy	X-monosomy (77.5)X mosaicism with structural ab-normalities of the second X (7.5)X-mosaicism with-out structural abnormalities of the second X (12.5) Structural abnormalities of the second X (2.5) (30)	1	40	2014
	Albania	The most frequent pathologies are thyroid autoim-mune disorders, cardiovascular anomalies, renal pa-thologies, hearing impairment and hypertension, 59%, 43%, 41%, 4.3% and 3,3% (31)	5	52	2015
	Sweden	64 women with TS between 25-38 yr old. 52% suf-fering from impaired hearing in at least one ear. (32)	-	64	2017

**Keys: NOS: number of samples, SP: study period in year*

Table 9: 45,X/46,XY mosaicisms cases (n=12)

Row	*Apparent symptoms*	*Sex*	*45, X (%)*	*46, XY (%)*
1	Blind	Male	75	25
2	-	Male	20	80
3	-	Female	70	30
4	Short stature	Female	30	70
5	Cryptorchidism	Male	60	40
6	Sexual ambiguity	Male	80	20
7	Primary amenorrhea	Female	30	70
8	Primary amenorrhea - Not growing	Female	75	25
9	Sexual ambiguity	Female	80	20
10	Low Growth	Male	80	20
11	Sexual ambiguity genitalia	ambiguous genitalia	10	90
12	Primary amenorrhea	Female	70	30

**Data obtained from the patient's documents that underwent karyotyping*

Conclusion

TS is one of the most common chromosomal disorders worldwide and prevalence is increasing yearly. Therefore, as expectedly, there is a continuous stream of referrals being made to specialist genetic clinics running cytogenetic tests. Diagnosis of TS at an earlier stage would be beneficial to the patient to allow identification of the presence of parallel disease and its treatment or prevention. Prenatal screening decreases the number of patients born with complex and potentially fatal genetic diseases.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declared no conflict of interest.

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