Clinical impact of parental consanguineous marriage in idiopathic nonobstructive azoospermia

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Objective: To determine the frequency of parental consanguineous marriages (PCMs) in men with diagnosed idiopathic nonobstructive azoospermia (INOA) and to compare clinical and pathological parameters between azoospermic men with and without PCM.
Design: Retrospective.
Setting: A private clinic.
Patient(s): Two hundred forty-six men with INOA. Patients were divided into two groups: group 1 with PCM and group 2 without PCM. Clinical parameters, surgical sperm retrieval rates, and pathological findings were compared between the groups.
Intervention(s): Surgical sperm retrieval.
Main Outcome Measure(s): PCM and clinical parameters.
Result(s): Among the 246 patients with INOA, 81 had PCM. Men with PCM had lower follicle-stimulating hormone (13.7 vs. 21.9 mIU/mL), higher testosterone (3.8 vs. 3.4 ng/mL), and larger testes (14.1 vs. 11.8 mL). In parallel with the clinical findings, the most common pathological pattern in men with PCM was maturation arrest. However, there was no difference in surgical sperm retrieval rate between men with (23.4%) and without (32.1%) PCM.
Conclusion(s): Our data showed that PCM was present for 33% of men with INOA. The clinical parameters of men with PCM and INOA were significantly different than those without PCM, primarily demonstrating maturation arrest in testicular pathology. Further genetic research in families who have infertile male siblings may elucidate underlying rare genetic abnormalities in spermatogenesis. (Fertil Steril Rep® 2020;1:209–12. ©2020 by American Society for Reproductive Medicine.)
Key Words: Idiopathic nonobstructive azoospermia, male infertility, parental consanguineous marriage

Azoospermia is the absence of sperm on microscopic evaluation of two consecutive centrifuged semen samples (1). Azoospermia is found in 1% of all men and up to 15% of infertile men (2). Men with azoospermia should be evaluated by a complete medical history and physical examination, along with measurements of follicle-stimulating hormone (FSH) and total testosterone to evaluate hormonal status and determine whether the cause is obstructive or nonobstructive. If testicular spermatogenetic failure is suspected, which is termed "nonobstructive azoospermia" (NOA), genetic testing is recommended (3). Up to 20% of men with testicular spermatogenetic failure have an identifiable chromosomal numerical abnormality (e.g., Klinefelter's syndrome), structural abnormalities (e.g., translocations and inversions), or Y chromosomal microdeletion abnormalities. However, most azoospermic men with NOA lack any detectable genetic disorders and are given a diagnosis of idiopathic azoospermia (4).

The term "consanguinity" refers to a relationship between two people who share a common ancestor or blood. The Middle East has high rates of consanguineous marriages, with overall rates reported at 20% to 50% and up to 80% in certain regions (5). Most consanguine marriages are between first cousins. Sharing a common genetic pool not only increases the risk of homozygotes for autosomal recessive genetic disorders but also appears to have an adverse effect on both male and female gametes (6). The prevalence of male factor infertility in Middle Eastern countries is thought to be

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increased compared with Western countries due to the high rate of consanguineous marriages (5, 7). In a study of 120 infertile and 100 fertile men in Lebanon, the rate of parental consanguineous marriage (PCM) and family clustering in azoospermic and severe oligospermic men ($<1 \times 10^6$ sperm/mL) was found to be 50% and 41%, respectively (8). Severe male factor infertility clustered among the men in these families, suggesting that a strong genetic transmission from their parents underlies spermatogenetic failure. In this study, we aimed to determine the frequency of PCM in men with diagnosed idiopathic NOA (INOA) and to compare clinical, surgical, and pathological parameters between azoospermic men with and without PCM.

MATERIALS AND METHODS

Data of men with azoospermia due to spermatogenetic failure who applied to our outpatient clinic for infertility between November 2014 and June 2018 were retrospectively analyzed. All patients were evaluated by medical history and genital physical examination. Testis volume was measured by testis orchidometer. All patients received serum FSH and total testosterone measurements along with genetic evaluations for karyotype and Y chromosome microdeletions. Azoospermic patients who were found to have obstructive causes, such as congenital vas agenesis or ejaculatory duct obstruction due to prostate cysts, or history of epididymitis or orchitis were excluded from the study. Patients were also excluded from the study if they had Y chromosome microdeletions, chromosomal abnormalities such as Klinefelter syndrome, or other structural chromosomal abnormalities, a history of cryptorchidism or cancer treatment with chemotherapy and/or radiotherapy, or hypogonadotropic hypogonadism. A total of 246 NOA patients with no known etiology were included in this study. The presence of PCM was based on marriage between first (children of the aunt or uncle) and second (children of parental cousins) cousins.

All patients underwent microdissection testicular sperm extraction (micro-TESE) operation for surgical sperm retrieval from testicular tissue. Testicular tissue was evaluated by an embryologist during the operation, and the presence of spermatozoa was reported. If no spermatozoa were detected in one testis, the contralateral testis was evaluated using micro-TESE. In patients who had no sperm recovery during the micro-TESE operation, a random biopsy was taken from each testis for pathological evaluation. Testicular biopsies were collected from a total of 138 patients with no sperm recovery during the micro-TESE operation. All biopsy samples were immediately fixed in Bouin's solution. Each testicular biopsy was categorized as having hypospermatogenesis, maturation arrest, or Sertoli cell–only syndrome according to the most advanced pattern of spermatogenesis on histological examination. Hypospermatogenesis is defined by the presence of a few spermatozoa in any tubule. Maturation arrest is used to describe patterns, whereby there is interruption of spermatogenesis to varying degrees in the seminiferous tubules. Sertoli cell–only patterns are defined by the absence of any germ cell within the seminiferous tubules.

Patients were divided into two groups according to PCM status and compared according to age, FSH, total testosterone, infertility duration, testicular volume, surgical sperm retrieval rates, and pathological findings. Statistically significant differences by univariate analysis between the two groups were investigated using Student's t test and χ^2 test. The data were analyzed using the Statistical Package for the Social Sciences version. $P < .05$ was considered statistically significant. This study was approved by Istanbul Bilim University Clinical Research Ethical Committee (no, 44140529/2018-10607).

RESULTS

In our cohort, 81 of 246 (33%, 95% confidence interval 0.271–0.389) men with INOA have PCM. In the PCM group, the rate of surgical sperm retrieval (23.4%, 19/81) was lower than those without PCM (32.1%, 53/165), but the difference did not reach statistical significance ($P = .18$). Clinical characteristics, mean age of the patients, and fertility duration were similar between the groups. However, testis volume (14.1 vs. 11.8 mL, $P = .0008$) and total testosterone level (3.8 vs. 3.4 ng/mL, $P = .02$) were significantly higher, while FSH levels (13.7 vs. 21.9 mIU/mL, $P = .0001$) were significantly lower in men with parental consanguinity (group 1; Table 1).

The distribution of testicular pathology in group 1 (n = 50) was as follows: hypospermatogenesis in two (4%), maturation arrest in 33 (66%), and Sertoli cell–only syndrome in 15 (30%) patients. In group 2 (n = 88), one patient had hypospermatogenesis (1.1%), 19 patients had maturation arrest (21.6%), and 68 patients had Sertoli cell–only syndrome (77.3%, $P < .00001$, Table 2).

TABLE 1

Comparison of clinical characteristics and surgical sperm retrieval rates between the groups 1 and 2.

Characteristic	All patients	Group 1 (%)	Group 2 (%)	*P* value
n	246	81 (33)	165 (67)	
Age, y	33.65 ± 5.6	33.2 ± 4.6	33.7 ± 5.9	.50
Infertility duration, y	5.8 ± 4.4	5.5 ± 4.4	6.0 ± 4.6	.41
Testis volume, mL	11.6 ± 5.0	14.1 ± 5.2	11.8 ± 4.9	.0008
FSH, mIU/mL	19.8 ± 12.9	13.7 ± 9.4	21.9 ± 13.9	.0001
Total T, ng/mL	3.65 ± 1.5	3.8 ± 1.35	3.4 ± 1.4	.02
SSR rate, n (%)	72/246 (29.3)	19/81 (23.4)	53/165 (32.1)	.18

Note: Data presented as mean standard deviation, unless noted otherwise. FSH = follicle-stimulating hormone; SSR = surgical sperm retrieval; T = testosterone.

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In total, 91 of 246 (37%) of the full cohort, including 36 of 81 (44.4%) men in group 1 and 55 of 165 (33.3%) men in group 2, had undergone previous micro-TESE operation in another center without successful sperm recovery. The distribution of patients with previous micro-TESE history was similar between the groups ($P = .09$). In the second micro-TESE operation attempt at our center, spermatozoa were successfully recovered in three (8.3%) and nine (16.3%) men in groups 1 and 2, respectively ($P = .35$). In group 1, 16 (19.7%) men had infertile siblings in their family compared with 11 (6.6%) men in group 2 ($P = .0039$).

DISCUSSION

The frequency of parental consanguinity was found to be 33% (95% confidence interval, 0.271–0.389) among our cohort of men with INOA. In Turkey, the rate of consanguineous marriage was found to be 24% in the general population (9). In the present study, the data indicate that the rate of PCM was higher in men with diagnosed INOA compared with the frequency of consanguineous marriage in the general population. Supporting our data, Inhorn et al. (8) reported that the rate of PCM was found to be 29.2% among men with severe oligozoospermia and azoospermia in the Lebanese population. In another study of 62 infertile men with a family history of consanguinity, severe sperm defects including the tail defect "stunted tail" and the acrosome defect "round head" were detected in 27% of the cohort (10). A recent study suggested that severe forms of male factor infertility may be more frequent in Eastern and Muslim populations, in which the practice of consanguineous marriage is very high compared with Western countries (5). Ethnical and sociocultural differences across Middle Eastern countries may contribute to regional variations in the frequency and the severity of male factor infertility compared with other parts of the world.

In this study, we identified differences in clinical parameters of patients with consanguineous parents compared with those without. Among this cohort with INOA, FSH levels were lower but testis volume and total testosterone levels were higher in men with PCM compared with men without PCM. In conjunction with the clinical parameters, pathological analysis showed a significantly higher incidence of maturation arrest patterns in men with consanguinity. Maturation arrest is defined as an absence of mature spermatozoa and is categorized into early and late maturation arrest, based on the most mature cell type in seminiferous tubules. Follicle-stimulating hormone has been shown to be the most useful variable for evaluating the severity of maturation arrest, and normal gonadal hormone concentrations in patients with maturation arrest suggest an abnormality outside the hypothalamo-pituitary-testicular axis (11). Beyond these pathological findings, immunofluorescence analyses of specific makers are used to determine meiotic arrest types in human spermatogenesis (12). Metaphase arrest was found to be the most frequent type of meiotic arrest, and uniform activation of meiotic checkpoints suggests a genetic cause of spermatogenic arrest. To investigate the basis of genetic abnormalities in INOA, whole exome sequencing was recently conducted on consanguineous families who have more than one azoospermic and/or oligozoospermic sibling. A mutation in the *TEX15* gene, which may be related to maturation arrest at the primary spermatocyte stage, was found with exome sequencing in one of three brothers who are all infertile and born into a consanguineous family (13). In another genetic analysis using the same technique, a novel homozygous mutation of the *GTF2H3* gene was identified in two azoospermic men with a history of PCM. Pathological examination of one of these men showed maturation arrest (14). In this study, the rate of infertile siblings in consanguineous families was significantly higher compared with families without consanguinity. Therefore, analysis of large families with well-documented male factor infertility and some degree of consanguinity may be an approach to identify rare genetic defects, especially those involved in spermatogenesis. Future efforts to understand the genetic basis of spermatogenesis may suggest new targeted treatment modalities as an alternative to surgical approaches and may be used to predict mature sperm production in testis of men with spermatogenetic failure.

The micro-TESE results in this study showed that surgical sperm retrieval was lower in men with PCM but did not reach statistical significance. In total, 72 of 246 (29.3%) men underwent successful surgical sperm retrieval by micro-TESE operation. These surgical sperm retrieval rates were lower than those reported in the literature (15, 16). One reason for the low rate of sperm recovery is that 91 of 246 (37%) patients had a repeated micro-TESE operation after a failed micro-TESE attempt (at least 6 months before) in another institution. Another reason may be that our study excluded known causes of azoospermia such as undescended testis or genetic abnormalities (Klinefelter syndrome) that have higher sperm recovery outcomes with micro-TESE.

TABLE 2

Comparison of groups 1 and 2 according to pathological results.

Pathological diagnosis	Group 1	Group 2	*P* value
n	50	88	
Sertoli cell only	15 (30)	68 (77.3)	< .00001
Maturation arrest	33 (66)	19 (21.6)	
Hypospermatogenesis	2 (4)	1 (1.1)	

Note: Data presented as n (%), unless noted otherwise. Group 1: patients with a parental consanguineous marriage; group 2: patients with no parental consanguinity.

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CONCLUSION

In our INOA cohort, 33% of the men had PCM. Testis volume and total testosterone levels were higher while serum FSH levels were lower in INOA patients with parental consanguinity compared with those no parental consanguinity. Pathological findings revealed that most of the men with consanguinity have a maturation arrest pattern. To better understand the genetic causes of male factor infertility, further research efforts on consanguineous families should be undertaken to elucidate features of spermatogenesis.

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