Original Article

Evaluation of Azoospermic Patients to Distinguish Obstructive from Non-Obstructive Azoospermia, and Necessity of Diagnostic Testis Biopsy: A Retrospective Study

Iman Shamohammadi, M.D.[1, 2], Mohammad Ali Sadighi Gilani, M.D.[1, 3*], Seyed Mohammad Kazemeyni, M.D.[1], Tara Hasanzadeh, M.D.[2], Ahmad Vosough Taqi Dizaj, M.D.[3], Alireza Dizavi, M.Sc.[3]

1. Department of Urology, Shariati Hospital, Tehran University of Medical Science, Tehran, Iran
2. Department of Urology, Nemazee Hospital, Shiraz University of Medical Science, Shiraz, Iran
3. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Background: Accurate etiology of azoospermia is required for optimal management of patients. The aim of this study was the determination of serum hormonal levels and testicular long axis cut off points to distinguish obstructive azoospermia (OA) from non-OA (NOA) in Iranian patients as well as the evaluation of the necessity of diagnostic testis biopsy in azoospermic patients.

Materials and Methods: In this retrospective study, data of 471 azoospermic patients such as history and physical examination, serum hormonal level, semen fluid parameter, and testicular long axis based on ultrasound were evaluated from 2016 to 2020. All patients were examined by a single urologist and underwent a diagnostic testis biopsy for a definite diagnosis. The diagnostic parameters were analyzed using Statistical Package for the Social Sciences (SPSS) version 16 with t test and chi-square test and receiver operating characteristic (ROC) curves to distinguish NOA from OA.

Results: A total of 127 patients with OA and 284 with NOA were included in this study. The mean serum testosterone level was significantly higher in OA than NOA (4.2 vs. 3.4 ng/ml), whereas the mean serum follicular stimulating hormone (FSH, 5.3 vs. 19.1 mIU/ml) and luteinizing hormone (LH, 5.3 vs. 11 mIU/ml) were lower in OA. ROC curve analysis showed that FSH and testicular long axis were the best diagnostic predictors. Using a combination of serum FSH (8.9 mIU/ml) and testicular long axis (39 mm), the positive predictive value for NOA was 97.02% and for OA was 78.8%.

Conclusion: Combination of serum FSH higher than 8.9 mIU/ml and testicular long axis lower than 39 mm were strong predictors to distinguish NOA from OA in Iranian participants in this study. In addition, diagnostic testicular biopsy seems to be necessary for patients with OA and NOA characteristics.

Keywords: Biopsy, Nonobstructive Azoospermia, Obstructive Azoospermia

Citation: **Shamohammadi I, Sadighi Gilani MA, Kazemeyni SM, Hasanzadeh T, Vosough Taqi Dizaj A, Dizavi A. Evaluation of azoospermic patients to distinguish obstructive from non-obstructive azoospermia, and necessity of diagnostic testis biopsy: a retrospective study. Int J Fertil Steril. 2022; 16(3): 156-161. doi: 10.22074/IJFS.2021.532258.1140.**

Introduction

Infertility is one of the major medical problems in the world (1). The prevalence of male infertility is unknown due to conflicting reports (2). One cause of male infertility is azoospermia, which is defined as a lack of sperm in semen analysis after centrifugation (3) and is confirmed by two consecutive semen tests (4). The prevalence of azoospermia is about 1 % in the general male population (5) and approximately 10% of infertile males (6). Azoospermia divides into two types, including obstructive azoospermia (OA) and non-OA (NOA) (7). About 40% of the azoospermic patient are in the OA group, which occurs secondary to a physical obstruction in sperm transfer from the testis to the urethra (8, 9). About 60% of azoospermic patients are in the NOA group, which occurs secondary to testicular failure in sperm production (10, 11). Accurate etiology of azoospermia is required for optimal management of patients (12). Sperm retrieval in NOA is done by microdissection testicular sperm extraction (mTESE) (13). OA is usually corrected by reconstructive microsurgery (14). There are several clinical and laboratory differences between OA and NOA that reduce the role of diagnostic testis biopsy

Received: 16/June/2021, Accepted: 29/November/2021
***Corresponding Address: P.O.Box: 1411713135, Department of Urology, Shariati Hospital, Tehran University of Medical Science, Tehran, Iran**
Email: masadighi@gmail.com

Royan Institute
International Journal of Fertility and Sterility
Vol 16, No 3, July-September 2022, Pages: 156-161

(15). According to studies, a combination of testicular long axis with a cutoff point of 4.6 cm and serum FSH with a cutoff point of 7.6 mIU/mL could predict the type of azoospermia with high sensitivity and specificity (16). However, those levels of the published data regarding the differentiation between OA and NOA weren't applicable in our population. Therefore, we decided to conduct a study with the aim of determining serum hormonal level and testicular long axis cut off points to distinguish OA from NOA in Iranian patients. The necessity of diagnostic testis biopsy azoospermic patients was also evaluated in this study.

Materials and Methods

Patients

In this retrospective study, from 2016 to 2020, a total of 471 azoospermic patients were evaluated. The present study is approved by the Ethics Committee of Tehran University of Medical Sciences with the code of ethics (IR.TUMS.MEDICINE.REC.1399.1005). All male participants in this study were examined by a single urologist and underwent a diagnostic testes biopsy as well as testis ultrasound by a single radiologist. 27 patients with a diagnosis of Klinefelter syndrome, 9 patients with a diagnosis of hypogonadotropic hypogonadism and 24 patients due to incomplete file information were excluded from this study. Finally, 411 azoospermic patients were evaluated, including 284 NOA and 127 OA. Data of patients include age, height, weight, history of herniorrhaphy, history of vasectomy, history of varicocelectomy, history of epididymo-orchitis or urinary tract infection, history of cryptorchidism and orchiopexy, testicular volume estimation with orchidometer, palpation of the vas, testicular long axis based on ultrasound, serum FSH level, serum LH level, serum testosterone level, semen fluid analysis report for fructose, pH and volume, final testicular pathology report and microscopic report during testis biopsy were extracted from the files and were entered into a checklist.

Statistical analysis

The collected data was analyzed using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 16, with t-test and chi-square test. $P<0.05$ were considered statistically significant. The receiver operating characteristic (ROC) curve (17) was also used to determine the appropriate cutoff points for the hormonal profile and testicular long axis to distinguish NOA from OA. Accuracy was assessed by the area under the ROC curve (AUC), and results were considered to be excellent (AUC 0.9-1), good (AUC 0.8-0.9), fair (AUC 0.7-0.8), poor (AUC 0.6-0.7) and failed (AUC 0.5-0.6).

Results

In this study, 411 azoospermic patients were analyzed. All of them underwent diagnostic testis biopsy. 284 cases (69.1%) were NOA and 127 cases (30.9%) were OA. Demographic information, history, and physical examination, testicular long axis based on ultrasound, hormonal profile, and semen fluid analysis are compared between NOA and OA in Table 1.

Table 1: Comparison of demographic and testis ultrasound and laboratory data between OA and NOA

Baseline characteristics of patients	OA	NOA	P value*
Patients (%)	127 (30.9)	284 (69.1)	
Age (Y)	33.10 ± 6.22	31.91 ± 5.03	0.06
BMI (kg/m^2)	26.70 ± 3.80	26.17 ± 4.80	0.24
Genitourinary tract infection	1 (0.7)	4 (1.4)	0.17
Epididymitis history	5 (3.9)	3 (1.1)	0.051
Unilateral UDT and orchiopexy	8 (6.2)	20 (7)	0.53
Bilateral UDT and orchiopexy	5 (3.9)	12 (4.2)	0.57
Unilateral herniorrahphy	10 (7.9)	18 (6.3)	0.57
Bilateral herniorrahphy	4 (3.1)	6 (2.1)	0.53
Unilateral varicocelectomy	15 (11.8)	33 (11.6)	0.96
Bilateral varicocelectomy	7 (5.5)	11 (3.9)	0.45
Bilateral vasectomy	4 (3.1)	0 (0.0)	0.003
At least one palpable vas	103 (81.1)	283 (99.6)	0.0001
Testis volume in P/E (ml)	19.06 ± 4.50	10.50 ± 5.45	0.0001
Testis length in P/E (cm)	3.89 ± 0.38	2.78 ± 1.13	0.0001
Testis longitudinal axis in ultrasound (mm)	39.83 ± 3.36	33.05 ± 6.00	0.0001
Volume of semen fluid (ml)	1.66 ± 1.51	2.85 ± 1.53	0.0001
PH of semen fluid	7.08 ± 0.61	7.72 ± 0.23	0.0001
Fructose of semen fluid (mg/dl)	77.04 ± 114.80	202.02 ± 91.50	0.0001
Serum LH (mIU/ml)	5.33 ± 3.05	11.05 ± 6.16	0.0001
Serum FSH (mIU/ml)	5.30 ± 4.11	19.11 ± 10.61	0.0001
Serum testosterone (ng/ml)	4.26 ± 1.86	3.40 ± 1.75	0.0001

Data are presented as mean ± SD or n (%). OA; Obstructive azoospermia, NOA; Non-obstructive azoospermia, BMI; Body mass index, UDT; Undescended testis, P/E; Physical examination, LH; Luteinizing hormone, FSH; Follicular stimulating hormone, and *; Analysed with t test or chi-square.

OA patients had a mean age (33.1 years) and BMI (26.7). NOA patients had a mean age (31.9 years) and BMI (26.1). There were no significant differences between the two groups about age, BMI, history of urinary tract infections and epididymo-orchitis, history of UDT and orchiopexy, history of varicocelectomy, and herniorrhaphy ($P>0.05$). Only 4 out of 127 OA patients had a history of vasectomy. Clinical examination findings including at least one palpable vas, testis size, testis volume, and also the testicular long axis base on

ultrasound were significant differences between the two groups (P<0.05). OA patients had significantly greater testis size than NOA patients (P<0.05). The mean serum testosterone level in OA (4.26 ng/ml) was significantly more than NOA (3.40 ng/ml). The mean serum FSH level in OA (5.3 mIU/ml) was significantly lower than NOA (19.11 mIU/ml), and similarly, the mean serum LH level in OA was significantly lower than NOA (P<0.05). Semen fluid analysis findings including volume, pH and fructose in OA were significantly lower than NOA (P<0.05). ROC curve analysis was performed to determine cutoff points for differentiation between OA and NOA. Figure 1 shows the ROC curve for serum FSH, LH, testosterone level, and testicular long axis.

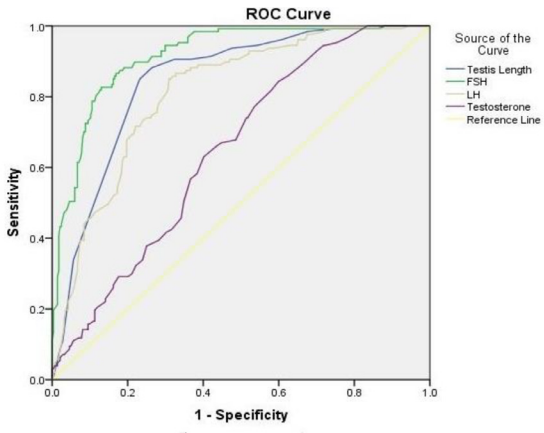

Fig.1: ROC curve for serum hormonal level and testicular long axis. Testis length (0.85), FSH (0.92), LH (0.81), and testosterone (0.65). ROC; Receiver operating characteristic, FSH; Follicular stimulating hormone, and LH; Luteinizing hormone.

According to Figure 1, serum FSH with a curved area (0.92), the testicular long axis with a curved area (0.85) and serum LH with a curved area (0.81) had excellent accuracy for differentiating OA from NOA, but serum testosterone with a curve area (0.64) had poor accuracy for differentiating OA from NOA. Based on the ROC curve by determining the cut off point 8.9 mIU/ml for serum FSH with the sensitivity of 85%, specificity of 77%, the positive predictive value of 92%, the negative predictive value of 62%, accuracy of 80%, and determining the cut off point 39 mm for testicular long axis with the sensitivity of 83%, specificity of 87%, positive predictive value 93%, the negative predictive value of 70%, accuracy of 84% can differentiate between OA and NOA. According to Table 2, by combination cut off points of serum FSH and testicular long axis with the highest accuracy, 69% of NOA patients had FSH ≥8.9 mIU/ml & testicular long axis <39 mm, and 76% of OA patients had FSH<8.9 mIU/ml & testicular long axis ≥39 mm. These differences were significant (P<0.05).

Based on the ROC curve, a combination of serum FSH level and testicular long axis had sensitivity (88.28%), specificity (94.17%), positive predictive value (97.02%), negative predictive value (78.86%) and accuracy (90.15%) for differentiating NOA from OA. According to Table 3, we can predict that azoospermic patients with serum FSH ≥8.9 mIU/ml & testicular long axis <39 mm are in the NOA group (positive predictive value 97.02%), and azoospermic patients with FSH <8.9 mIU/ml and testicular long axis ≥39 mm are in OA group (positive predictive value 78.86%).

Table 2: Combining testis longitudinal axis size and FSH for differentiation NOA from OA

FSH and testis length of patients	OA	NOA	P value*
FSH<8.9 and testis length≥39 mm	97 (76.4)	26 (9.2)	0.0001
FSH<8.9 and testis length<39 mm	11 (8.7)	40 (14.1)	0.12
FSH≥8.9 and testis length≥39 mm	13 (10.2)	22 (7.7)	0.40
FSH≥8.9 and testis length<39 mm	6 (4.7)	196 (69)	0.0001

Data are presented as n (%). FSH; Follicular stimulating hormone, NOA; Non-obstructive azoospermia, OA; Obstructive azoospermia, and *; Analysed with chi-square.

Table 3: Positive predicting value for NOA or OA using testicular long axis and FSH

FSH and testis length of patients	OA	NOA	PPV for NOA	PPV for OA
FSH<8.9 and testis length≥39	97 (76.4)	26 (9.2)	-	78.86
FSH≥8.9 and testis length<39	6 (4.7)	196 (69)	97.02	-

Data are presented as n (%) or %. NOA; Non obstructive azoospermia, OA; Obstructive azoospermia, FSH; Follicular stimulating hormone, and PPV; Positive predictive value.

The final pathological reports of azoospermic patients are shown in Table 4. Sertoli cell-only syndrome pattern was the most common pathology in azoospermic patients (37%).

According to Table 4, normal spermatogenesis pattern in the group of azoospermic patients with FSH <8.9 mIU/ml and testicular long axis ≥39 mm, and Sertoli cell only syndrome pattern in the group of azoospermic patients with FSH ≥8.9 mIU/ml and testicular long axis <39 mm were most common pathological reports (P<0.05). Photomicrographs describing pathological reports are shown in Figure 2.

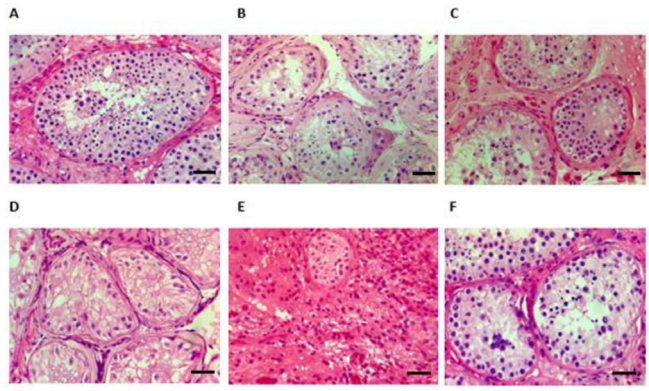

Fig.2: Photomicrographs pathological reports of testis biopsy. **A.** Normal spermatogenesis, **B.** Hypo spermatogenesis, **C.** Maturation arrest, **D.** Sertoli cell only syndrome, **E.** Lydig cell hyperplasia, and **F.** Mixed pattern (scale bar: 20 µm).

Table 4: Pathological report of testis biopsy specimen in azoospermic patients

Report of pathology	Azoospermic patients (n=411)	FSH<8.9 and testis length<39 (n=51)	FSH<8.9 and testis length≥39 (n=123)	FSH≥8.9 and testis length≥39 (n=35)	FSH≥8.9 and testis length<39 (n=202)
Normal spermatogenesis	127 (30.8)	11 (21.6)	97 (78.9)	13 (37.15)	6 (3)
Hypo spermatogenesis	29 (7.1)	5 (9.8)	4 (3.3)	6 (17.15)	14 (6.9)
Germ cell maturation arrest	62 (15.1)	9 (17.6)	15 (12.2)	7 (20)	31 (15.3)
Sertoli cell only syndrome	152 (37)	19 (37.3)	4 (3.3)	5 (14.3)	124 (61.4)
Lydig cell hyperplasia	12 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	12 (5.9)
Hypo spermatogenesis and maturation arrest (mixed)	29 (7.1)	7 (13.7)	3 (2.4)	4 (11.4)	15 (7.4)
P value*	0.0001	0.16	0.0001	0.015	0.0001

Data are presented as n (%). FSH; Follicular stimulating hormone and *; Analysed with chi-square.

Discussion

Azoospermia is one of the most common male infertility factors evaluated in various studies (18). Determination type of azoospermia helps urologists to treat patients appropriately. OA patients will undergo reconstructive surgery if possible. Otherwise, they will undergo sperm retrieval by testis biopsy for in vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI). NOA patients are candidates for sperm retrieval by testis biopsy (6, 19). If sperm was not found, they are candidates for sperm donation (20, 21). Several studies show that azoospermic patients can be correctly sorted into OA or NOA groups with multiple diagnostic parameters. Usually, testis volume in OA is normal, whereas in NOA is less than normal (7). The results of our study support this finding significantly. The average testis volume with an orchidometer in OA is 19.06 ml and in NOA is 10.5 ml. The average testicular long axis measured in ultrasound for OA is 39.83 mm and for NOA is 33.05 mm. A history of genital infections or epididymo-orchitis can obstruct an inflammatory process and the production of antibodies (22). However, in this study, a history of epididymo-orchitis or genitourinary system infections were no significant association with OA or NOA. The history of an undescended testis (UDT) and orchiopexy is a risk factor for azoospermia. In fact, UDT is associated with spermatogenic dysfunction (23), but findings in the present study show that the history of UDT and orchiopexy cannot differentiate OA from NOA. In the absence of bilateral vas deferens, evaluation of the CFTR panel for cystic fibrosis is recommended (24). Our finding similar to other studies show that lack of bilateral palpation of vas deferens is associated with OA significantly. In these patients spermatogenesis is normal and IVF/ICSI can be used for fertilization (25, 26). Usually, OA patients with ejaculatory ducts obstruction have semen analysis with features of low volume, low pH, and low fructose level. Trans-rectal ultrasound is helpful for the diagnosis of these patients (27). The results of this study also confirm that in OA patients compared to NOA, the mean semen parameters, including volume, pH, and fructose are significantly lower than normal. NOA includes primary testicular failure (high serum FSH with small testis size), secondary testicular failure (low serum FSH with small testis size), or incomplete features such as elevated FSH with normal testis, small testis with normal FSH, and normal testis with normal FSH. Maturation arrest histopathology is associated with normal serum FSH and normal testis size, while Sertoli cell only histopathology is associated with high serum FSH and small testis size (28). The results of our study also confirm this finding, since the most common pathology in NOA with normal serum FSH and normal testis size was maturation arrest and the most common pathology in NOA with high serum FSH and small testis size was Sertoli cell only.

According to Schoor et al. (16) study, differentiating with high accuracy between OA and NOA is possible by a combination of serum FSH and testis size. Their results showed that mean serum FSH, LH, and testosterone levels in NOA were significantly higher than OA. According to the results of this study, similar to Schoor's study data, the mean serum FSH and LH in NOA were significantly higher than OA, however, the mean serum testosterone in NOA was significantly lower than OA which is in contrast with Schoor's results. Based on the ROC curve in this study, similar to Schoor et al. (16) and Huang's study (29), serum FSH level and testis size have been identified as the best criteria for predicting the differentiation of OA from NOA. The best serum FSH level cut off points for differentiating OA from NOA was 7.6 mIU/ml (sensitivity 77%) in Schoor's study, 9.2 mIU/ml (sensitivity 89%) in Huang's study, and 8.9 mIU/ml (sensitivity 85%) in the present study. The best testicular long axis cut off point for differentiating OA from NOA were 46 mm (sensitivity 72%) in Schoor's study and 39 mm (sensitivity of 83%) in our study. This discrepancy between testicular long axis cut off points is likely due to ethnic and racial differences. For example, testis size in Asian races was significantly smaller than in Caucasians (30, 31). Azoospermic patients with testicular long axis lower than cut off point and serum FSH level higher than cut off point, based on Schoor's study (89% probability), Huang's study (99% probability), and our study (97% probability) are in NOA group. Azoospermic patients with testicular long axis greater than cut off point and serum FSH level lower than cut off point, based on Schoor's study (96% probability),

Huang's study (81% probability), and our study (78% probability) are in OA group.

The role of diagnostic testicular biopsy in these patients is questionable. According to Shoor's study in the Campbell-Walsh-Wein urology textbook (16, 32), patients with a high level of serum FSH and small testis size are NOA with 89% probability. These patients are candidates for mTESE and IVF/ICSI or sperm cryopreservation without diagnostic testicular biopsy. However, due to the impossibility of using sperm donation in some centers such as our center, a diagnostic testicular biopsy to find sperm for cryopreservation is recommended with our team. Then, if sperm are found, their female partners are candidates for IVF/ICSI. Therefore, inappropriate treatment costs and medical complications for the patient's female partners are reduced. Also, according to Shoor's study, patients who have low serum FSH and normal testis size are in the OA group with 96% probability. These patients are candidates for reconstructive surgery or testis biopsy and sperm extraction alone depending on their reproductive goals. According to our results, 23.6% of patients in the OA group did not have serum FSH levels less than 8.9 mIU/ml and a long testicular axis of more than 39 mm. If a diagnostic testicular biopsy is not performed in this group of OA patients, they may be misclassified in the NOA group and are candidates for mTESE with ICSI, which increases related risks of ovulation induction for patients' female partners and also wastes unnecessary treatment costs. On the other hand, 21.1% of patients with serum FSH levels less than 8.9 mIU/ml and a long testicular axis of more than 39 mm were in the NOA group. If a diagnostic testicular biopsy is not performed in this group of NOA patients, they may be misclassified in the OA group and are candidates for reconstructive microsurgery or are recommended for MESA or TESA with ICSI. Therefore, diagnostic testicular biopsy seems to be necessary for patients with OA and NOA characteristics.

This study has several limitations. First, we did not evaluate mTESE results of NOA patients with negative TESE. Second, since the spermatogenesis is not uniform in the testis and it could be patchy so the single testis biopsy cannot show the whole pattern of the spermatogenesis. Third, data of some patients were incomplete and we had to exclude these patients from the study.

Conclusion

In Iranian patients, azoospermic patients are predictable by the higher level of serum FSH than 8.9 mIU/ml as well as testicular long axis lower than 39 mm with 97% probability in NOA group. Azoospermic patients with serum FSH levels lower than 8.9 mIU/ml and testicular long axis greater than 39 mm with 78% probability in the OA group are also predictable. Findings in this study also emphasize the need for adjusting cut off points based on patients' ethnicity and race to improve diagnosis. In addition, diagnostic testicular biopsy seems to be necessary for patients with OA and NOA characteristics.

Acknowledgements

The authors would like to thank Dr. Goodarzian for providing photomicrographs describing the pathological report. We thank all individuals who provided help in this study, especially the Royan Institute staff. There is no financial support associated with this study. The authors declare no conflict of interest.

Authors' Contributions

I.S.; Participated in study design, data collection, evaluation, and interpretation of data. S.M.K.; Participated in study design and interpretation of data. A.V.T.D.; Participated in testicular ultrasound and data collection related to testicular size. T.H.; Participated in the collection, interpretation of data, and drafting the manuscript. A.R.D.; Participated in the pathological data gathering and statistical analysis. M.A.S.G.; Participated in both study design and interpretation of data, and was responsible for overall supervision. All authors read and approved the final manuscript.

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