MOLECULAR GENETIC AND LABORATORY FINDINGS IN INFERTILE MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA

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ABSTRACT:

Objectives: To study the relationship between Y-chromosome microdeletions with clinical and laboratory findings in infertile men with non-obstructive azoospermia (NOA).

Design: Cross-sectional study.

Patients: Infertile men with non-obstructive azoospermia (n = 146).

Methods: Clinical evaluation and scrotal colour Doppler ultrasonography were evaluated. Standard semen analysis and serum levels of hormones (FSH, LH, total testosterone and prolactin) were performed. Multiplex PCR was done for detection of Y chromosome microdeletions.

Results: AZF deletions were detected in 9.59% of azoospermic men. Complete AZFc was detected in 2.05% of azoospermic men. Partial AZFc deletions were found in 7.5% of azoospermic men, with gr/gr deletion in 6.85% and b2/b3 deletion in 0.69%. There was no significant difference between patients with AZF deletions and azoospermic men without deletions as regards testicular volume and serum levels of reproductive hormones.

Conclusions: Microdeletions of Y chromosome may play a role in pathogenesis of NOA. The testicular volumes as well as levels of reproductive hormones were not correlated with the finding of Y chromosome microdeletions.

Key words: non-obstructive azoospermia, Y chromosome microdeletion.

INTRODUCTION:

Azoospermia is defined as the absence of spermatozoa in the ejaculate, after analysis of a centrifuged specimen and such evaluation should be repeated on at least 2 occasions. This condition affects approximately 1% of men in the general population, and 10% to 15% of infertile men [(1, 2)]. The cause of azoospermia, whether secondary to spermatogenic failure or to obstruction of the excurrent ducts of the testis, is a key determinant of the management of these patients [(3)]. At least 15% of cases with NOA are related to genetic disorders, including both chromosomal and single-gene alterations [(4)].

Microdeletions of Y chromosome are the second most frequent genetic cause of NOA after [(5)]. Azoospermia Factor (AZF) has been identified on the long arm of the Y chromosome and is subdivided into three regions, AZFa, AZFb, and AZFc [(6)]. The overall

frequency of Y chromosome microdeletions varies from 1 to 55% in the different published studies [7-9]. The most frequent deletion type is the AZFc region deletion [10].

Identification of AZF deletion can provide valuable prognostic information. Complete deletions of the AZFa or AZFb regions indicate that finding sperm at the time of testicular sperm extraction (TESE) is impossible, whereas deletions in AZFc indicated a 50% possibility of finding sperm on microTESE [11]. Testing of Yq microdeletions should be offered for infertile men undergoing intra-cytoplasmic sperm injection to rule out the possibility of transmission of Y microdeletions to their male offspring [12].

The objective of this study was to evaluate the clinical and laboratory findings in relation to AZF deletions in infertile men with non-obstructive azoospermia (NOA).

PATIENTS AND METHODS:

This study included 146 infertile men with NOA attending Andrology clinic at Sohag University Hospitals. The study was approved by Ethical and Research committees at Faculty of Medicine, Sohag University. All patients assigned an informed written consent.

Exclusion criteria:

Patients having numerical chromosomal anomalies and those with evidence of varicocele or obstruction of seminal tract were excluded. Exclusion criteria also included patients with history of testicular maldescent, genital infection, trauma, testicular torsion or treatment with chemotherapeutic agents or radiotherapy.

Methods: Patients were evaluated as follow:

I- Initial evaluation: Personal data (age, residency and occupation) and marital history were obtained from all patients. The family history regarding the fertility status of the relatives was reported. General examination was done to detect features of hypogonadism. Genital examination was performed to detect abnormality of penis, testes or epididymis or spermatic cord. Scrotal colour Doppler ultrasonography was performed for all patients.

II- Laboratory investigations:

1) Semen analysis:

Semen analysis was performed according to World Health Organization 2010 guidelines [13]. Azoospermia was defined as complete absence of spermatozoa even after centrifugation at 3000*g* for 15 minutes for at least 2 times, 2 weeks apart.

2) Hormonal profile:

Venous blood sample was drawn from the cubital vein in the morning and was incubated at 37°C water bath for 10 minutes, and centrifuged at 3000 g for 10 minutes. Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone and prolactin were measured using an enzyme linked fluorescent immune-

assay (BioMERIEUX, USA). The normal reference ranges were: FSH (1.7- 12.0 mIU/ml), LH (1.1- 7.0 mIU/ml), total testosterone (3.0- 10.6 ng/ml) and prolactin (3.0- 25.0 ng/ml).

3) Y-chromosome microdeletion analysis:

It was done via polymerase chain reaction (PCR) according to the European Academy of Andrology and the European Molecular Genetics Quality Network (EAA/EMQN) 2014 guidelines [(10)].

Statistical analysis:

Data were recorded in Excel data sheet and analyzed using Statistical Package for Social Sciences soft ware program (SPSS, version 24). Qualitative variables were recorded as frequencies and percentages and were compared by chi-square test. Quantitative variables were presented as means ± standard deviation (SD) and were compared by independent *t*-test. *P* value < 0.05 was considered statistically significant.

RESULTS:

The mean age ± SD of the patients was 35.12±6.1 years, with 75 (51.4%) of them from urban areas. The mean duration of marriage ± SD of the infertile patients was 5.60 ± 3.96 years. The mean volume ± SD of the right testis was 11.29 ± 2.85 ml and that of the left testis was 10.17 ± 2.87 ml. The mean serum level ± SD of hormones were FSH (21.89 ± 10.23 mIU/ml), LH (14.12 ± 6.19 mIU/ml), testosterone (5.38 ± 1.37 ng/ml), and prolactin (6.88 ± 1.60 ng/ml).

Micodeletions of Y chromosome were found in 14 of the 146 azoospermic patients (9.59%). Complete AZFc was detected in 2.05% and partial AZFc deletions were found in 7.54% of azoospermic men, with gr/gr deletion in 6.85% and b2/b3 deletion in 0.69%.

The study populations were classified according to results of microdeletions into two groups: azoospermic men with positive microdeletion (n= 14) and azoospermic men with negative microdeletion (n= 132). The demographic data of the study groups are shown in table 1. The clinical and laboratory data in the study groups are shown in table 2.

Table 1: Demographic data in the study population (n= 146).

Item		**Azoospermic men with positive microdeletion (n= 14)**	**Azoospermic men with negative microdeletion (n= 132)**	***P* value**
Age (years)		36.6 ± 5.9	34.2 ± 6.5	0.17
Residence	Urban	6 (43%)	69 (52.3%)	0.51
	Rural	8 (57%)	63 (47.7%)	
Occupation	Employee	2 (14.3%)	30 (22.7%)	0.96
	Farmer	4 (28.6%)	47 (35.6%)	
	Worker	7 (50%)	51 (38.6%)	
	Others	1 (7.1%)	4 (3%)	
Duration of current marriage (years)		5.43 ± 2.34	5.65 ± 4.46	0.77
Family history of infertility		1 (7.1%)	10 (7.6%)	0.64

* P value < 0.05 was considered significant.

Table 2: Clinical and laboratory data in the study population (n= 146).

Item		Azoospermic men with positive microdeletion (n= 14)	Azoospermic men with negative microdeletion (n= 132)	*P value
Testicular volume (ml)	Right testis	11.71 ± 1.44	10.80 ± 3.13	0.62
	Left testsis	10.43 ± 1.70	9.92 ± 3.10	0.35
Hormonal profile	FSH (mIU/ml)	21.29 ± 7.27	22.5 ± 11.04	0.69
	LH (mIU/ml)	14.26 ± 4.83	14.08 ± 6.57	0.92
	Testosterone (ng/ml)	5.38 ± 1.39	5.39 ± 1.41	0.98
	Prolactin (ng/ml)	7.07 ± 1.62	6.77 ± 1.90	0.52
FSH: Follicle stimulating hormone; LH: Luteinizing hormone				

* P value < 0.05 was considered significant.

DISCUSSION:

The Y chromosome Microdeletions are the second most frequent genetic cause of male infertility [(5)]. A correlation between Y chromosome deletions in AZF regions and male infertility was first documented in 1976 [(14)]. Partial AZFc deletions were identified, with gr/gr deletion as the most common [(15)]. The prevalence and effect of the deletion is variable according to the ethnic and geographic origin of the study population [(10)].

In the current study; microdeletions were found in 9.59% of azoospermic men, which is less than that previously reported in Egyptian studies; 15% [(16)], 39.3% [(17)], 20.4% [(18)], and 10.3% [(19)]. These differences may be related to different inclusion criteria, sample size and the used technique.

Complete AZFc deletion was found in 2.05% of the azoospermic men. This was higher than previously reported in Germany (1%) [(20)], South Iran (1.25%) [(21)], and India (0.97%) [(22)]. However; This prevalence was less than previous studies on Egyptian azoospermic men; 5% [(16)], and 9.2% [(18)]; and also patients from other countries; 7.4 in a Han-Chinese population [(23)], and 9.17% in Dravidian-Indian [(24)].

In the present study; partial AZFc deletions were found in 7.54% of azoospermic men, with gr/gr deletion in 6.85% and b2/b3 deletion in 0.69%. This was near that was previously reported; gr/gr deletions in 7.6% and b2/b3 deletions in 0.85% of azoospermic Indian men [(25)], and gr/gr deletions in 7.48% and b2/b3 deletions in 5.6% in Dravidian-Indians [(24)].

However; higher prevalences were previously reported: gr/gr deletions in 9.2% of azoospermic Egyptian men [(18)], gr/gr deletions in 12.5% and b2/b3 deletion in 9.3% in China [(23)], gr/gr deletions in 8.5% and b2/b3 deletions in 5.8% of infertile Korean men [(26)], gr/gr deletions in 10% and b2/b3 deletions in 5% of infertile men from Iran [(27)], and gr/gr deletions in 12.4% and b2/b3 deletions in 4.96% of infertile Chinese men [(28)].

In a previous study on infertile men from five different locations (India, Poland, Tunisia, United States and Vietnam); ***Rozen*** and colleagues reported gr/gr deletions in 2.4%, b2/b3 deletion in 1.1%, and b1/b3 in 0.1% of the studied populations [(29)]. Another

study on Italian azoospermic men documented gr/gr deletion in 3.2% and b2/b3 deletion in 0.5% of them [30]. A more recent study on Spanish azoospermic men demonstrated gr/gr deletion in 3.9% and b2/b3 deletion in 1.3% of patients [31]. The variation in the frequency may be related to genetic background, ethnic variation and Y haplotypes.

In the present study; the difference in testicular volume between azoospermic patients with AZF deletions and azoospermic men without deletions was not significant. This is in accordance with a previous report [19]. This finding implied that AZF microdeletions in infertile patients are not related to the testicular volume.

In the current study; there was no significant difference in the serum levels of FSH, LH, testosterone and prolactin between azoospermic men with and without AZF deletions. These findings are in accordance with previous studies [19, 20, 24, 32, 33]. These results implied that AZF microdeletions in azoospermic patients were not be related to the levels of reproductive hormones.

To the contrary; in a previous study; the serum levels of FSH and testosterone were significantly lower in patients with microdeletion, while serum level of LH was significantly higher in patients with microdeletions [34]. This may be related to different inclusion criteria.

This study provided further evidence that partial deletions of the AZFc region are a risk factor for NOA. Several partial deletions of AZFc were found to be associated with impaired spermatogenesis, suggesting multiple genes related to this process are located in this region. These findings reinforce the necessity of AZF microdeletion testing among infertile males prior to employment of assisted reproduction techniques.

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النتائج الوراثية الجزيئية و المعملية لدى الرجال غير المخصبين عديمى الحيوانات المنوية غير الإنسدادى

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الهدف من البحث:

تحليل علاقة الفقد الدقيق للجسيم الصبغى "واى" بالخصائص الإكلينكية و المعملية فى الرجال غير المخصبين عديمى الحيوانات المنوية غير الإنسدادى .

المرضى وطرق البحث:

ضم هذا البحث مجموعة من المرضى عديمى الحيوانات المنوية بعيادة الذكورة بمستشفيات سوهاج الجامعية (العدد= ١٤٦) وذلك بعد موافقة لجنة أخلاقيات البحث العلمى بالكلية. تم أخذ موافقة كتابية مبنية على المعرفة من جميع المشاركين فى البحث.

تم أخذ التاريخ المرضى للمرضى مع فحصهم إكلينكياً. تم عمل تحليل سائل منوى لجميع المشاركين فى البحث. تم عمل تحليل لمستوى الهرمونات بالدم (الهرمون المحفز للحويصلات و الهرمون المنشط للتبويض و هرمون الذكورة و هرمون البرولاكتين). تم أخذ عينة من الدم و فحصها لتحديد الفقد المجهرى للجسيم الصبغى "واى" عن طريق تفاعل البوليميراز المتسلسل.

تم استبعاد المرضى الذين يعانون من إنسداد أو إلتهابات فى الأعضاء التناسلية، وجود خلل فى الكروموسومات، و جود دوالى الخصتين وكذلك وجود تاريخ مرضى للعلاج الكيماوى أو الإشعاعى.

نتائج البحث: تمثلت نتائج البحث في الملاحظات الاتية:

- ٩.٥٩% من الرجال عديمى الحيوانات المنوية يعانون من فقد فى منطقة عامل النطاف.
- الفقد الكامل لجزء من منطقة عامل النطاف كان موجود فقط فى المنطقة c (فى ٢.٠٥% من عديمى الحيوانات المنوية).
- الفقد الجزئى فى منطقة عامل النطاف c كان موجود فى ٧.٥% من الرجال عديمى الحيوانات المنوية (٦.٨٥% فى منطقة gr/gr ، و ٠.٦٩% فى منطقة b2/b3).
- لم يكن هناك فروق ذات دلالات احصائية بين المرضى عديمى الحيوانات المنوية سواء حاملى الفقد فى منطقة عامل النطاف أو غير حامليها فيما يتعلق بحجم الخصتين أو مستوى الهرمونات.

الخلاصة:

- وجود فقد مجهرى فى منطقة عامل النطاف قد يكون أحد العوامل المهمة لعدم الانجاب خاصة عند الرجال الذين يعانون من انعدام فى الحيونات المنوية.
- لا يمكن توقع الفقد فى منطقة عامل النطاف عن طريق حجم الخصتين أو مستوى هرمونات الخصوبة بالدم.