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Y Chromosomal Microdeletion; SY127 Gene Related Male Infertility Screening in Iraq

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Abstract--- *SY127 is one of the main genes on AZFb region that locus on the long arm of human Y chromosome and have essential role for fertility because deletions of AZFb region cause stopping of spermatogenesis.*

Materials & Methods: *Subjects and semen analysis: In this study, 126 infertile males were analyzed who were diagnosed and treated at the Department of Al-Hillah Educational Hospital and in Babil, Iraq, from September 2019 to January 2020. The DNA was extracted using DNA extraction kits (Invitrogen, USA). Then DNA amplification by multiplex PCR was performed using STS primers for the AZFb sub-region (SY127).*

Results: *In this study SY127 gene was found in 73 (48.3%) case from 151 samples that detected by polymerase chain reaction "PCR" technique. Its revealed that microdeletions involving sY127 is significantly related to azoospermia. The high frequency of sY127 deletion 9.59% (7/73) among azoospermic patients and the significant difference between azoospermic group and oligozoospermic group in the deletion of sY127 may indicate that, microdeletions involving sY127 may associated with severe spermatogenic failure. The present study concluded that, microdeletions involving sY127 are related to azoospermia. Deletions of sY127 as deletions of sY84, or sY86 alone may be a high risk factor of azoospermia. Conclusion: SY127 gene was found in higher percentage among Oligo–asthenozoospermia and Asthenozoospermia than other cases involved in this study.*

Keywords--- *AZFb, SY127, Male Infertility, PCR, Oligozoospermia, Theratozoospermia, Azoospermia, Asthenozoospermia.*

I. INTRODUCTION

Male factor infertility accounts for 40–50% of all infertility cases. Deletions of one or more AZF region parts in chromosome Y are one of the most common genetic causes of male infertility. Microdeletions of AZF regions in Y chromosome occur as de novo event in 2–10% non-constructive azoospermia or oligospermia cases (1–3). **1**

Several factors have been implicated in male infertility such as hormonal abnormalities, erectile dysfunction, infections, antisperm antibodies, exposure to chemical agents and radiations, testicular cancer, varicocele, genetic factors, and others (4, 5). Thus, male infertility is a multifactorial syndrome encompassing a wide variety of disorders. However, in about 30%-50% of male cases, the etiology of infertility is still unknown. Microdeletion of the azoospermia factor (AZF) region located on the long arm of the Y chromosome (Yq11) is considered the most common genetic cause of male infertility (6,7). **2**

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The association between Y-chromosome microdeletion and defective spermatogenesis has been studied previously, and the frequency of Y-chromosome microdeletions has been reported to account for 5–10% in azoospermic and 2–5% in severely oligozoospermic men (8,9). As the frequency of Y-chromosome microdeletions may vary among different ethnical populations, we conducted this study to evaluate azoospermic/severely oligozoospermic infertile men in our local community. Several Sequence-Tagged Site (STS) marker sequences on the long arm of the Y chromosome (AZF sub-regions) were investigated. Furthermore, testicular biopsies were processed for histological examination to evaluate possible morphological abnormalities.

Several reasons were proposed for male infertility including anti-sperm antibody production, defective delivery of sperm, obstruction of seminal tract, etc.; among these, 40-90% of male infertilities are said to be related to impaired spermatogenesis, which is a significant rate (10). Semen analysis of men suffering from impaired spermatogenesis shows abnormal semen parameters manifested as azoospermia, oligozoospermia, theratozoospermia, and asthenozoospermia.

Among these, genetic factors are the most important factors in male infertility, affecting wide physiological processes such as hormonal homeostasis, spermatogenesis, and quality of sperms (11). Up to now, about 200 genes have been detected which can control spermatogenesis, among which 30 genes are located on Y chromosome. Two important genetics factors in men infertilities are Y chromosome microdeletions and chromosomal abnormalities; although there are other factors such as protamine deficiency, sperm DNA damage, single gene mutation, etc.

II. MATERIALS & METHODS

Subjects and semen analysis: In this study, **126** infertile males were analyzed who were diagnosed and treated at the Department of Al-Hillah Educational Hospital and in Babil, Iraq, from September 2019 to January 2020. All patients underwent physical examination, semen analysis and Y chromosome microdeletion analyses.

Semen samples were obtained by masturbation into a sterile container after 3-5 days of sexual abstinence. Specimens were sent at room temperature to the laboratory and analyzed for sperm count, sperm volume, pH, motility, morphology, and fructose concentration was measured according to the guidelines of the World Health Organization (WHO) (12). All subjects underwent semen analysis at least twice.

Blood and semen samples were taken from all participants (Oligo–asthenozoospermia, Asthenozoospermia, Normozoospermia, Oligozoospermia, Azoospermia, Astheno – teratozoospermia, Oligo - astheno-teratozoospermia) for DNA analysis. The DNA was extracted using DNA extraction kits (Invitrogen, USA). Then DNA amplification by multiplex PCR was performed using STS primers for the **AZFb** sub-region (**SY127**). Samples showing microdeletions on the first screening were verified by subsequent multiplex PCR amplification for another two times, and the deleted loci were confirmed by simplex PCR. The complete description of primers used for detecting Y-chromosome microdeletion and the amplification sets condition was optimized as follows: initial denaturation in 94 ◦C for 5 min, followed by 32 cycles of 94 ◦C for 30 s, 57 ◦C for 30 s and 72 ◦C for 90 s; with a final extension at 72 ◦C for 7 min.

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Optimal electrophoresis conditions were achieved after performing various optimizations. The gel was used at a concentration of 10% (29 ml of milli-Q H2O, 2.5 ml of Tris-aceta-EDTA 10%, 5 ml of glycerol, and 12.5 ml of 40% Acrylamide/Bis-acrylamide, 300 µl of 10% ammonium persulfate, and 32 µl of Tetramethylethylenediamine. The gel was then Redsafe stained and visualized.

III. Results & Discussion

SY127 is one of the main genes on AZFb region that locus on the long arm of human Y chromosome and have essential role for fertility because deletions of AZFb region cause stopping of spermatogenesis. In this study SY127 gene was found in 73 (48.344%) case from 151 samples that detected by polymerase chain reaction "PCR" technique (table1).

Table 1: Percentage of SY127 Gene with PCR

%	*NO.*	*SY127*
48.34	73	Positive
51.66	78	Negative
100	151	Total

The results show (table2) that the percentage of SY127 in primary infertile men was about (52.1 %) , and that was higher than secondary infertile men (31.5%) and control group (16.4) ,also in patient with (20-29) years was about (35.62%) and that was higher than the other ages.

Table 2: SY127 Gene Related to Type of Infertility

%	*NO.*	*Type of Infertility*
52.1	38	Primary infertility
31.5	23	Secondary infertility
16.4	12	fertile(control)
100	73	Total

Screening for Yq microdeletion allowed us to identify four severely infertile subjects with apparently similar breakpoint deletion in AZFb not removing the candidate AZFb gene sy127. This study dealt with the frequency and the patterns of Y chromosome microdeletions in infertile men in Babil. The results have shown that micro deletions were detected among 73 male patients out of 151 infertile men, with a prevalence of 48.34% (73/151).

Compared with previous studies this findings revealed that, microdeletions are more prevalent among Chinese population as compared with Korean population where a percentage of 7.7% has been reported (13). The prevalence is similarly higher among Northeastern China population when compared with findings in Brazilian (7.5%) and Serbian population (5.4%) (14, 15).

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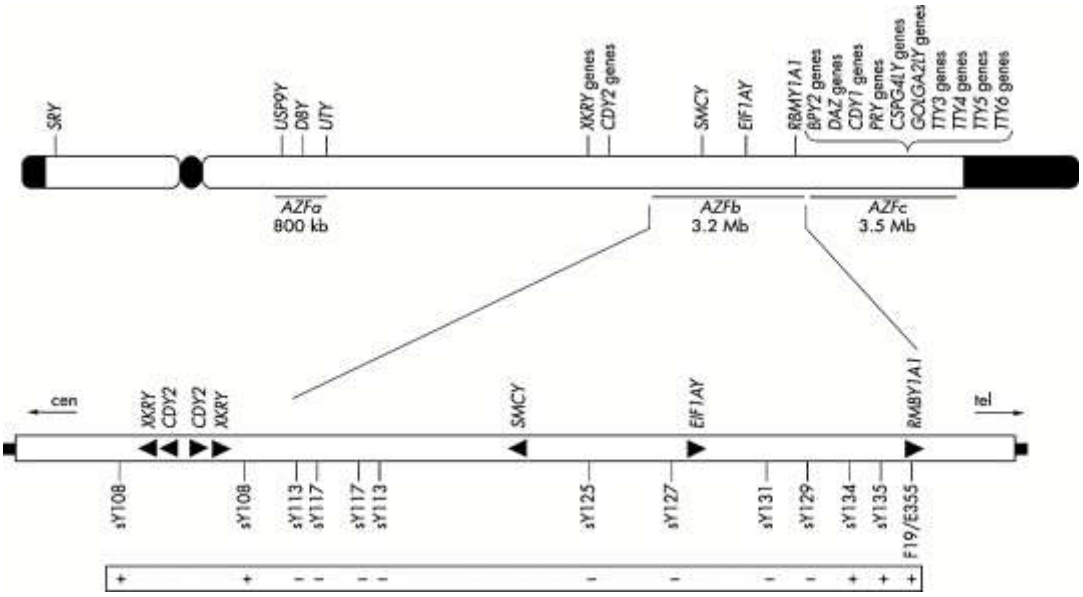

Figure 1: Schematic picture of the human Y chromosome and AZFb deletion found in the four subjects. Top: representation of the Y chromosome with previously mapped genes. AZF regions are indicated. Bottom: magnification of AZFb and surrounding regions with the relative position of the STSs used for the preliminary screening. Genes are indicated in the 5′-3′ orientation by black triangles. Immediately below are indicated the results of PCR analysis (plus, normal amplification; minus, no amplification).

Table 3: Age Distribution related with SY127

%	*NO.*	*Ages*
4.1	3	< 20 years
35.62	26	20 – 29 years
34.25	25	30 – 39 years
17.81	13	40 – 39 years
6.85	5	50 – 59 years
1.37	1	> 50 years
100	73	Total

This study has revealed presence of sY127 in all patients. Moreover, the present study also showed that, absence of sY127 with other sequence tagged sites results in azoospermia. This finding is in agreement with the report of Hopps *et al* who noted that, men with microdeletion of the AZFa or AZFb regions of the Y chromosome have very poor amount of sperm, whereas the majority of male with AZFc deletion have sperms within the semen or testes (16).

The study results showed microdeletions involving sY127 is significantly related to azoospermia. The high frequency of sY127 deletion 9.59% (7/73) among azoospermic patients and the significant difference between azoospermic group and oligozoospermic group in the deletion of sY127 may indicate that, microdeletions involving sY127 may associated with severe spermatogenic failure. The present study concluded that, microdeletions involving sY127 are related to azoospermia. Deletions of sY127 as deletions of sY84, or sY86 alone may be a high risk factor of azoospermia.

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AZFb showed proximally a structure that resembles that of AZFc, (17) with large direct and inverted repeats organized in palindromes, but the most part of it consists of single copy sequence. AZFb as former defined 13 actually extends for 3.2 Mb. By using a number of already described and novel markers, we tried to determine the deletion breakpoints, mapping the distal one in a 173 bp region between sY1211 and sY1207. Identification of the proximal breakpoint was more difficult because the presence of the repeats prevents detailed analysis. We were unable to isolate and sequence the junction fragments, which is usually required for the identification of deletion breakpoints.

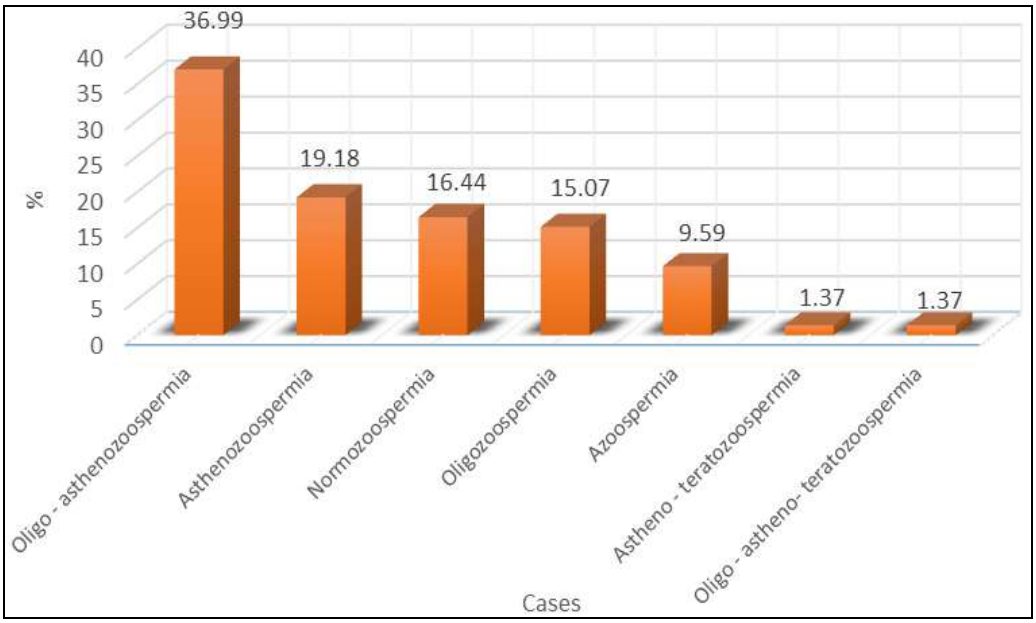

Figure 2: Results of SY127 related to the of Patients

Table 4: Frequencies of Infertile men Groups and Control

Total	***No.***	***Group***	***Specimens***
126	57	Oligo - asthenozoospermia	**Patients**
	24	Asthenozoospermia	
	23	Oligozoospermia	
	11	Azoospermia	
	10	Oligo-astheno- teratozoospermia	
	1	Astheno - teratozoospermia	
25	25	Normozoospermia	**Control**
151	151	**Total**	

Yq microdeletion ; The AZF region in chromosome Y, at a molecular level, and according to the certain deletion pattern of microdeletions which observed, was divided into AZFa, AZFb, and AZFc sub regions (18). Further, a fourth region named AZFd, located between AZFb and AZFc was also reported. However, whether AZFd truly existed is still debatable. There are variable phenotypes regarding the deletions in different regions of AZF; as the entire deletion of AZFa region leads to SOCS (lack of germ cells in seminiferous tubules and presence of Sertoli cells only syndrome) and azoospermia (19). Complete deletions of AZFb and AZFb+c show a histological picture of SCO or spermatogenic arrest resulting in azoospermia (20).

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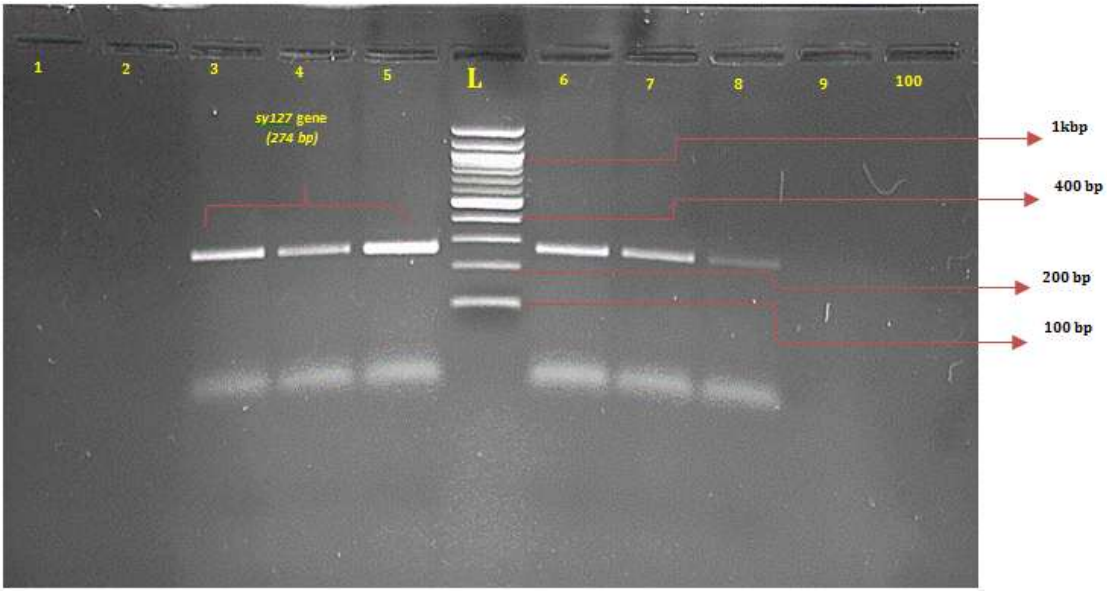

Figure 3: Electrophoresis of sy127 (274 bp) gene products. L lane contain the 100 bp DNA Ladder, 5 % NuSieve® 3:1 agarose gel in 1X TBE buffer containing 5µl Redsafe; (3-8) positive results; (1,2) and (9,10) negative results

According to the results of the investigators worldwide, deletions mainly involve AZFc, less frequently AZFb and only rarely the AZFa regions, the frequency of AZFc, AZFb, and AZFa deletions in men with Yq microdeletions is estimated to be about 60%, 16%, and 5% respectively (21, 22). It is believed that the majority of Y microdeletions arise through the distant homologous recombination between specific palindromic sequences; although, deletions generating by the non-homologous recombination were also recognized (23-25). Considering the results of the papers, studying the incidence of microdeletions in these regions among Iranian infertile men, there is a discrepancy among the frequency of deletions in AZF regions reported by different researchers.

In a study by Omrani et al, the incidence of AZF loci microdeletion was investigated among 99 azoospermic or severe oligospermic Iranian men from West Azarbaijan. The deletions were mainly occurred in the AZFc region (87.5%) and with less or no occurrence in the AZFb (29.2%) and AZFa locus, respectively (26). Also, Keshvari and coworkers (2011) showed that microdeletions mostly involved AZFc (100%), less frequently AZFb (50%), and AZFa (25%) regions (27). The small sample size in this study might be the reason for these high frequencies since only 4 patients were found to have AZF microdeletion. In another study, Totonchi and colleagues showed among the 185 patients with AZF microdeletion 147 cases suffered from azoospermia and 38 patients from severe oligozoospermia (28).

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CONFLICT OF INTEREST

The authors had no conflicts of interest to declare in relation to this article.

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