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Role of DAZgene microdeletionandAMH changesin Azoospermia and Oligozoospermia patients

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ABSTRACT--*This study aimed to detect the microdeletions ofDAZin azoospermic and oligozoospermic patients and assessment of anti-müllerian hormone (AMH). One hundred patients included in this study,amplification of PCR by specific primer sets. All the samples tested for the presence of STS markers sY254 to detect AZFcDAZ gene deletions.The processed DNA was subjected to monoplex PCR, each specific region of the AZF locusamplifies by primer pair. AMH hormone level measured by ELISA kit.microdeletion of DAZ found in 20 of samples, present in 10(50%) in azoospermic patients with decreased in AMH level and oligozoospermic patients had 7(35%) while 3(15%) in normozoospermic men.Based on results, this study has shown DAZ genes microdeletion and decrease in AMH level are a major causes of male infertilityin this study and this microdeletions were more incidences in azoospermia more than oligozoospermia.*

Keywords*--DAZ, anti-mullerian hormone, microdeletion.*

I. INTRODUCTION

Spermatogenesis directly encoded by genes; some of these genes are located within the region known as azoospermia factors (AZF) on Y-chromosome which consider as a hot spot for deletion mutations. There are three intervals of azoospermia factors; AZFa, AZFb and AZFc(1). *AZFc*sub-region has genes which show testis specific expression. The most important candidate gene of DAZ (deletion-in-azoospermia)is sub-region ofAZFc, most men with infertility suffer from deleted in this sub-region,*DAZ* was the most important candidate gene and first identified family in AZFc region,if this locus had spontaneous mutation in the paternal germ line will be disturbed the Spermatogenesis (2).DAZ protein was contained in the inner layer of the male germs and sperm cell tissue. In infertile men observed high incidence of *DAZ* gene deletion (3).Anti-müllerian hormone (AMH), also named müllerian inhibiting substance (MIS), is a member of the transforming growth factor β (TGF- β) land it is 140-kD a dimeric glycoprotein (4).On the chromosome 19p13.3 the AMH gene is found, while on the chromosome 12 the AMHR2 gene that codes for its receptor(5).At the early stages of development, in utero, fetuses of both sexes have two pairs of müllerian forming wolffian ducts, upper third of the vagina, uterusandthe fallopian tubes. In females, AMH is expressed from the onset of puberty until menopause and regulates follicular recruitment and development within the ovary (6). It is secreted by Sertoli cells and it has the main role in male sexual differentiation which responsible for the inhibition of Müllerian ducts in the male fetus, as part of the sexual differentiation (7).AMH is

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also importantin testicular development and function [8]. When the AMH increase consider as a marker of good spermatogenic response.In mammals, Spermatogenesis demands peptide and steroid hormones to be compiled, each of which is essential to the normal functioning of the epithelium[9].

II. MATERIALS AND METHODS

Estimation of DNA concentration

The DNA concentration was determined by using nano drop, one microliter of sample put on instrument after measured DNA rehydrolyze solution as control to compare with samples. NAS-99 is a nucleic acid spectrophotometer designed for complex measurements (Figure 1). The light source is UV-LED (UV light-emitting diode). It can accurately measure nucleic acid samples without diluting high concentrations of samples. Simply add 1.5 - 2.5 µl of sample to the base and lower the sampling arm. The NAS-99 can automatically measure within 3 seconds with computer software to achieve control and data storage capabilities.

DAZ microdeletions analysis by PCR-based STS

Amplification of PCR was carried out by specific primer sets,using STS markers *sY254*for all the samples to detect AZFc*DAZ* gene deletions.Monoplex PCR was subjected to processed DNA of patients, specific region of the AZF locus which located in the long arm of Y chromosome was amplifies byeach primer pair, the sequence of these primer were explained in (Table 1)

Table (1): The sequence of STS primers used in this study.

STS	**Size**	**Forward**	**Reverse**
sY254	380	GGGTGTTACCAGAAGGCAAA	GAACCGTATCTACCAAAGCAGC

1. Mixture A: In this mixture we used STS markers DAZ (sY254).The components and program of mixture B are presented in tables 2, 3

Table (2): The component of mixture B

Chemicals	**Conc.**
F-DAZ(Y254)	1µl
R-DAZ(Y254)	1µl
Master mix	10µl
D.W	6 µl
DNA sample	4 µl
Final volume	22 µl

Table (3): Polymerase chain reaction program for mixture A.

No.	Steps	Temperature	Time	No. of cycles

1	Denaturation 1	95C°	10min	1 cycle
2	Denaturation 2	94C°	30sec	35cycles
3	Annealing	57 C°	1min	
4	Extension 1	72C°	90sec	
5	Extension 2	72C°	10min	1 cycle
6	Holding	4 C°	15min	1 cycle

PCR *products electrophoretic detection*

PCR yield product contain0.3 μg/ml ethidium bromide for each 2% (w/v) agarose gel in 1x TBE buffer. As well as, a 100 bp DNA ladder was run at the same time with each electrophoretic run to reveal product sizes. Electrophoresis was done at 70 v/cm for 1 hour, byUV transilluminator documentation system the results were visualized and documented.

Evaluation of anti-Müllerian hormone

The MIS / AMH ELISA is a three-stage immunoassay quantitative. Unknown samples and calibrators were added to the microtiter wells covered with AMH antibody in the first step controls and incubated then. Then the wells were washed and incubated with AMH antibody solution for biotinylating applications. Wash and incubate a horseradish peroxidase conjugate (SHRP) solution for the second time with streptavidin. The wells are incubated with substrate solution tetramethylbenzidine (TMB) after the third incubation and washing step, followed by an acidic stopping solution. The antibody-biotin conjugate binds to the streptavidin-enzyme conjugate which in turn binds to thesolid phase antibody-antigen complex. This complex bound to the well and detected by enzyme-substrate reaction.

III. RESULT

The microdeletion of *DAZ* and decreased in AMH level found in 20 of samples, present in 10of azoospermic patients and oligozoospermic patients had 7while 3of normozoospermic men as shown in table (4) and figure (1).

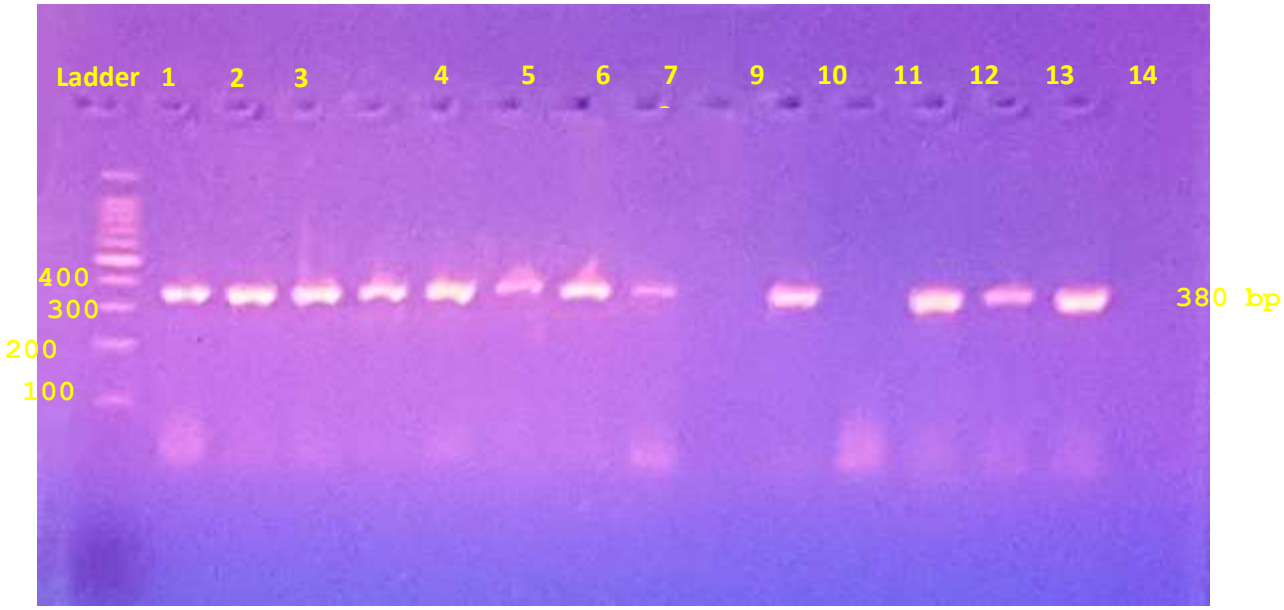

Figure (3-10):Agarose gel electrophoresis of the PCR product of AZFa (*DAZ*gene)deletion was occured in case no.9 and 11. 100 bp DNA Ladder, Lane 1-14 samples with 277 bp

Table (4): The study of samples with *DAZ* gene microdeletion and low level of AMH.

Subjects type	**Gene**	**No. of cases**	**AMH (ng/ml)**
Azoospermia	***DAZ***	**10**	5.34 ± 0.75 b
Oligozoospermia	***DAZ***	**7**6.31 ± 0.73 b	
Normozoospermia	***DAZ***	**3**	15.05 ± 0.95 a
LSD value		2.303 **	
P-value		0.0002	

IV. DISCUSSION:

Microdeletions AZFc are more common than the others, and almost reports consider that *DAZ* microdeletions and AZFc deletions are physiologically the same [(10)]because a large number of an abnormal sperms and a small amount of active sperm may result from DAZ microdeletions. A number of studies have found that AZFc contains 60 percent Y chromosome microdeletions[(11)]. AZFc interval contains many genes, *DAZ* gene plays a major role in spermatogenesis. The world frequency of these microdeletions in infertile males is about 10-15% in azoospermia males[(12)]. This study had shown that (20 out of 100) had *DAZ* microdeletion, among (33)

azoospermic patients 10(30.3%) had detectable *DAZ*microdeletion with decreased in the level of AMH and oligozoospermic patients had 7(12.1%) while 3(8.82%) of normozoospermic men.

AZFc microdeletions can produce a small quantity of active sperms and a large number of abnormal sperms, Dhanoa*et al*. (2016) reported that microdeletion of *DAZ* is associated with spermatogenic impairment[13]. Also, Ambulkar and Pande (2017) found the microdeletion of *DAZ* was associated with arrest of germ cells at spermatid stage and hypospermatogenesis with sperm counts and reduction of copy number lead to an increase risk in infertility can cause azoospermia or severe oligozoospermia[14]. Different types of rearrangement resulting in changes in the progressive copy number of the *DAZ* gene family were reported to be associated with diseases such as testicular germ cell tumors and male infertility, because contains massive sequence repeats called amplicons[15], the amplicons are organized in palindromic domains that harbour a group of genes for spermatogenesis which are highly sensitive to intra- and inter-chromosomal recombinations, microdeletions considerably occur in the regionof*DAZ*, often leading to failure ofspermatogenesis in men[16].

Indicated from the previous hormonal levels there was severe testiculopathy in AZF microdeletion patients involving the spermatogenic systemcauses of testicular damage, such as cryptorchidism or varicocele[17].The concentration of AMH and testosterone are low, May be the hormones levels of patients and testicular volumes affected by severe testiculopathy with AZF deletions (18), may be indicated to the primary testicular failure because of non-increased in Inhibin that make the pituitary gland to arrest the secretion of testosterone(19).

V. CONCLUSION

This study has shown that *DAZ* gene microdeletion with decreased in the level of anti-mullerian hormone are a major causes of male infertility in this study and this microdeletion were more incidences in azoospermia more than oligozoospermia.

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