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AZFc deletion assignment in fertile male

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Abstract: Background: Infertility due to male factor, is considered as a major contributor in the prevalence picture of the infertility in the world. Spermatogenic process & its products are considered the backbone of male fertility. This process is directly related to the genes expression which are located on the long arm of Y-chromosome (Yq). These genes are occupy the three AZF (Azoospermia factors;AZFa,AZFb and AZFc) regions which proved to be a hot spot for deletion mutations. AZFc intervals deletion is the most prominent deletion with less penetrance that ranging from mild oligospermia to moderate oligospermia. To assess AZFc deletion correlation with male fertility status and to evaluate the rate of AZFc deletion within primary fertile males population. Cross sectional study was adopted to select 110 child fathering fertile males who are subjected for conventional infertility investigations and molecular genetic analysis (AZFc deletion). Sixteenth cases (16/110 (14.5%)) shows poor semen parameters (oligospermia) but with normal genetic analysis (no AZFc deletion). one case out of which (1/16 (6.25%)) complaining of secondary infertility. 5 cases (5/110(4.5%)) shows AZFc deletion with variable range of oligospermia. 4(4/5(80%) out of which complaining secondary infertility but with primary fertile status (fathering a child). Conclusion: These results denoting that, AZFc delation mostly associated with spermatogenic failure (oligospermia) but not necessarily with absolute male infertility.

Key wards: fertility, Male infertility, AZF deletion, oligospermia and Azoospermia

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Introduction:

Spermatogenesis is a crucial stage in male gamete development regulated by many Y chromosome specific genes Known as azoospermic factors genes (AZF) that located on the long arm of Y chromosome (Yq) (1). Many researcher claim AZF deletion to idiopathic male infertility. Hence AZF deletion emerged as the most frequent structural chromosome anomaly associated with the oligo and /or azoospermic condition(2).

AZF (AZFa, AZFb, and AZFc) are three deletion intervals, each associated with specific infertility phenotype. AZFa deletions were expressed as Sertoli cell-only syndrome (SCOS) and AZFb deletions to maturation arrest at the spermatocyte stage. Hence patients complaining of AZFa or AZFb deletions are Azoosperic with primary infertility, whereas AZFc deletions are mostly associated with oligospermia and could be transmitted to the progeny(3).

AZFc deletions are of variable degree of deletions (gr/gr, DAZ, b2/b312, u3-gr/gr13 or g1/g1). AZFc deletion frequency is considered high if compared to AZFa and AZFb. However complete AZFc and gr/gr deletions are

manifest-able with varying degree spermatogenic failure whereas any other partial deletions are, seems to have no effect on fertility status in association with a certain Y chromosome background commonly present in northern Eurasian populations (Y haplogroup)(4).

AZFc deletions are known to be associated with a moderate oligozoospermia to azoospermia that, phenotypic range may pointing out the extent of deletion. Environmental factors or different genetic backgrounds may account for these phenotypic variability for instance, in certain men a compensatory effect for the absence of Yq genes, by autosomal or X linked factors could explain the cases where the father showed a sustained fertility over several years (5). Moreover, even sustained fertility does not necessary mean normal spermatogenesis. In the paper by Chang et al. the father conceived his youngest son (the fourth son) at 38 years old but at the time of the observation he was azoospermic (6).

Material and methods:

Based on study strategy, one hundred and thirteen fertile males were selected randomly and the final male fertility status decision confirmed by child fathering.

However blood sample were collected from all selected male (113) ,who they are present in different job sectors during the study period (2015-2016).

All fertile males (child fathering males) sample were subjected to full extensive questioner about their fertility status, gonadal examination, hormonal levels , seminal analysis(according to the WHO 1999 guidelines) and to AZFc Y-chromosome microdeletion screening test . According to the EAA/EMQN guidelines (Simoni *et al*., 1999), A minimal tow primers were selected for analysis of AZFc which are sufficient to determine the presence of Y-chromosome micro-deletion (STS deletion) AZFc (7, 8).

This study select molecular screening test only for AZFc because AZFa and AZFb are mostly associated with sertoli only syndrome(infertile males with azoospermia) and most of the Y chromosome deletion is within AZFc region(9).

	Left arm	***Right arm***		
SY254	***5-GGG TGT TAC CAG AAG GCA AA-3***	***5-GAA CCG TAT CTA CCA AAG CAG C-3***	***AZF***c	400 bp

The following program was adopted.

Table (1): PCR program

No.	Steps	Temperature	Time	No. Of cycles
I	Denaturation 1	95C°	3min.	1
First loop				
II	Denaturation 2	94C°	1min.	35
III	Annealing	55C°	1min.	
IV	Extension 1	72C°	1min.	cycles
IVI	Extension 2	72C°	5min.	1

Results:

Virtually only 110 cases of 113 were complete this study. Three cases were dropout of the study .The study revealed that, 89 (80.9%) cases are completely normal (normal; clinical examination, hormonal levels, seminal analysis and molecular analysis (AZFc)). sixteenth (16/110 (14.5%)) cases shows poor semen parameters (oligospermia) but with normal genetic analysis (no AZFc deletion). one case out of which (1/16 (6.25%)) presented with secondary infertility .

The 5 (5/110(4.5%)) remaining cases shows AZFc deletion with poor semen outcome (fig (1)). 4(4/5(80%) out of which complaining secondary infertility but with primary fertile status (fathering a child).

Unfortunately those males (secondary infertile males) have no previous semen analysis history to confirm the diagnosis of oligo and /or azoospermia and its correlation with genetic backbone (AZFc deletion).

The secondary infertile males 5 (4.5%) shows variable degree of sperm concentration ranging from moderate oligospermia to sever oligospermia and have no history of assisted reproduction.

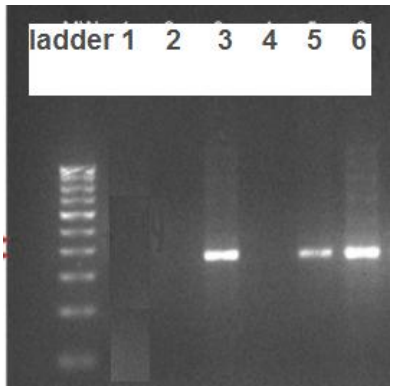

Figure (1): Agarose gel electrophoresis analysis shows , the microdeletion in AZFc marker SY 254 of Y chromosome in fertile male.ladder: 100 BP ;lane one :water ; lane 2 : female ;lane 3,4and 6 : fertile male ; lane 4 : microdeletion in fertile male .

Discussion:

The results is in consistent with this hypothesis; a progressive decrease of sperm number over time has also been reported in men with AZFc deletions (10, 11).

The highlights of these results are; although most of idiopathic infertile cases complaining of AZF deletion but not necessarily these deletions lead to infertility but for less extent fertile male with AZFc deletion is complaining of compromised sperm output. Moreover male fertility status not necessarily means normospermia. This picture displayed the variable rates of AZF deletion penetrance within certain population. However AZF region has variable copies of the tandem on Y chromosomes, moreover these tandems are overlapped .Therefore the fertility status is firmly correlated to the gene type deletion and the extent that affect

theses copies. The DAZ (Deleted in azoospermia) gene of AZFc region consists of four identical copies, but different combinations of partial deletions of these gene copies may result in impaired fertility or may have little or no effect on fertility (12). So rarely within a family, the same deletion of the Y chromosome has been reported to occasionally cause infertility in some males but not in others (13, 14, 15 and 16). Which increases the complexity of the current image that spermatogenesis process assessed by thousands of genes encoded on the X and Y chromosomes, as well as the autosomes that influence the process of spermatogenesis. Many of these genes may be expressed not only in the germline but also in the Sertoli cells. Studies of gene inactivation or deletions in knockout mice have shown that more than 200 genes are directly or indirectly involved in male fertility (17).

Regarding the female partner role in expression of male infertility is that, male fertility outcome share and depends on the potential fertility of his female status. Compensation of male subfertility by female `super' fertility is a well-known phenomenon and it must be considered whenever a genetic defect, such as a Y microdeletion is found in a `fertile' male with an unknown sperm count (18, 19).

This study concludes that; many primary fertile males might be complaining of variable degree of AZFc deletion. However AZFc deletion expression is totally dependent on the extent of AZFc interval deletion and on the general fertility status of male and his female partner fertility status. Post primary fertile males may complaining of de-novo AZFc deletions which causes secondary infertility status.

Virtually It is important to adopt molecular analysis in case of idiopathic infertility as routine laboratory examination.

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