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Original Article

Relationship of Azoospermia Factor (AZF) and Inhibin B Level in Patients with Non-Obstructive Azoospermia

Ahmed Gamal El-Metwaly Ghanem[1]*, Mohamed Ali Atwa Barakat[2], Reham Mohammed El-Farahaty[2], Youssef Elbayoumi Youssef Soliman[3], Sohier Yahia[4], Hosam Abdel Twab[5]

[1]*M.B.B.Ch, M.S. Clinical Pathology, Specialist of Clinical Pathology, Mansoura Fever Hospital, Egypt*
[2]*Professor of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt*
[3]*Professor of Dermatology, Andrology and STDs, Faculty of Medicine, Mansoura University, Egypt*
[4]*Professor of Pediatrics and Genetics, Faculty of Medicine, Mansoura University, Egypt*
[5]*Lecturer of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt*

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ABSTRACT

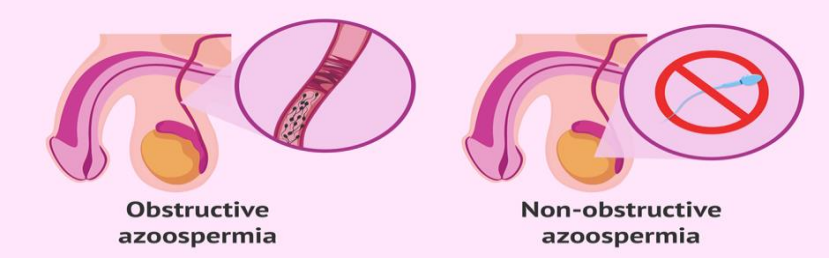
Background: Non-obstructive azoospermia (NOA) is a challenging subset in management of infertile males. Micro-deletion of the azoospermia factor (AZF) region located on long arm of the Y-chromosome (Yq11) is considered as the commonest observed genetic cause among infertile males. Inhibin-B was identified to be a more sensitive factor in evaluating azoo-spermic males than FSH and testicular biopsies.

Patients and Methods: In this prospective case-control study, 150 participants were separated into two groups: group (A) consisted of 75 patients with non-obstructive azoospermia, and group (B) consisted of 75 age-matched, fertile men having a child in the year prior to the study as a control group. Entire cases were subjected to full history taking and complete examination followed by laboratory analysis which comprised semen analysis, hormonal assays, including TSH, FSH, LH, E2, prolactin, total testosterone, and inhibin-B and detection of AZF micro-deletion.

Results: PRL, FSH, and LH were significantly higher (P 0.028, <0.001, <0.001), while total testosterone, inhibin-B level, TT/FSH, and inhibin-B/FSH ratios were significantly lower (p<0.001 for each) among azoospermia cases compared with control group. The frequency of AZF micro-deletions among azoospermia cases was 40/75 (53.3%). AZFc deletion was the most frequent type of Y-chromosome micro-deletion.

Conclusion: AZF and inhibin B levels were demonstrated to be significantly correlated in patients with non-obstructive azoospermia. Higher FSH, lower testosterone, and lower inhibin-B levels could be considered as risk predictors for azoospermia, but could not be considered as risk predictor of AZF deletion among azoospermia cases.

GRAPHICAL ABSTRACT



* Corresponding author: Ahmed Gamal Elmetwaly Ghanem
✉ E-mail: Email: ahmedg.ghanem@gmail.com

Introduction

Infertility has become a major worldwide public health problem according to the WHO, due to its direct effect on society and economy. Infertility is the failure to conceive after 12 months of unprotected coitus (WHO, 2018). It was revealed that approximately 50 million couples might be influenced all over the world, with male infertility representing around 50% of these cases [1]. Azoospermia is defined by the WHO as absence of sperms in the seminal fluid even after extended centrifugation. Non-obstructive Azoo-spermia (NOA) is the most severe form of male infertility, involving about 1% of male population and 10% of infertile males [2].

Azoospermia is caused by various etiologies including endocrinal and chronic diseases affecting hypothalamic–pituitary–gonadal axis (e.g., hypogonadism or DM), and also pathological syndromes associated with disrupting sperm transport within vas deferens (e.g., undescended testes, varicoceles, cancer, or urogenital infection). However, in one-third of azoospermic males, there is a noticeable genetic abnormalities resulting in defective spermatogenesis [3]. Diagnosis of genetic causes of azoospermia often depends on identification of structural and numerical chromosomal anomalies, Y-chromosome micro-deletions, as well as cystic fibrosis transmembrane conductance regulator mutations [4, 5].

Micro-deletion of the azoospermia factor (AZF) is considered as the commonest genetic cause observed in infertile males [6]. AZF region implies three non-overlapping sub-regions known as AZFa, AZFb, and AZFc, all of them are necessary for normal spermatogenesis. AZF micro-deletions are linked to several spermatogenetic defects such as Sertoli cell-only syndrome, maturation arrest, and hypo-spermatogenesis [7]. The early detection of AZF micro-deletion among infertile males can clarify the cause of oligo-spermia and azoo-spermia, and also help in management of infertile cases and saving their male offspring [8].

Sertoli cells have a vital role in regulating spermatogenic process and testicular signaling through acting as targets for FSH and testosterone hormones [9]. FSH activates Sertoli cells to release inhibin, androgen-binding protein, and also has a leading role in initiating and progressing of the spermatogenic process [10]. Inhibin-B was identified to be a more sensitive factor in evaluation of azoo-spermic males than testicular size, FSH, and testicular biopsies. It reveals the function of whole testicular tissue, while biopsy does not represent the total testicular tissue. Moreover, inhibin-B level is considered as an index of Sertoli cell numbers and integrity [11].

Thus, the effects of chromosomal abnormalities in NOA on spermatogenic process might be reflected by serum inhibin-B concentrations. The current study was carried out to focus on the correlation of AZF micro-deletions and inhibin-B in male patient with NOA.

Patients: This was a prospective case control study that comprised 150 subjects, divided into 75 patients having NOA (mean age was 31.4 ± 6.1 years old) and 75 age-matched normal fertile men having child in the last year before the study as a control group (mean age of 31.2 ± 4.3 years old).

Patients with HIV, HBV and obstructive azoospermia were not included in the study.

Written informed consents were taken from every participant prior to participation in this study. The Mansoura Faculty of Medicine's Institutional Review Board approval for this study (IRB: MD.20.09.361).

Sampling

Five ml of blood were drawn from the patient under complete aseptic conditions. 3 ml on plain tube, and then centrifuged and the supernatant was stored at -20 °C for hormonal analysis (inhibin-B, TSH, FSH, LH, E2, PRL, and total testosterone) and 2 mL on EDTA tube were kept frozen at -20 °C till DNA extraction and analysis using real-time PCR genotyping kit for molecular investigation of Azoospermia Factor (AZF).

Materials and Methods

Full history taking, complete clinical examination and laboratory investigations were carried out for all participants. At least two semen samples,

at one month interval for confirmation of azoospermia and excluding of obstruction according to WHO guidelines for semen analysis (WHO, 2010). Levels of TSH, FSH, LH, E2, PRL, and total testosterone were measured using Electro-Chemiluminescence immunoassay (Cobas e411). Furthermore, serum inhibin-B was measured by (ELISA) Cat. No E0984Hu. Intra-Assay: CV<8% Inter-Assay: CV<10%.

Molecular analysis for AZF Micro-deletions was carried out according to the recommended guidelines of the European Academy of Andrology and the European Molecular Genetics Quality Network [12]. DNA extraction was done using PREP-RAPID DNA Extraction Kit according to the manufacturer's instructions.

Following DNA extraction, the AZF Micro-deletions REAL-TIME PCR genotyping kit was used to identify the Y-chromosome loci AZFa, AZFb, and AZFc based on fluorescence PCR modification. Two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules are included in the PCR-mix. These probes are activated after hybridization to a target sequence, and the rise in fluorescence that follows is proportional to the target sequence amplification. Fluorescence intensity was estimated at each cycle of reaction using PCR thermal cycler data collection unit and was analysed by software provided.

The following AZF micro-deletions were detected in azoospermic cases (SY-84, SY-86, SY-127, SY-134, SY-142, SY-242, SY-254, SY-255, SY-615, SY-Y1125, SY-1197, SY-1206, and SY-1291) in addition to sex determining region Y protein (SRY gene) which indicates sex and additional genomic target that serve as sample intake control (SIC) intended for sample quality assurance.

Statistical analysis

Data were reviewed, coded and tabulated using statistical package for Social Science (Version 25.0. Armonk, NY: IBM Corp.). Parametric numerical data were expressed using the mean and standard deviation, while for non-parametric numerical data, the median and range. For non-numerical statistics, percentage and frequency were employed. The difference between the means of the two groups was assessed using Student-T tests. Mann Whitney and Kruskal-Wallis tests were done to evaluate the difference of non-parametric variables in two or more groups, respectively. The intensity and direction of the linear relationship between two variables are described by the correlation coefficient. Receiver operating characteristic curve offered a valuable method to assess the sensitivity and specificity for quantitative diagnostic data to discriminate between studied groups. To determine which independent factors had a discernible influence on the result, a logistic regression analysis was conducted. A p-value was deemed significant if it was less than 0.05 at a 95% confidence interval.

Results and Discussion

PRL, FSH, and LH were significantly higher in azoospermia group compared with control group (*P* 0.028, <0.001, <0.001), while T.Testosterone, inhibin-B level, TT/FSH, and inhibin-B/FSH ratio showed significantly lower level in azoospermic cases compared to control group (p<0.001 for each). No significant differences were detected in TSH and E2 levels between azoospermia and control groups (p>0.05 for each).

Azoospermia group was subdivided into Azoospermia with AZF deletion (53.3%) and Azoospermia without AZF deletion (46.7%).

FSH and LH level were significantly higher (*P*1, *P*2, and *P*3), while Inhibin-B level T. Testosterone level, TT/FSH, and inhibin-B/FSH ratios were significantly lower (*P*1, *P*2, and *P*3) in Azoospermia without AZF deletion and Azoospermia with AZF deletion compared with control group. PRL showed significantly higher level in Azoospermia with AZF deletion compared with control group (P3 0.025). Otherwise, no significant difference was found regarding studied hormones or ratios (*P*4) between azoospermia without AZF deletion and azoospermia with AZF deletion.

ROC was conducted to validate the ability of inhibin B to discriminate between different studied groups. Inhibin B level had moderate accuracy to discriminate between controls and azoospermia (AUC=0.796), as well as between

controls and cases without AZF deletion (AUC=0.787), and between controls and cases with AZF deletion (AUC=0.804). However, inhibin B level failed to discriminate between cases with and without AZF deletion (AUC= 0.542). Best cutoff values and performance characteristics are showed in Table 1.

Among all azoospermia cases, AZF-A deletion was found in (5.3%), AZF-B deletion in (12%), AZF-C deletion in (53.3%), AB in (2.7%), AC in (5.3%), BC in (12%), and ABC in (2.7%) (Table 2).

Inhibin B level showed significant (positive) correlation with total Testesterone (P 0.022) and significant (negative) correlation with FSH (P 0.017). Otherwise, no significant correlations were found regarding inhibin B with studied parameters among studied cases (Table 3).

Logistic regression analysis was conducted for predicting azoospermia susceptibility, using age, FSH, TSH, LH, E2, PRL, t.testosterone, and inhibin-B level as covariates. Higher PRL, FSH, LH, E2, lower total testosterone, and inhibin-B levels were associated with risk of azoospermia in univariable analysis, while in multivariable analysis, only higher FSH, lower testosterone, and inhibin B levels were considered as risk predictors for azoospermia susceptibility (Table 4).

Logistic regression analysis was done for predicting AZF deletion among azoospermia cases, using age, FSH, TSH, LH, E2, PRL testosterone, and inhibin level as covariates. None of them was considered as a risk predictor of AZF deletion among azoospermia cases.

Concerning hormonal status, the current study demonstrated that Prolactin (PRL), Follicular Stimulating Hormone (FSH), and Luteinizing Hormone (LH) were significantly higher in azoospermia group compared with control group (P 0.028, <0.001, <0.001). On the other hand, T.Testosterone and TT/FSH showed significantly lower level in azoospermia cases compared with control group (p<0.001 for each). In the same line, Gangwar *et al.* [13] have displayed that LH, FSH, and PRL levels were markedly elevated in azoo-spermic, oligo-zoospermic, and astheno-zoospermic infertile cases whereas FSH and PRL were significantly elevated in normo-zoospermic infertile cases compared with control group, while testosterone level was significantly reduced among infertile cases compared with control group of fertile males. Also, recorded significantly higher PRL levels in cases with azoospermia than those with normozoospermia ($P < .001$) [14]. In addition, Elsaid *et al.* have recorded that PRL, LH, and FSH concentrations in cases with azoospermia were more higher than cases with oligozoospermia. Hence, there was a relationship between high prolactin concentrations and azoospermia [15].

The current study demonstrated that azoospermia group was associated with a significant lower Inhibin-B level compared to control group (median 71 versus 120 pg/ml; p<0.001), and also inhibin B/FSH ratio were significantly lower in azoospermia group compared with control group (p<0.001), as provided in Table 5.

Table 1: Validity of inhibin B for discrimination between azoospermia and control groups

	Control and NOA	Control and NOA with no deletion	Control and NOA with deletion	NOA with no deletion and NOA with deletion
AUC	0.796	0.787	0.804	0.542
Cutoff value	78.5	78.5	78.5	70.5
Sensitivity (%)	70.7	65.7	75	55
Specificity (%)	88	88	88	60
PPV (%)	85.5	71.9	76.9	61.1
NPV (%)	75	84.6	86.8	53.8
Accuracy (%)	79.4	80.9	83.5	57.3

AUC, ROC, and PPV; positive predictive value, NPV; negative predictive value.

Table 2. Correlation of inhibin B with studied parameters among studied cases

	Inhibin B	
	R	*P*
Age (year)	0.018	0.825
TSH *(ulU/mL)*	0.119	0.147
PRL (ng/mL)	-0.007	0.930
FSH (mIU/mL)	-0.195	0.017
LH (mIU/mL)	-0.088	0.285
E2 *(Pg/mL)*	0.003	0.970
T.Testosterone *(ng/mL)*	0.187	0.022

Correlation coefficient. Spearman's correlation was used.

Table 3: Regression analysis for prediction of azoospermia

	Univariable				Multivariable			
	P	OR	95% CI		P	OR	95% CI	
Age (year)	0.465	1.276	0.849	2.387				
TSH *(ulU/mL)*	0.594	0.960	0.826	1.115				
PRL (ng/mL)	0.005	1.113	1.033	1.198	0.084	1.239	0.972	1.579
FSH (mIU/mL)	<0.001	1.333	1.209	1.470	0.002	1.858	1.261	2.738
LH (mIU/mL)	<0.001	1.189	1.095	1.292	0.154	1.103	0.642	1.204
E2 *(Pg/mL)*	0.015	1.032	1.006	1.058	0.203	1.123	0.942	1.210
T.Testosterone *(ng/mL)*	<0.001	0.738	0.669	0.813	<0.001	0.546	0.403	0.739
Inhibin B (Pg/ml L)	<0.001	.987	0.982	0.992	0.001	0.949	0.920	0.978

OR, odds ratio; CI, confidence interval. Logistic regression test was used.

Table 4: Regression analysis for prediction of AZF deletion among azoospermic cases

	P	OR	95% CI	
Age (years)	0.379	1.022	0.974	1.071
TSH (*ulU/mL)*	0.763	0.963	0.755	1.229
PRL (ng/ml)	0.213	1.048	0.973	1.129
FSH (mIU/mL)	0.205	1.016	0.991	1.041
LH (mIU/mL)	0.808	1.006	0.958	1.057
E2 *(Pg/mL)*	0.096	1.014	0.998	1.031
T.Testosterone *(ng/mL)*	0.055	1.122	0.998	1.261
Inhibin B (Pg/mL)	0.858	0.999	0.993	1.006

OR, odds ratio; CI, confidence interval. Logistic regression test was used.

Table 5: Comparison of TSH, PRL, FSH, LH, E2, TT, TT/FSH ratio, inhibin-B, and inhibin-B/FSH among control and azoospermia with and without AZF deletion groups

	Control group			Azoospermia group					P1	P2	P3	P4
	N=75			Without AZF deletion			with AZF deletion					
	n=75			n=35			n=40					
	Median	Range		Median	Range		Median	Range				
TSH *ulU/mL*	2.16	0.41	7	2.38	0.67	7.42	2.22	0.38	6.15	0.992	0.908	0.930	0.958
PRL ng/mL	6.61	2.96	11.8	7.4	2.78	14.6	8.14	3.02	60.21	0.061	0.194	0.025	0.329
FSH mIU/mL	4	1.84	7.1	12.6	0.25	40.5	13.74	0.67	57	<0.001	<0.001	<0.001	0.387
LH mIU/mL	4.84	1.18	8.21	5.93	0.1	27.1	6.49	1.15	24.4	<0.001	0.001	<0.001	0.815
E2 *Pg/mL*	23.08	16.94	33.78	20	20	63.2	21.1	20	132	0.204	0.091	0.766	0.131
T.Testosterone *ng/mL*	7.63	2.9	10.3	4.18	1.27	8.79	4.82	1.6	12.3	<0.001	<0.001	<0.001	0.102
TT/FSH ratio	1.82	0.53	4.67	0.32	0.06	13.92	0.38	0.05	13.96	<0.001	<0.001	<0.001	0.949
Inhibin B Pg/mL	120	70	228	73	10	201	69.5	27	207	<0.001	<0.001	<0.001	0.531
InhibinB/FSH ratio	28.97	16.20	82.61	5.15	0.97	288	5	0.85	43.28	<0.001	<0.001	<0.001	0.414

*P*1, comparison between control and azoospermia without AZF deletion and azoospermia with AZF deletion using Kruskal Wallis test.

*P*2, comparison between control and azoospermia without AZF deletion using Mann Whitney test.

*P*3, comparison between control and azoospermia with AZF deletion using Mann Whitney test.

*P*4, comparison between azoospermia with and without AZF deletion using Mann Whitney test.

Likewise, Andersson *et al.* (2004) reported that, inhibin B concentrations and inhibin B/FSH ratios were significantly lower in infertile males in comparison with fertile male. Accordingly, Alhalabi *et al*. has recorded that inhibin-B was increased in patients with successful sperm retrieval compared with patients with failed sperm retrieval 71.77 vs. 27.49 pg/mL, respectively [16].

In the current study, regarding validity of inhibin B for discrimination between azoospermia and control groups, The cutoff value for inhibin B level to diagnose NOA is 78.5 pg/mL, also the current study demonstrated that Inhibin B level had moderate accuracy to discriminate between controls and NOA (AUC=0.796) (sensitivity 70.7 %, specificity 88 %), as well as between controls and cases without AZF deletion (AUC=0.787) (sensitivity 65.7 %, specificity 88 %), and between controls and cases with AZF deletion (AUC=0.804) (sensitivity 75 %, specificity 88 %). However, inhibin B level failed to discriminate

between azoospermia cases with and without AZF deletion (AUC= 0.542) (sensitivity 55%, specificity 60%), as depicted in Table 1 and Figures 1, 2, 3 and 4.

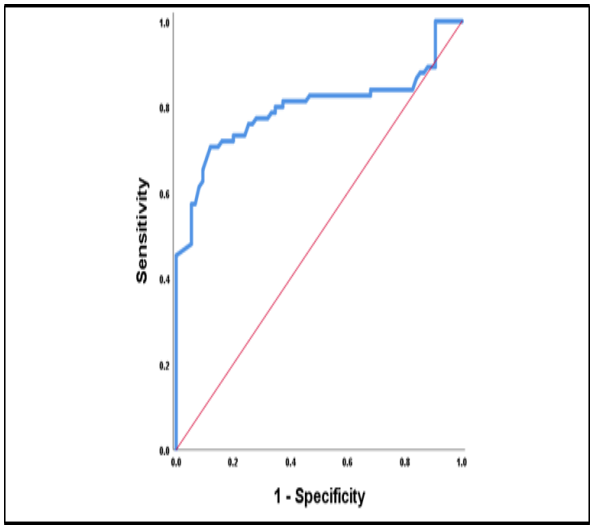


Figure 1: ROC curve of inhibin-B for discrimination between azoospermia and control groups

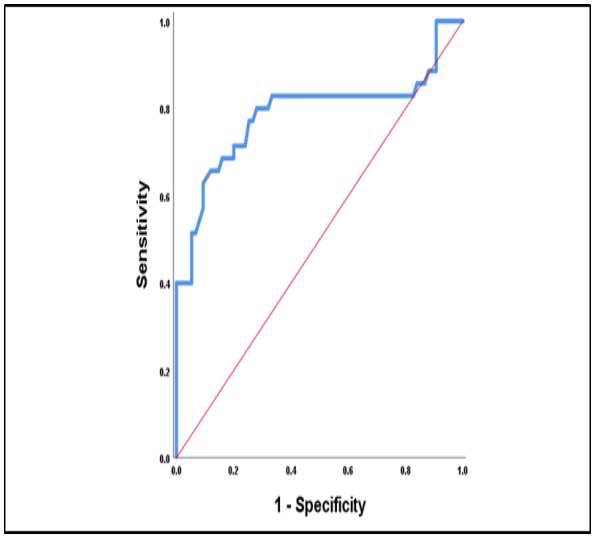


Figure 2: ROC curve of inhibin-B for discrimination between azoospermia without AZF deletion cases and control groups

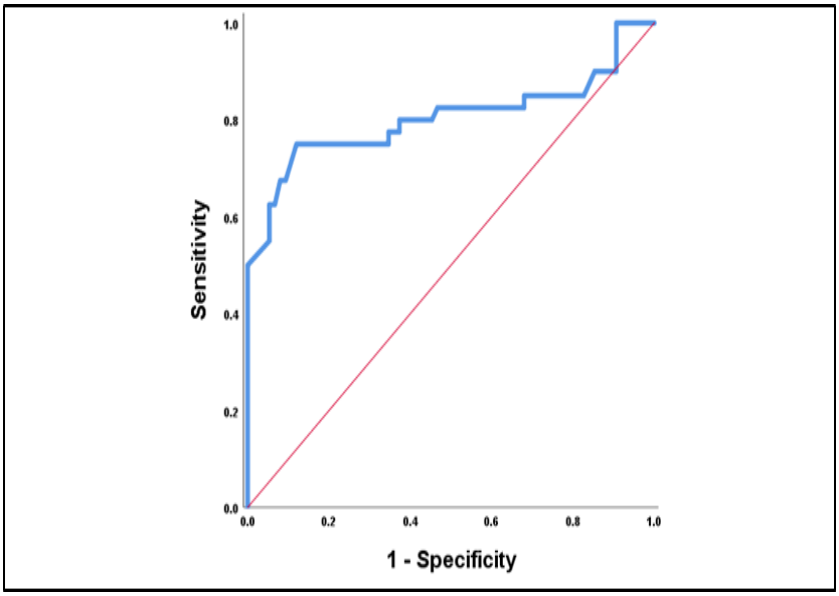


Figure 3: ROC curve of inhibin-B for discrimination between azoospermia with AZF deletion cases and control groups

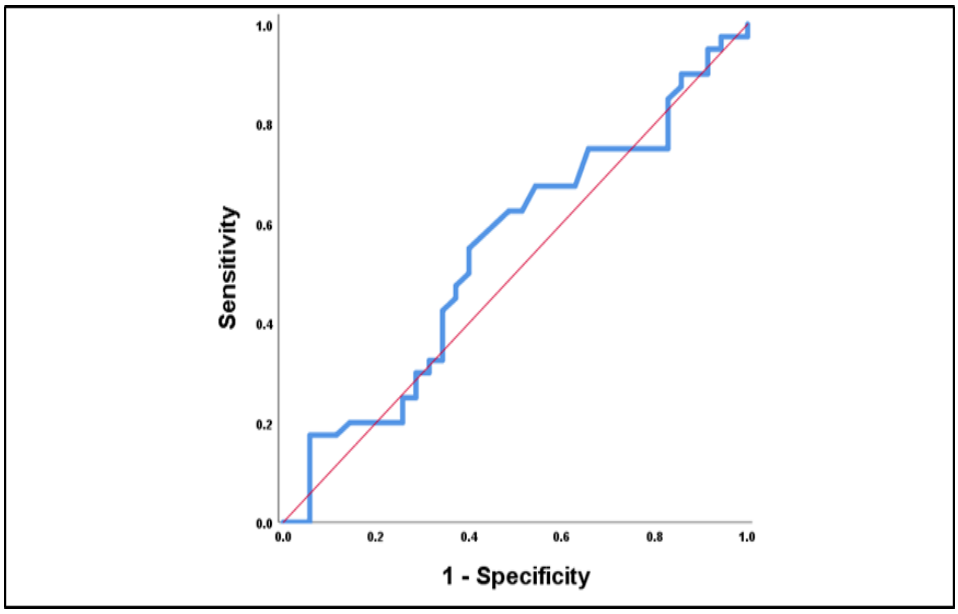


Figure 4: ROC curve of inhibin-B for discrimination between azoospermia with and without AZF deletion cases

Likewise, Wang *et al.* (2020) documented that, the best cutoff level of inhibin-B for predicting the successful sperm retrieval outcome was above 77.72 pg/mL (sensitivity 59%, specificity 92%, and AUC = 0.801).

On the other hand, Ballesca *et al.* (2000) found a cutoff level of 40 pg/mL for inhibin-B levels and also, Sikaris *et al.* (2005) reported 48 pg/mL. However, Pierik *et al.* (1998) demonstrated 139 pg/ml as a proper cutoff level for inhibin-B.

Nagata *et al.* have found that the best cut-off for inhibin-B concentration was 34.0 pg/ml in serum (specificity 95.6%, sensitivity 70.6%) [17]. Similarly Alhalabi *et al.* recorded that the best cutoff for serum inhibin-B was 35pg/ml for discrimination between +ve and -ve sperm retrieval and for the prediction of the existence of testicular spermatozoa (specificity 80.85, sensitivity 75.86) [16]. Thus, they concluded that Inhibin-B may be a predictive marker of spermatogenesis in the males with NOA. In addition, Kong *et al.* have demonstrated that the cutoff for inhibin B to diagnose NOA is 45.9 pg/ml (97% PPA and 85% NPV) [18]. This significant difference of inhibin B cutoff levels might be due to differences in ethnicities, environmental backgrounds in each study, inclusion criteria and the methodologies used in different labs (*Wang et al.*) [19].

The current study demonstrated that azoospermia group was subdivided into Azoospermia with AZF deletion 53.3% (40/75) and Azoospermia without AZF deletion 46.7% (35/75), as indicated in Table 6. Likewise Elsaid *et al.* came in accordance with current study regarding frequency as they demonstrated that AZF micro-deletion was detected in 64% (16/25) of cases with azoo-spermia and 53.8% (14/26) of cases with oligo-spermia [15]. In addition, Atia *et al.* have demonstrated that; 22% (11/50) of the study cases had a minimum one or more AZF micro-deletion [20]. However, a Japanese large-scale study screened 1030 infertile males for Y-Chromosome Micro-deletions revealed a prevalence of about 7% (including all AZF abnormalities) among severe oligo-zoospermic or azoo-spermic males [21].

Variable frequencies of AZF micro-deletions were reported in previous studies [22] reported 10.93% (134/1,226), while Ambulkar *et al.* [23] reported 8.33% (13/156). Regarding AZF micro-deletions frequency in Arab populations between infertile men, Morocco reported (18.83%) [24], Kuwait reported 2.6% [25], Tunisia reported 6.85% [26] Saudi Arabia reported (3.2%) [27] and also Abdel-Razek *et al.* (2017) estimated the frequency of AZF micro-deletions among Egyptian infertile males with NOA (6.1%) and (3.16%) in patient with sever oligo-zoospermia (SOZ).

Table 6: Frequency of AZF deletion types

Types of AZF deletion		Azoospermia group n=75	
	SY	N	%
A	84	1	1.3%
	86	2	2.7%
	615	3	4.0%
B	127	3	4.0%
	134	6	8.0%
	142	4	5.3%
C	242	14	18.7%
	254	9	12.0%
	255	8	10.7%
	1125	4	5.3%
	1197	6	8.0%
	1206	7	9.3%
	1291	22	29.3%
A		4	5.3%
B		9	12.0%
C		40	53.3%
AB		2	2.7%
AC		4	5.3%
BC		9	12.0%
ABC		2	2.7%

Concerning the frequency of AZF deletion types, the current study demonstrated that; AZF-C deletion type in all cases with micro-deletions (53.3%), AZF-B deletion in (12%) and AZF-A deletion was found in (5.3%), as presented in Table 3. Similar results reported by Ambulkar *et al.* (2014) who recorded that AZFc micro-deletion was the most frequent 84.6% (11/13) followed by AZFb 15.4% (2/13) and AZFa 15.4% (2/13). Furthermore, Elsaid *et al.* reported that; AZFc micro-deletion was the commonest 56.3% (9/16) in cases with azoo-spermia [15]. Meanwhile, AZFa micro-deletion was the commonest 71.4% (10/14) in cases with oligo-spermia. Singh and Raman (2005) evaluated the frequency of AZF micro-deletions and they found that AZFc micro-deletion was the commonest type of deletions detected between infertile males. The documented ratios of AZF micro-deletions in India range from 2-10% [28]. Most of the recorded data in Asian countries including India and Japan showed that AZFc micro-deletions is the most common type of AZF micro-deletions followed by AZFa and AZFb micro-deletions [21, 29, 30].

The current study demonstrated that the most frequent combination was AZFbc (12%), and then AC (5.3%) and lastly AB and ABC (2.7%), as listed in Table 3. This was in agreement with Elsaid *et al.* [15] who documented that the commonest combinations were AZFbc microdeletions (18.8%), and then AZFbcd (12.5%) and lastly AZFabc (6.3%). Comparable outcomes were observed in Iranian records as regards frequency of AZF micro-deletion and revealed high frequencies of AZFbc micro-deletions (20%), and then AZFabc (5%) [31]. Moreover, Fu *et al.* (2012) reported that**,** AZFbc micro-deletions (18.75%) was the commonest combination followed by AZFabc (3.47%) and lastly AZFac and AZFab with the same frequency (2.08%), While Kim *et al*. [22] revealed that AZFc micro-deletion was the commonest (51.49%), and then AZFbc micro-deletion (20.90%), AZFb micro-deletion (8.21%), AZFa micro-deletion (7.46%), and AZFabc micro-deletion (5.22%).

In the current study, inhibin B showed a significant positive correlation with total testesterone (r = 0.187; p= 0.022) and a significant negative correlation with FSH (r = -

0.195; p = 0.017), as listed in Table 2. Otherwise, no significant correlations were found regarding inhibin B with studied parameters among all cases. In the same line, Al-Chalabi *et al.* (2019) reported that serum inhibin B showed a strong negative correlation with FSH level (r = -0.46, p=0.000) and a strong positive correlation with testosterone levels (r = 0.326, p=0.001) in oligo-spermic and azoo-spermic cases. Likewise, Corinne *et al.* (2020) demonstrated a significant negative correlation between FSH and Inhibin B in the normo-zoospermic control group (r = -0.367; p = 0.011), and in the infertile cases (r = −0.511; p < 0.0001).

Through performing a logistic regression analysis, the current study revealed that higher PRL, FSH, LH, E2, lower testosterone, and inhibin levels were associated with risk of azoospermia in univariable analysis. While in multivariable analysis, only higher FSH, lower testosterone, and inhibin B levels were considered as risk predictors for azoospermia susceptibility. On the other hand, all hormones (TSH, FSH, LH, E2, PRL, T. testosterone, and inhibin B level) could not be considered as risk predictor of AZF deletion among azoospermia cases, as indicated in Tables 3 and 4.

Kim *et al.* demonstrated [22] that FSH and LH levels among infertile cases with AZF micro-deletions were associated with a significant increase compared with cases without AZF micro-deletions. High FSH levels was correlated with AZF micro-deletions and sever testicular failure [31]. In addition, the adequate FSH levels regulate and maintain the proper production of sperms. It was demonstrated that azoospermic cases with high FSH level was associated with decreased success rate of assisted reproductive techniques [32, 33]. In the same line, Wang *et al.* [34] demonstrated that AZF micro-deletion was associated with a high FSH level. Elsaid *et al.* [15] did not recorded any association between FSH, LH, and prolactin as well as the presence or absence of the AZF micro-deletions and this in agreement with our study result that revealed no significant difference was found in any of the studied hormones FSH, LH, total testosterone, and inhibin B between azoospermia without AZF deletion and azoospermia with AZF deletion.

Conclusion

The current study emphasizes the importance of evaluation of Y-chromosome micro-deletions for infertile men with abnormal semen parameters. Azoospermia was associated with increased rate of AZF micro-deletions. It was found that AZFc deletion was the most frequent type of Y chromosome micro-deletion followed by AZFb and AZFa. Non obstructive azoospermia was associated with a significant decrease of inhibin B and total testosterone levels, and also associated with higher PRL, FSH, and LH levels. No significant difference was found regarding the studied hormones FSH, LH, total testosterone, and inhibin B between azoospermia without AZF deletion and azoospermia with AZF deletion.

Higher FSH, lower testosterone, and lower inhibin B levels were considered as risk predictors for azoospermia but could not be considered as risk predictor of AZF deletion among azoospermia cases.

Recommendation

Advanced studies are needed on a larger group of patients to validate the results of this study to evaluate the frequency and types of AZF micro-deletions as well as their relation to FSH, LH, Prolactin, total testosterone, and inhibin B with careful consideration to geographical areas, environmental factors, occupational exposures, and different ethnic populations because infertility is a multifactorial problem. Genetic counseling and evaluation of Y chromosome micro-deletions should be done prior to assist the reproductive techniques for infertile patients to prevent transmission of AZF micro-deletions to their male offspring.

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ORCID

Ahmed Gamal Elmetwaly Ghanem
https://orcid.org/0000-0002-4292-0142
Reham Mohammed El-Farahaty
https://orcid.org/0000-0001-8878-7095
Sohier Yahia
https://orcid.org/0000-0002-6974-5997
Hosam Eldin Abdel Twab Abdel Twab
https://orcid.org/0000-0003-0684-4151

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