Histopathological Evaluation of Non-Obstructive Azoospermic Males Using Testicular aspirate (TESA) biopsy

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Abstract

Background: Male infertility considers a real diagnostic challenge because of several factors have been involved in its pathogenesis. Histopathology of the testicular biopsy consider the corner stone in the diagnosis. The aim of this study is to report the utility of using of Testicular aspirate biopsy (TESA) for the evaluation of the male infertility & compare the outcomes with different parameters.

Materials & methods: we design prospective study for 205 infertile males with Non -Obstructive azoospermia who referred to the Teeba IVF & genetic center for the period from December 2016 to December 2018, they underwent bilateral TESA for histopathological evaluation & 45 patients out of 205 male had previous open testicular biopsy. The hormonal profile for all patient. were done. **Results:** among 205 patients underwent TESA procedure the commonest histopathological pattern was spermatocyte maturation arrest, present in 113 patients (55.1%) followed by round maturation arrest in 41 (20%). There was a good degree of agreement between the spermatogenesis scoring according to TESA and open surgical biopsy (P=< 0.001). The plasma levels for both LH & FSH were statistically showing significant differences in their levels according to the TESA spermatogenesis scoring. (P<0,001).**Conclusion:** the utility of TESA biopsy in the evaluation of testicular biopsy from Azoospermic males is significantly associated with the histopathological pattern of spermatogenesis & hormonal profile.

Key words: *TESA, Non -obstructive azoospermia (NOA), histopathology, microscopic testicular sperm extraction (TESE), spermatogenesis score.*

Introduction

Infertility in general can be defined as failure to achieve conception after twelve months of sexual unprotected intercourses.[1] Approximately 10% of couples unable to conceive and asked treatment for primary infertility with equal percentage unable to achieve a second conception . [2,3]

Male infertility causes can be subdivided into three main categories; pretesticular, testicular & post testicular.[4]

Azoospermia simply defined as complete absence of spermatozoa in the ejaculated semen for at least two times & its account for about 1% of male population & 10 % of infertile male.[5]

For male infertility evaluation, it's very important to have a comprehensive clinical history & complete physical examination along with hormonal profile and semen analysis [3], however different testicular phenotype changes can't be predicted accurately through these parameters mentioned above only, so clinicians recommended testicular biopsy and / or testicular sperm aspiration (TESA) to the accurate evaluation of the spermatogenesis and any pathological conditions.[6,7]

The aim of the present study is to evaluate a sample of Iraqi azoospermic males clinically & pathologically & to compare the efficacy of TESA biopsy versus open surgical testicular biopsy in evaluating the process of

spermatogenesis.

Materials and Methods

Between the period from December 2016 to December 2018 a prospective study for205 infertile azospermic men was done, a detailed history & physical examination for all patients were done to exclude obstructive azoospermic ones by specialist in the center, and hormonal assessment for testosterone, LH, and FSH were performed.

All patients then were subjected to bilateral testicular sperm aspirate (TESA) procedure under general anesthesia, the samples that obtained were divided into two parts; one to be fixed in liquid nitrogen when it was positive for mature sperms, the other part preserved into Boun's fixative for histopathological assessment.

Only 45 men who undergone TESA have pervious open testicular biopsies, the slides for those biopsies together with those from TESA biopsies were examined by two expert pathologists and the scoring for spermatogenesis was performed according to Johnsen 1970 modified by Holestein et. al. [8] And then the patients were subdivided into six groups according to the histopathological patterns.

Statistical analysis: Statistical analysis was carried out using SPSS version 20. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). ANOVA test was used to compare means between three groups or more. Kappa statistics was used to assess the degree of agreement between paired categorical readings. A p-value of ≤ 0.05 was considered as significant.

Results

The histopathology of 205 TESA biopsies were reviewed retrospectively with reviewing the results of open testicular biopsies for 45 patients. the age range of the patients from 19-60 years, 195 of patients were of primary infertility (95.1%), 10 patients were of secondary infertility (4.9%), with 76.6% of the total patients being with elevated FSH, 64.9% elevated LH and 89.3% with low testosterone (table 1).

Table 1: The Distribution of patients according to study variables

Study variables			
Age (years)		(36.89± 7.64)	(19-60)
Type of infertility	**Primary**	195	95.1%
	Secondary	10	4.9%
	Total	205	100.0%
FSH (IU/L)	**Normal**	48	23.4%
	Elevated	157	76.6%
	Total	205	100.0%
Testosterone (ng/dl)	**Normal**	15	7.3%
	Low	183	89.3%
	Elevated	7	3.4%
	Total	205	100.0%
LH (IU/L)	**Normal**	133	64.9%
	Low	2	1.0%
	Elevated	70	34.1%
	Total	205	100.0%

In this study, the most common pattern of spermatogenesis, according to TESA scoring was the spermatocyte maturation arrest, present in 113 patients (55.1%) fallowed by round maturation arrest in 41 (20%) (table 2) and according to testicular biopsy the spermatocyte maturation arrest pattern of spermatogenesis is present in 18 patients (40.0%) (table.3).

Pattern			
	Spermatogenesis score	**No.**	**%**
Normal spermatogensis	10	0	0%
Hypospermatogensis	9 and 8	8	3.9%
Round spermatid Maturation arrest	6 and 7	41	20%
Spermatocytes maturation arrest	3, 4 and 5	113	55.1%
Sertoli cell only	2	19	9.3%
No germ cells and no sertoli cells	1	24	11.7%
Total		205	100.0%

Table 2: distribution of Azoospermic males according to histopathological pattern in TESA biopsies. (n=205)

Table 3: The classification of patients according to open biopsy score (n=45)

Pattern	**Histopathological classification of testicular biopsies**		
	spermatogenesis score	**No.**	**%**
Normal spermatogenesis	10	0	0%
Hypospermatogensis	9 and 8	3	6.7%
Round spermatid Maturation arrest	6 and 7	11	24.4%
Spermatocytes maturation arrest	3, 4 and 5	18	40.0%
Sertoli cell only	2	6	13.3%
No germ cells and no sertoli cells	1	7	15.6%
Total		45	100.0%

Table 4 & Fig. 1 show the degree of agreement between the spermatogenesis scoring according to TESA and open biopsy. The value of Kappa equal (0.701) which indicate good agreement between two types of score. (N=45, P=< 0.001*).

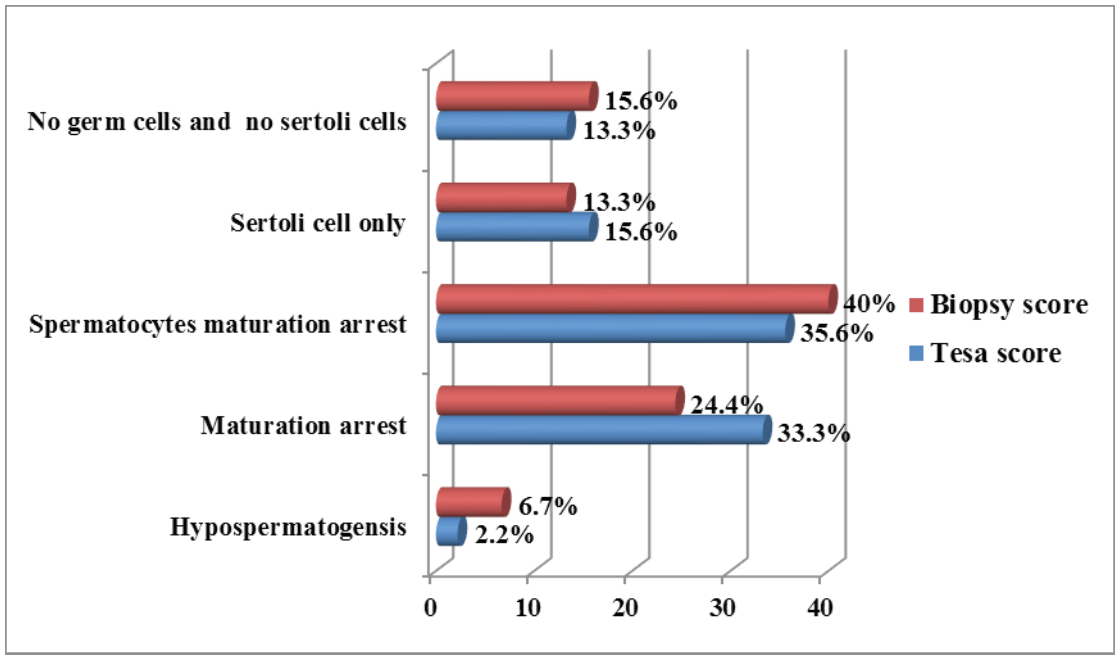

Figure 1: Comparison between spermatogenesis scoring using TESA biopsy & open surgical biopsy (n=45)

Table 4: Degree of agreement between diagnosis of patients according to TESA and open surgical biopsy scores.

Results according to TESA score	Results according to surgical biopsy score		Total	Proportion in agreement	Kappa	P-value
	6-9	1-5				
6-9	12 (26.7%)	4 (8.9%)	16 (35.6%)	0.867	0.701	< 0.001*
1-5	2 (4.4%)	27 (60.0)	29 (64.4)			
Total	14 (31.1%)	31 (68.9%)	45 (100.0%)			

The mean plasma levels for all patients for testosterone, LH and FSH were 360 ng /dl, 8.18mIU/L and 24.20mIU/L respectively. There were no statistically significant differences among the groups of spermatogenesis scoring according to the plasma levels of testosterone, while the plasma levels for both LH & FSH were statistically showing significant differences in their levels according to the TESA spermatogenesis scoring. (P<0,001) as shown in Table 5.

Table 5: The mean differences of testosterone, LH and FSH according to TESA biopsy score.

Pattern (according to TESA score)	No.	Testosterone ng /dl		LH mIU/L		FSH mIU/L	
		Mean	SD	Mean	SD	Mean	SD
Hypospermatogensis	8	362.00	154.22	7.4250	6.48311	20.52	16.25
Round spermatid Maturation arrest	41	395.21	187.85	5.2976	3.40533	13.80	10.03
Spermatocytes maturation arrest	113	360.04	158.58	7.8628	4.39440	23.52	11.69
Sertoli cell only	19	357.47	184.20	11.7263	7.21563	38.37	14.78
No germ cells and no sertoli cells	24	301.04	184.04	12.0417	7.63151	35.18	20.38
Total	205	360.00	170.33	8.1800	5.49107	24.20	15.01
P - value	0.329			<0.001*		<0.001*	
F- value	1.162			9.172		16.284	

Discussion

Infertility is not uncommon health problem in multiple societies & male infertility account for about quarter of the causes of infertility. [9]

In this study evaluation of non - obstructive azoospermic males were done by TESA mainly & in 45 cases the TESA were preceded by open surgical biopsy, the most common pattern for spermatogenesis was spermatocytes maturation arrest similar to the results obtained by several studies although none of them use TESA like Al- Dabbgh et al [10] that is carried in Iraq Using testicular FNA for assessment of spermatogenesis & also Khalifa et al [11] , Seo and Ko [12], Amer et al [13], and Tusjimura et al [14]. while other studies reported lower frequency of spermatocytes maturation arrest like those carried in Saudi Arabia by Molham M et al [15] & in Jordan by Haddad et al [16] revealed that the most prevalent pattern of spermatogenesis among azoospermic males was normal spermatogenesis followed by hypospermatogenesis putting in our minds that those studies were not excluding patients with Azoospermia due to obstructive causes as in ours, Beside that germ cell maturation arrest may be due to variety of causes like genetic factors or secondary causes such as toxic substances (chemotherapy or radiotherapy), or due to certain chronic renal or liver diseases.

In this study there was good agreement in spermatogenic scoring between TESA and open biopsy, this result indicate that TESA can be used as a substitute to open biopsy because its less invasive and eliminate the need for incision, it's also simple & cost effective than those of TESE & open surgical biopsy which report higher incidence of intra- testicular hemorrhage, a more important advantage of TESA procedure is low complication rate when it compared with other procedures.

However, the testicular architecture is well preserved in TESA biopsy than in testicular FNA .

These results agrees with Abraham Kurien et al [17] Sridevi et al[18], who found no significant differences between spermatogenic patterns in testicular biopsy and

testicular FNA, this result indicates that TESA can be used as a substitute to FNA which is less representative.

Our present study revealed that most of the patients were of primary infertility type (95.1%) with 76.6% of them being elevated FSH, 64.9% elevated LH and 89.3% with low testosterone, these results agree with Ramesh Babu et al[19] , and Accordingly, most of the patients in the current study are with hypergonadotrophic hypogonadism which may be due to different etiologies ; congenital or acquired causes (due to damage to or dysfunction of the gonads) include orchitis, trauma, surgery, autoimmunity, chemotherapy, radiation, infections (e.g., sexually-transmitted diseases), toxins (e.g., endocrine disruptors), and drugs (e.g., antiandrogens, opioids, alcohol). [20,21,22]

Preoperative assessment for FSH levels in azoospermic men has long been used as an indicator for prognosis & to correlate with spermatogenesis score as established by several studies using different methods like Ezeh et al[23] showed that there were significant differences between means of FSH level and pattern of spermatogenesis according to testicular biopsy and similar results obtained by Tunc L et al [24] with TESE biopsy, our study revealed similar outcomes with significant differences between FSH levels & spermatogenesis score (P<000.1). according to these results, FSH level can be used as indicator or predictor for patients pattern according to TESA scores.

Our study also revealed the same correlation between LH levels & TESA scores with the highest levels being with those of spermatogenesis score one & two , in contrast to other studies carried by Elchanan Bar-On et al [25] and Tomomoto Ishikawa et al [26] , who revealed that serum LH level appears to have no significant association with spermatogenic pattern in testicular biopsy, while our results were in agreement with the results obtained by Ramesh Babu et al [19] and Micic S et al [27] who revealed that mean serum LH levels were significantly associated with spermatogenesis score with the highest level in Sertoli- only categories (score 2).

Similar to several studies carried worldwide, we didn't notice significant differences between spermatogenesis scoring obtained by TESA scoring & serum testosterone level: Elchanan Bar-On et al [24] showed that plasma testosterone level is of no diagnostic value in predicting any specific spermatogenic pattern in testicular biopsy. Sultan A. Althakafi et al [28] and Reifsnyder JE et al [29] showed that serum testosterone level appears to have no significant association with microdissection testicular sperm extraction (microTESE) outcomes in non-obstructive azoospermia. Tomomoto Ishikawa et al [26] concluded that testosterone level was unrelated to the stage of maturation arrest in testicular biopsy .

In summary, We demonstrate that TESA biopsy is an efficient method for assessment of spermatogenesis in NOA males, its valuable surgical method as its faster, less invasive &less coasty than TESE, and can cover several areas of the testis more than those covered by open surgical biopsy, with keeping the testicular architecture well preserved if it compared with testicular FNA.

Ethical Clearance: the research was prepared and done according to standardized national law of research committee of ministry of higher education and scientific research of Iraq.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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