Validity of Testicular Biopsy Among Azoospermic Patients Prior to Intracytoplasmic Sperm Injection

Dr. Abdulaali Hussain Salman, [*]
Dr . Mundhir T.Ridha AlBarzanchi, [**]
Dr. Sabah Mahdi Hussain [*]
Dr. Ziyad T.H.Al-Naeib [*]

الخلاصة

الهدف:كان الهدف من هذا البحث هو تقييم الأهمية السريرية الطبية لدور الخزعه النسيجية التشخيصية للخصية كمؤشر للتنبوء بنجاح عملية استخلاص الحيامن من خزعة الخصية العلاجية (TESE) من المرضى المصابين باللانطفية (Azoospermia) قبل الاشتراك في برامج عمليات طفل الأنبوب المجهري الإجباري ونقل الأجنة (ICSI).

المنهجية : شملت الدراسة ستون مريضا مصاب بالعقم بسبب باللانطفية (عدم وجود النطف في السائل المنوي). اجري التقييم ألسريري والمختبري الشامل لجميع الأشخاص الذين تشملهم الدراسة. فحص مستوى(الهرمون المنبه للجريب (FSH)، الهرمون اللوتيني (LH) والهرمون الذكري (التيستوستيرون) وهرمون البرولاكتين)بالدم كما تم قياس حجم الخصية.[*]

أخذت الخزع من نسيج الخصى لستين مريضا مصابين باللانطفية لإجراء الفحص النسيجي التشخيصي وقيمت الحالة النسيجية لغرض التصنيف واعتمادًا على نوع حالات الإنطاف الأكثر تقدما(المرحلة المتقدمة من التكوين النطفي) وعلى وجود بؤر إنطاف حاوية على النطف و على نوع الإيقاف النطفي في حالة الإيقاف النطفي التام(CMA).

النتائج : أظهرت الدراسة عدم وجود اختلاف ذا قيمة معنوية بين معدل حجم الخصية ومعدل تركيز الهرمونات التناسليةبالدم في حالة الإيقاف النطفي التام(CMA) مقارنة مع حالة الإيقاف النطفي مع وجود بؤر إنطاف (MAFS) وكذلك بين معدل حجم الخصية و معدل تركيز الهرمونات التناسلية في حالة وجود خلايا سرتولي التام (CSCO) مقارنة مع حالة خلايا السرتولي المترافقة بوجود بؤر انطاف (SCOF) .

إن نتائج هذه الدراسة بينت إن الفحص النسيجي التشخيصي لخزعة الخصية المستحصلة جراحيا ذو فائدة كبيرة في التنبؤ بنجاح عملية استخلاص النطف جراحيا بعملية الخزعه النسيجية العلاجية للخصية (TESE) مقارنة مع معدل حجم الخصية ومعدل تركيز الهرمون المنبه للجريب(FSH)بالدم حيث إن الفحص النسيجي التشخيصي اظهر وجود نطف في حالاتNS,HS,MAFS and SCOF ولم توجد في الفحص النسيجي لحالاتCMA,CSCO and TF .

التوصيات : إن الطريقة التصنيفية النسيجية الفسلجية الجديدة لنسيج الخصية سوف تساعد في تقييم الحالات اللانطفية التي يمكن علاجها بواسطة عملية طفل الأنبوب ألمجهري الإجباري.

Abstract

Objective: To evaluate the clinical significance of open diagnostic testicular biopsy as prognostic predictor of successful sperm retrieval among azoospermic infertile patients**.**

Design: Prospective study.

Setting: Infertility clinic and assisted reproduction unit at the institute of embryo research and infertility treatment, Baghdad University.

Patients: Sixty infertile azoospermic patients.

Intervention: Pieces of testicular tissue taking during open diagnostic multiple bilateral testicular biopsies was prepared for histological examination and grouped according to well-defined histopathological patterns. Measurement of testicular size and serum reproductive hormones (FSH, LH, Testosterone, and PRL) were done for all these sixty azoospermic patients.

Main Outcome Measures: Sperm found with a new histopatholigical categorization and sub categorization.

Results: Our study showed no significant difference between mean testicular size and mean serum reproductive hormonal (FSH, LH, T and PRL) concentrations of MAFS compared to CMA and that of SCOFS compared to SCO. The sperm found with open diagnostic bilateral biopsy was positive in transverse section of seminiferous tubules of NS, HS, MAFS, and SCOFS, where as it was negative in CMA, CSCO, and TF.

Conclusions: It was concluded from the results of the work that the new histological categorization of open testicular biopsies was found to be practical, informative, and most useful diagnostic and prognostic predictor to select the patients for TESE-ICSI.

Key Words: Azoospermia, testicular biopsy, testicular histology, FSH, LH, T, PRL.

[*] Institute for Embryo Research and Infertility Treatment, Baghdad Teaching Hospital, University of Baghdad
[**] Department of Urology, Baghdad Teaching Hospital, College of Medicine, University of Baghdad
[*]

Introduction

Male factor infertility is responsible in about 20% to 40% of infertile couples [1] , the most identifiable cause is the varicocele which found in approximately 40% of infertile men [2]. Azoospermia presents in about 10% to 20% of infertile men, other isolated causes include oligospermia, asthenospermia, teratospermia, low semen volume and ejaculatory problems can cause male infertility as well [3].

Conventional intracytoplasmic sperm injection (ICSI) using ejaculated spermatozoa is now routinely recommended for most men with either a low sperm count ,poor motility , high percentage of abnormal forms or spermatozoa that in previous in vitro fertilization (IVF) attempts have failed to fertilize oocytes [4]. Intracytoplasmic sperm injection (ICSI) of surgically retrieved sperm has proved to be a successful procedure [5].

In cases of obstructive azoospermia, sperm retrieval is simple and predictable. However, in cases of non-obstructive azoospermia, the chance of sperm retrieval is less. The sperm can be retrieved from testes of azoospermic men by testicular sperm extraction technique (TESE) for use with ICSI, with high chance of achieving pregnancies, and deliveries of normal children [6].Spermatogenesis may vary geographically within the failing testis, depending on the severity of germ cell injury, so testicular recovery may not always be successful in all patients with non-obstructive azoospermia [7].Testicular sperm extraction technique must be restricted to those patients who have the best chance of extracting testicular spermatozoa [8], accordingly the identification of prognostic indicators of sperm retrieval success with TESE is clearly needed. Unfortunately previous studies shown that none of biological or clinical parameters are predicting successful sperm retrieval with testicular biopsy [9, 10, 11].

The objective of this study was to evaluate the clinical significance of open diagnostic testicular biopsy as prognostic predictor of successful sperm retrieval among azoospermic infertile patients for use in ICSI.

Methodology

This study was carried out at the Institute of Embryo Research and Infertility treatment, Baghdad University. Sixty infertile men diagnosed with azoospermia based on details of history, physical examination and at least two standard semen analyses found to be devoid of sperm cells, moreover examination of post ejaculate urine in case of aspermia was performed to exclude retrograde ejaculation as sperm may be recovered from urine. Physical examination and mostly concentrated on examination of the external genitalia, size of testis was estimated by ruler measurement of longitudinal axis of testis.

Blood samples obtained from all patients, were immediately centrifuged and the plasma was separated and stored at (-20°C) until it was analyzed. Plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T) ,and prolactin (PRL) were determined by minividas instrument (BioMercux company, model VIDAS , France).

Open diagnostic bilateral testicular biopsy was performed for all sixty azoospermic patients under general anaesthesia.The testicular biopsy specimens were fixed in Bouin's solution. During histopathological evaluation about 50 to 100 seminiferous tubules were counted and assessed and the numbers of the tubules containing germ cells in any stage of maturation were recorded.

Depending on the identification of the most advanced pattern of the spermatogenesis and on the presence or absence of the sperm focus, testicular histology was modified into the following suggested histopatholigical categories:

1. Normal spermatogenesis (NS) {this pattern showed complete spermatogenesis and perfect tubules in 60% or more of the tubules}.
2. Hypospermatogenesis (HS) {: reduction in the degree of normal spermatogenic cells}.
3. Maturation arrest with focal spermatogenesis (MAFS) {in this pattern some of tubules yielded sperm represented by presence of sperm focus}.
4. Complete maturation arrest (CMA) {in which none of tubules showed sperm}.
5. Sertoli cell only with focal spermatogenesis (SCOFS) {in this pattern there is focus of spermatogenesis}.
6. Complete Sertoli cell only (CSCO): {this pattern showed only Sertoli cells and germ cells were completely absent}.
7. Tubular fibrosis (TF) {no germ cells or Sertoli cells in the seminiferous tubules}.

The rest of testicular biopsy specimen may processed to confirm the presence of motile spermatozoa that may be used in the procedure of injection by ICSI technique and the remaining testicular tissue that contain motile spermatozoa was ready for cryopreservation.

All values were presented as mean ± standard error of the mean (SEM). Student's t-test and chi-squared test were used for analysis of the data for all evaluation. Statistical significance was defined as P-value of less than 0.05.

Results

The mean age of sixty infertile azoospermic patients was 38.41 years (Ranged from 27 to 58 years). The mean duration of infertility was 10.37 years (Ranged from 1 to 30 years). The frequencies of various histopathological patterns observed during evaluation of the specimens of infertile azoospermic patients were recorded. The means of testicular size of normal spermatogenesis, hypo spermatogenesis, maturation arrest with focal spermatogenesis, complete maturation arrest, Sertoli cell only with focal spermatogenesis, complete Sertoli cell only, and tubular fibrosis, were 4.16±0.13, 3.68±0.20, 3.29±0.10, 3.17±0.12, 3.20±0.06, 3.16±0.05, 2.38±0.15 cm respectively. Serum FSH, luteinizing hormone (LH), serum testosterone (T), and prolactin (PRL) concentrations are shown in Table ١.

Table 1: Serum reproductive hormone concentrations among azoospermic patients with various histopathological patterns.

	NS	HS	MAFS	CMA	SCOFS	CSCO	TF	P value
No .of patients n=60	10 (16.66 %)	5 (8.33%)	17 (28.33 %)	17 (28.33 %)	3 (5 %)	5 (8.33%)	3 (5 %)	
FSH (mIu/ml) N.V=(1.7-12.0mIu/ml)•	8.85±1.58 *	13.25±1.7	19.37±2.02	22.58±2.61	19.8±11.6	24.9±3.99	45.56±10.3	<0.01
LH (mIu/ml) N.V=(1.1-7.0 mIu/ml) •	7.43±1.26**	6.43±1.38	8.39±1.20	8.07±1.05	10.42±1.1	11.77±1.52	20.31±7.83	<0.05
T (ng/ml) N.V=(3.0-10.6ng/ml) •	4.75±0.50 †	3.65±0.31	3.45±0.28	4.14±0.55	3.34±0.43	2.55±0.24	2.36±0.14	<0.05
PRL (ng/ml) N.V=(1.5-19.0ng/ ml) •	7.73±1.04††	7.10±1.15	9.52±1.20	9.52±1.12	8.42±0.92	10.01±1.76	17.37±1.09	<0.05

NS : Normal spermatogenesis , **HS** : Hypospermatogenesis , **MAFS** : Maturation arrest with focal spermatogenesis, **CMA** : Complete maturation arrest ,**SCOFS** : Sertoli cell only with focal spermatogenesis , **CSCO** : Complete Sertoli cell only, **TF** :Tubular fibrosis, **T**:Testosterone, **PRL**:Prolactin . **• N.V:** Normal value according to (BioMercux company, model VIDAS, France).
Data are mean ± standard error of the mean (SEM).
Parentheses represent the percent of patients of each group.
* $P < 0.01$ significantly different from other groups except HS group.
** $P < 0.05$ significantly different from TF group.
† $P < 0.05$ significantly different from TF and CSCO groups.
†† $P < 0.05$ significantly different from TF group.

There were significant difference ($p<0.001$) between the means of testicular size, serum FSH of normal spermatogenesis compared to other groups except hypospermatogenesis. The mean of luteinizing hormone (LH) and PRL hormone concentrations of normal spermatogenesis was significantly lowers ($P < 0.05$) than tubular fibrosis. Lastly the mean of testosterone concentration of NS was significantly higher ($P < 0.05$) compared to CSCO and to TF.

The sperm found with open diagnostic bilateral biopsy was positive in transverse section of seminiferous tubules of NS, HS, MAFS, and SCOFS, where as it was negative in CMA, CSCO, and TF.

Discussion

The elevated FSH levels may indicate decreased germinal cell mass, diminished Sertoli cell function and consequently primary testicular failure [(12)].The concentration of FSH rises with increasing testicular destruction because FSH is under the negative feedback control of the seminiferous epithelium and when it is severely damaged it will result in a decrease of inhibin production and elevated FSH. Normal FSH in azoospermia patients was compatible with obstruction or malformation of the efferent duct system [(13)]. Elevated LH concentration in this study indicates a severe degree of germ cell depletion [(14)].

The mean testosterone concentration of NS was higher ($P < 0.05$) than that of CSCO and TF. This finding reflects Leydig cell dysfunction. The Leydig cell dysfunction may be secondary to variable causes that may induce spermatogenic damage accompanied by Leydig

cell dysfunction and suppression of androgen production, associated with defect of spermatogenesis [15].

Elevated PRL due to various etiologies is often leading to reproductive dysfunction. In men, hyperprolcatinaemia causes hypogonadotropic hypogonadism with decreased LH and FSH secretion (16), and low serum testosterone concentration [17].

Now it is possible to actually extract sperm from the testis of azoospermic patients, and inject them into their wives eggs by intracytoplasmic sperm injection. Testicular sperm extraction combined with intracytoplasmic sperm injection (TESE-ICSI) is promising treatment for many azoospermic men.[18] .

The TESE-ICSI is costly and an invasive procedure which might have complication and because failure in TESE is as many as 52% of patients resulting in an unnecessary ovarian stimulation cycle for their partner [9]. Accordingly the identification of prognostic indicators of sperm retrieval success with TESE is clearly needed, in order to restrict TESE to those patients with azoospermia who have the best chance of yielding testicular spermatozoa and this was the major objective of the present work.

There were many patients with very small testis and high FSH levels yielded sperm on diagnostic testicular biopsy. This finding was encouraging to perform bilateral open diagnostic testicular biopsy as a most reliable predictor of sperm retrieval success with TESE.

Azoospermic men in this study exhibit different histopathological syndrome with open diagnostic testicular biopsy varying from tubular fibrosis, Sertoli cell only syndrome, spermatogenic maturation arrest, hypospermatogenesis and normal spermatogenesis. Our study showed no significant difference between mean testicular size and mean serum reproductive hormonal (FSH, LH, T and PRL) concentrations of MAFS compared to CMA and that of SCOFS compared to SCO and this results mean that neither testicular volume, nor serum reproductive hormones levels especially FSH were a reliable criteria for identifying patients who fail to have sperm retrieval with TESE .These finding are compatible with previous studies [10, 11, 19].

Conclusions

According to our study we suggest the following histopathological categorization and subcategorization depending on the most advanced pattern of spermatogenesis and on the presence or absence of focal area of spermatogenesis and on the type of maturation arrest seen on diagnostic testicular biopsy:[1] normal spermatogenesis(NS);[2] hypospermatogenesis(HS) ;[3] partial spermatogenic maturation arrest(PMA);[4] spermatogenic maturation arrest with focal spermatogenesis(MAFS) ;[5]complete spermatogenic maturation arrest(CMA). This might further subcategorized into late spermatid maturation arrest, intermediate spermatid maturation arrest, early spermatid maturation arrest, secondary spermatocyte maturation arrest, primary spermatocyte maturation arrest., and spermatogoneal maturation arrest(23**)**; [6] Sertoli cell only with focal spermatogenesis (SCOFS); [7] complete Sertoli cell only (CSCO);[8] tubular fibrosis with focal spermatogenesis(TFFS) ; and [9] tubular fibrosis with no focal area of spermatogenesis(TF)**.**

It was concluded from the results of the present work that the open bilateral diagnostic testicular biopsies were informative and the most useful and helpful diagnostic and prognostic predictors of successful sperm retrieval with sperm extraction.

Recommendations

The following suggestions for further studies are recommended depending on the results of the present study:

1- We suggest the application of the new testicular histopathologic classification of the present study in TESE–ICSI program. Further study is suggested to study the TESE–ICSI outcome in comparing the results of large sample size in patients with various histopathologic patterns by using this new histopathologic classification.

2- To study the clinical significance of non-invasive prognostic predictors such as serum inhibins levels, Doppler ultrasonographic studies of the testis and to be correlated positively and significantly to predict sperm retrieval in azoospermic infertile patients undergoing TESE – ICSI program.

References

1. Thonneau P, marchand S, Tallec A , Ferial ML, Ducot B, Lansac S, et al**. Incidence and main causes of infertility in a resident population** (1,850,000) of three French regions (1988-1989). Hum Reprod 1991; 6: 811-6.
2. Dubin L and Amelar RD. **Etiologic factors in 1294 consecutive cases of male infertility.** Fertil Steril 1971; 16: 735-57.
3. Jarow JP, Espeland MA, Lip Shultz LI. Evaluation of the azoospermic patient**. J Urol** 1989; 142: 62-5.
4. Palermo G, Joris H , Devroey P, Van steirtegham AC. Pregnancies after intracytoplasmic sperm injection of single spermatozoon into an oocytes .**Lancet** 1992; 340 : 17 .
5. Silber SJ,Van Steirtegham AC, Liu J, Nagy Z, Tournaye H, and Devroey P. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy **.Hum Reprod** 1995 ; 10 : 2031.
6. Schoysman R, Vander Zwalmen P, Nijs M , Segel L , Segal –Bertin G, Geert L, et al . Pregnancy after fertilization with human testicular spermatozoa (letter). Lancet 1993; 342: 1237.
7. Jow WW, Stekel J, Schlegel PN, Magid MS, and Goldstein. Motile sperm in human testis biopsy specimens**. J Androl** 1993; 14: 194.
8. Tournaye H, Verhyen G, Nagy PZ, Ubaldi F, Goosens A , Silber S , et al . Are there predicative factors for successful testicular sperm recovery in azoospermic patients. **Hum Reprod** 1997; 12: 80-6.
9. Kahraman S ,Ozgur S, Alatas C, Akosy SA , Balaban B, et al. Fertility with testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermic men. **Hum Reprod** 1996; 11:756 – 700.
10. Schlegel PN, Palermo GD, Goldstein M, Menendez S, Zaninovic N, Veeck LL , Roseenwaks Z . Testicular sperm extraction with intracytoplasmic sperm injection for non-obstructive azoospermia**. J. Urol** 1997; 49: 435 -40.
11. Vernaeve V,Staessen C, Veheyen G, Steirtegham A, Devroey P, and Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47, XXY Klinefelter's syndrome patients?. **Hum Reprod** 2004; 19:1135 – 1139.
12. Battlgia C, Giulini S, Regnani G , Modgar I, Facchinetti F, and Volpe A. **Intratesticular Doppler flow, seminal plasma nitrites/nitrates, and non-obstructive sperm extraction from patients with obstructive and non-obstructive azoospermia** . Fertil Steril 2001; 75: 1088-1094.
13. Pryor P.J. Azoospermia. In: Hargreave TB. (ed). **Male Infertility.** Berlin: Springer-Verlag 1983; 211 – 226.

14. Wu FCW, Edmond P, Raab G, and Hunter WM. Endocrine assessment of the sub fertile male**. Clin Endocrinol** 1981; 14: 493-507.
15. Kerr JB, Rich KA, de Kretser DM .Alterations of the fine structure and androgen secretion of the interstitial cells in the experimentally cryptorchid rat testis. **Bio Reprod** 1979; 20:409-422.
16. Segal S, Polishukuk WZ,, Ben–David M. **Male hyperprolactinemic male infertility** . Fertil Steril 1979; 32: 556-561.
17. Rubin RT, Poland RE, Tower BB. Prolactin-related testosterone secretion in normal adult men. **J Clin Endocrinl Metab** 1976; 42:112-116.
18. Craft I, Bennett V, and Nicholson N. Fertilizing ability of testicular spermatozoa. **Lancet** 1993; 342: 864.
19. Mulhall JP, Burgess CM ,Cunningham D, Carson R , Harris D, Oates RD. Parenchyma of men with non-obstructive azoospermia : Prevalence and predictive factors. **J. Urol** 1997: 49.