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EVALUATION OF THE CLINICAL ROLE OF EXTRACELLULAR MATRIX 1 PROTEIN FOR DIAGNOSIS OF OBSTRUCTIVE AZOOSPERMIA

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ABSTRACT

Background: The seminal plasma protein assessment of epididymal specific protein 1 or extracellular matrix protein 1(ECM1) is already discovered and under final development for clinical use . Immunoassays of ECM1 has the potential to roll out most of the histopathological diagnosis of testicular biopsies and testicular sperm extraction (TESE) procedures for patients with azoospermia, and to reduce the total cost of azoospermia diagnosis. **Aim of study:** Evaluation clinical role of ECM1 in the diagnosis of obstructive azoospermia in relevance to its histological findings. **Patients and Methods:** The case control study conducted on 65 azoospermia male in the period from January 2018 to February 2019 at the High Institute for Infertility Diagnosis & Assisted Reproductive Technologies at Al-Nahrain University in Baghdad-IRAQ, all the 65 patients were undergone clinical examination and laboratory investigation such as hormonal, seminal fluid analysis is and seminal plasma collection and freezing to be thawed later for assessment seminal plasma proteins ECM1by ELISA technology as well as testicular biopsy with histopathological diagnosis. A blood sample was taken for all patients for assessment serum level of LH, FSH and testosterone. Written informed consent was obtained from all patients. **Results:** the mean age was recoding 33.37 ± 6.99 years. Histopathological findings were recording 10 (15.38%) cases of normal spermatogenesis obstructive azoospermia (OA) and 55(84.62%) cases of abnormal spermatogenesis or non-obstructive azoospermia(NOA) and Serum level of follicle stimulating hormone(FSH) and Luteinizing hormone(LH) were highly significantly lowest ($P \leq 0.01$) in men with normal spermatogenesis. Seminal plasma level of ECM1 was significantly ($P< 0.001$) higher in men with abnormal spermatogenesis than men with normal spermatogenesis, 1629.10 (458.13) pg/ml versus 469.60 (737.29) pg/ml respectively. Receiver operating characteristic curve(ROC) analysis was carried out and the results are shown the ECM1cutoff value was > 943.11pg/ml with a sensitivity rate of 87.3% and specificity rate of 90%. In addition the accuracy rate was 87.1% and P < 0.001. **Conclusion**: Seminal plasma ECM1 protein can be used for diagnosis of obstructive azoospermia especially if combined with reproductive hormones.

KEYWORDS: Seminal Plasma, Proteomics, ECM1, Obstructive Azoospermia.

INTRODUCTION

Obstructive azoospermia (OA) is absence of spermatozoa detection in the ejaculate semen despite of normal spermatogenesis. It is accounts for 6.1% to 13.6% of patients presenting with azoospermia.[1-2]

The most frequent etiology of OA were vasectomy[3], infection, iatrogenic injury, and genetic and congenital conditions.[4] Because of some of these conditions are amenable to surgical correction whereas other will require sperm retrieval technique combined with assisted reproduction such as intracytoplasmic sperm injection.[5] The OA correction is necessary when couples tried to return back their fertility so that preoperative diagnosis of obstructive azoospermia from other type of azoospermia was mandatory.[6]

Surgical reconstruction may be a viable treatment for some patients with OA while not amenable for other but sperm is readily retrievable from these patients via sperm retrieval techniques[2], so that diagnosis and treatment were invasive and cost procedure with their possible complication, so the treatment should be tailored to the individual and which one was candidate for either to sperm retrieval techniques or vasography and surgical correction of OA cases. The researchers tried to find investigation which is easy, available, cost effective and

non-invasive, seminal plasma proteins is an substitute, non-invasive procedures for diagnosis of Obstructive azoospermia(OA) of male infertility.

Seminal plasma (SP) is originated from male reproductive system which is rich with epididymis proteins. It has been used as a suitable clinical sample for the non-invasive diagnosis of a wide range of male reproductive system disorders.[7]

The Sp composed of 3200 proteins secreted by testes, epididymis, prostate, seminal vesicles, and Cowper's glands and these are directly involved in the production and maturation of sperm or in the interaction with the zona pellucida and fusion with oocytes.[8]

Epididymal specific biomarkers are not found in other biological fluid like blood due to stringent blood–epididymis barriers, semen and SP remain the only available fluids for the non-invasive diagnosis of male infertility.[9]

The seminal plasma protein assessment of epidydmal specific protein 1 or extracellular matrix protein 1(ECM1) are already discovered and under final development for clinical use.[10] Immunoassays of ECM1 has the potential to roll out most of the histopathological diagnosis of testicular biopsies and TESE procedures for patients with azoospermia ,and to reduce the total cost of azoospermia diagnosis.

AIM OF STUDY

Clinical role of ECM1in the diagnosis of obstructive azoospermia and its cutoff values within obstructive azoospermia diagnosis an its histological findings.

PATIENTS AND METHODS

The case control study conducted on 65 azoospermia male in the period from January 2018 to February 2019 at the High Institute for Infertility Diagnosis & Assisted Reproductive Technologies at Al-Nahrain University in Baghdad-IRAQ, all the 65 patients were undergone clinical examination and laboratory investigation such as hormonal, seminal fluid analysis is and seminal plasma collection and freezing to be thawed later for assessment seminal plasma proteins ECM1by ELISA technology as well as testicular biopsy with histopathological diagnosis. A blood sample was taken for all patients for assessment serum level of LH, FSH and testosterone. Written informed consent was obtained from all patients.

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. The following statistical tests were used: **Chi-square test** was use to evaluate association between any two categorical variables provided that less than 20% of cells have expected count of less than 5. However, **Fischer exact test** was used instead when chi-square test was not valid (in case that more than 20% of cells have expected count of less than 5). **Independent samples t-test** was used to evaluate the difference in mean of numeric variables between any two groups provided that these variables were normally distributed; otherwise **Mann Whitney U test** would be used instead if those variables were not normally distributed.**One way analysis of variance (ANOVA)** was used to evaluate difference in mean of numeric variables among more than two groups provided that these numeric variables were normally distributed; but **Kruskal Wallis test** was chosen in case of non-normally distributed variables. **One way ANOVA** was followed by **pos hoc LSD** test to evaluate individual differences in mean values between any two groups among groups tested primarily using **one way ANOVA**; whereas, **Kruskal Wallis test** was followed by **Mann Whitney U** test for the same purpose in case of non-normally distributed numeric variables. **Spearman correlation** was used to evaluate the correlation between any 2 numeric variables and the results were expressed as correlation co-efficient (*r*) and the level of significance (*P*). In order to detect the cutoff value that predict a positive finding, **receiver operator characteristic (ROC) curve analysis** was used with its corresponding **area under the curve (AUC)**, **accuracy level**, **sensitivity, specificity** and level of significance (*P*). The level of significance was considered at *P*-value of equal or less than 0.05. The level of high significance was considered at *P*-value of equal or less than 0.01.

RESULTS

The mean age of 65 cases of azoospermia patients was recording 33.37 ± 6.99 years. Histopathological finding was recording 10 (15.38%) cases of normal spermatogenesis OA and 55(84.62%) cases of abnormal spermatogenesis or non-obstructive azoospermia(NOA) as in table 1.

Serum level of FSH & LH were highly significantly lowest($P \leq 0.01$) in men with normal spermatogenesis 6.86 (5.15)mIU/ml, 5.51 (3.96)mIU/ml than abnormal spermatogenesis(hypo-spermatogenesis, maturation arrest and sertoli only syndrome) 20.76 (20.37)mIU/ml and 10.18 (12.91)mIU/ml respectively, while no significant was seen in serum level of testosterone in both types as shown in table 2.

To test the predictive value of FSH and LH in the differentiation between normal spermatogenesis and abnormal spermatogenesis a receiver operating characteristic (ROC) analysis was carried out and the results are shown in figure 1 and table 3. The serum level of FSH cutoff value, area under curve (AUC), Accuracy, 95% confidence interval (CI), P-value, Sensitivity and Specificity were recorded>12.05mIU/ml, 0.853, 85.3%, 0.743 to 0.928, p <0.001, 74.6% and 100.0% respectively. Whereas serum level of LH cutoff value, AUC, Accuracy, 95% confidence interval (CI), P-value, Sensitivity and Specificity were recorded as following

>8.87 mIU/ml, 0.780, 78.0%, 0.660 to 0.873, p<0.001, 58.2% and 100.0% respectively.

Seminal plasma level of ECM1 was significantly (*P*< 0.001) higher in men with abnormal spermatogenesis than men with normal spermatogenesis, 1629.10 (458.13) pg/ml versus 469.60 (737.29)pg/ml respectively as shown in table 4.

To test the validity of ECM1 in the differentiation between normal spermatogenesis of obstructive azoospermia(OA) and abnormal spermatogenesis of non obstructive azoospermia(NOA) a ROC analysis was carried out and the results are shown in figure 1 and table 5. ECM1cutoff value was > 943.11pg/ml with a sensitivity rate of 87.3% and specificity rate of 90 %. In addition the accuracy rate was 87.1% and P < 0.001.

A positive significant correlation of Seminal plasma level of ECM1 to serum level of FSH, no other significant correlation was seen in ECM1 to age, duration of infertility and serum hormone levels, as shown in table 6.

Table 1: General characteristic of the study sample.

Characteristic	Value
Sample size	65
Age (years)	
Range (min.-max.)	26 (22-48)
Mean ±SD	33.37 ±6.99
Type of azoospermia or state of spermatogensis	
Normal spermatogenesis (Obstructive Azoospermia), *n* (%)	10 (15.38)
Abnormal spermatogenesis (Non-obstructive Azoospermia), *n* (%)	55(84.62)

min.: minimum; max.: maximum; SD: standard deviation; IQR: inter-quartile range;

Table 2: Serum hormonal levels in patients according to type of histopathology of azoospermia.

Hormonal levels	Normal spermatogensis *n* = 10	Abnormal spermatogensis *n* = 55	*P* *
FSH (mIU/L), median (IQR)	6.86 (5.15)	20.76 (20.37)	<0.001 HS
LH (mIU/L), median (IQR)	5.51 (3.96)	10.18 (12.91)	0.005 HS
Testosterone (ng/ml), median (IQR)	2.905 (2.11)	3.42 (2.93)	0.827 NS

n: number of cases; FSH: follicle stimulating hormone; LH: luteinizing hormone; IQR: inter-quartile range; HS: highly significant at $P \leq 0.01$; NS: not significant at $P \leq 0.05$; *: Mann Whitney U test.

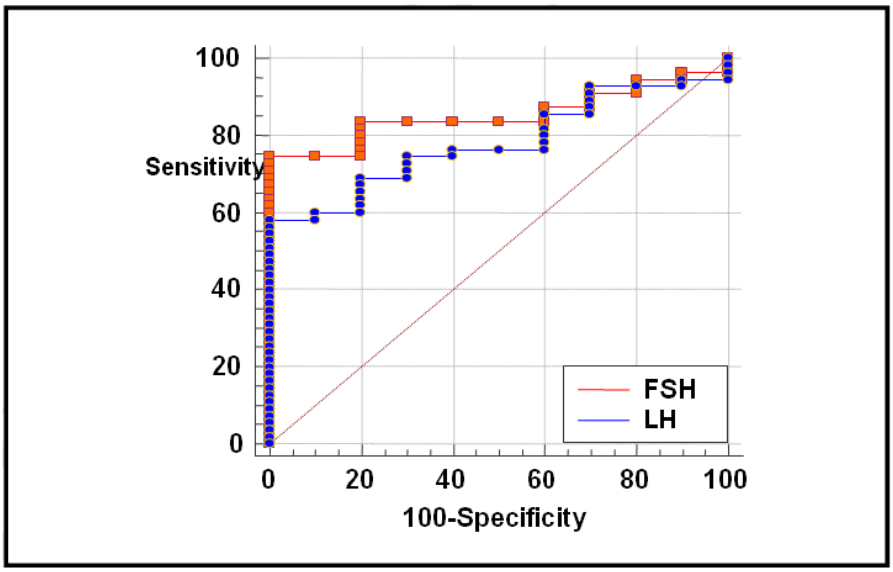

Figure 1: ROC analysis to find FSH and LH cutoff values that predict obstructive azoospermia versus non-obstructive azoospermia.

Table 3: Characteristics of the ROC curve.

Characteristic	FSH	LH
Cutoff value	>12.05	>8.87
Area under the curve (AUC)	0.853	0.780
Accuracy	85.3 %	78.0 %
95 % confidence interval (CI)	0.743 to 0.928	0.660 to 0.873
P	<0.001	<0.001
Sensitivity	74.6 %	58.2 %
Specificity	100.0 %	100.0 %

Table 4: ECM1 in azoospermia men.

Variable	Total *n* = 65	Normal spermatogenesis *n* = 10	Abnormal spermatogenesis *n* = 55	*P*
ECM1Pg/ml	1530.30 (857.99)	469.60 (737.29)	1629.10 (458.13)	<0.001 HS

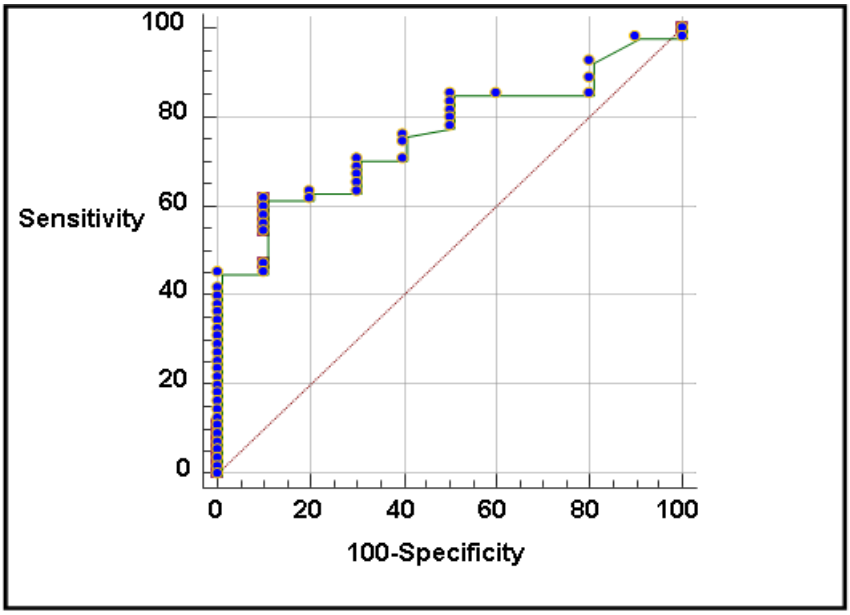

Figure 1: ROC analysis to find ECM1 cutoff values that predict abnormal spermatogenesis of NOA versus normal spermatogenesis of OA.

Table 5: Characteristics of the ROC curve.

Characteristic	ECM1
Cutoff value	> 943.11
Area under the curve (AUC)	0.871
Accuracy	87.1 %
95 % confidence interval (CI)	0.765 to 0.941
P	<0.001
Sensitivity	87.3 %
Specificity	90.0 %

Table 6: Correlation of SP ECM1 protein to age, and serum hormone levels.

Characteristic	ECM1pg/ml	
	r	*P*
Age (years)	0.088	0.488 NS
FSH (mIU/ml)	0.270	0.030 S
LH (mIU/ml)	0.239	0.055 NS
Testosterone (ng/ml)	0.000	0.998 NS

r: Correlation coefficient according to Spearman bi-variate correlation test; ECM1: ng/ml; NS: not significant at $P \leq 0.05$; S: significant difference at $P \leq 0.05$.

DISCUSSION

The age of patients were important parameter in the evaluation of male infertility, Abdullah L.*et al*, found a mean age of 24.5 years which was slightly lower than mean age of current study, he also reported the histopathological patterns recording 14% cases as normal spermatogenesis and 71% abnormal spermatogenesis or NOA which was approximately same result of presented study.[11]

In contrast the other studies were found OA is a common urologic condition and accountsfor 6.1%[1] and Although there are many causes of azoospermia, obstruction of the ductal system is responsible for approximately 40% of cases.[12]

Parikh U.R *et al* was reported a normal spermatogenesis was the most common finding encountered in 29 out of 80 cases, comprising approximately 36.25% while 63.75% abnormal spermatogenesis[13] as well as Kurien *et al* was found 50% of the patients had normal spermatogenesis on fine needle aspiration cytology examination. These findings suggest that the obstructive etiology is one of the major causes responsible for male infertility and has a good prognosis.[14]

Which were higher than the result of presented study this may be due to technical or inter laboratory variation and experience as well as type sperm retrieval procedure.

Obstructive azoospermia (OA) is less common than non-obstructive azoospermia (NOA), and accounts for 15 to 20% of all men with azoospermia[15] which was similar to finding of presented study.

Testicular biopsy plays an important role in the diagnosis of OA, however hormone profiles was used to predict the types of azoospermia. Concerning hormonal estimation the study showed Serum level of FSH & LH were highly significantly lowest($P \leq 0.01$) in men with normal spermatogenesis than abnormal spermatogenesis, while no significant was seen in serum level of testosterone in both types. In agree with Gudeloglu A and Parekattil SJ[16] were found the normal levels of LH and FSH are expected in normal spermatogenesis (OA); however, LH and FSH can be low or elevated in abnormal spermatogenesis(NOA).

I-Shen Huang *et al*[17] was enrolled 51 patients with OA and 156 with NOA, the mean levels of testosterone (4.5 vs. 3.4 ng/ml) and was significantly higher in the OA group, whereas the levels of Follicle Stimulating Hormone or FSH (FSH) (5.6 vs. 25.4 mIU/ml) and Luteinizing Hormone or LH (LH) (3.7 vs. 11.6 mIU/ml) were lower which is approximately same with current study except with regard to serum level of TT was non-significant difference between normal spermatogenesis and abnormal spermatogenesis in the presented study.

As well as I-Shen Huang and coworker were reported thereceiver operating characteristic curve analysis revealed that FSH was the best individual diagnostic predictors with cutoff value of FSH >9.2 mIU/ml, AUC (with a 95% CI for the area being between 0.9253 and 0.9897) and with a sensitivity of 89.7% and a specificity of 90.2%(17). whereas Schoor *et al*. recording that a precise cutoff value of FSH ≤7.6 mIU/ml allowed for a detection rate of 96% of patients with OA, 77% sensitivity, and 93% specificity.[18]

Ari Basukarno *et al* was reported The cutoff value of FSH with highest specificity and sensitivity is 10.36 mIU/ml. This value have specificity of 79.5% and sensitivity of 82.1%, for differentiationbetween both types of azoospermia.Unfortunately, Testosterone could not be used in predicting azoospermia classification.[19]

Chen *et al* study was reported a cutoff value of FSH at the level of 13.7 mIU/ml in order to differentiate between azoospermia with normal spermatogenesis and failure of spermatogenesis[20] which was approximately same the current study.

It was stated abnormal spermatogenesis is often associated with altered serum gonadotropins and testosterone. FSH, LH and testosterone levels were estimated in infertile men especially the azoospermic males.[21]

The optimal cutoff value for FSH in the current study was >12.05 mIU/ml, which is higher compared to previous studies, which may be due to patient selection and different ethnicity.

Regarding azoospermia types several epididymis-specific proteins would found as biomarkers for differentiation NOA versus OA. The ECM1, a protein secreted into semen predominantly by epididymis supports this hypothesis.[10]

The ELISA technology is used for quantitative detection of ECM1 is range 31-2000 pg/ml and sensitivity < 18.75pg/ml, this protein (ECM1) a significantly (P = 0.007), higher in men with Non obstructive azoospermia than men with Obstructive azoospermia, which is agree to other literatures that reported, analysis of seminal plasma proteins has shown the absence of certain proteins responsible for sperm function and proteins were absent in azoospermic patients such as Seminal plasma level of ECM1 were significantly higher in men with NOA than men with OA.[22-23] Obstructive azoospermia is may be due to physical or functional obstruction in the male genital tract, whereas NOA azoospermia is mainly due to the arrest or defect of the spermatogenesis process.[24]

Drabovich and coworker were found that ECM1 and TEX101 SP proteins could be used to as a test for differential diagnosis of azoospermia. Testing such SP, may be able to distinguish patients with OA and NOA as well as other types of NOA.[10]

Analysis of SP proteins has shown the absence of certain proteins in the seminal plasma, however many proteomic analysis were perform to determine the differential expression of proteins in azoospermia.[25]

The result of presented study is similar to Drabovich AP *et al*. 2013 was reported that extracellular matrix protein 1 was able to differentiate NOA and post-vasectomy men with a threshold value of 2.3 l ng/mL.[10]

In humans, several seminal plasma proteins were found which serve as diagnostic markers of spermatogenesis, seminiferous epithelium state and azoospermia.[26]

So that from these previous and current observation, high SP level of protein in NOA versus low level in cases of OA, this fact due to a focal spermatogenesis of deferent score in between NOA as mention above.[27]

Regarding to azoospermia types the presented observation shows, SP level of ECM1 was significantly lowest in men with OA ($P< 0.05$) so the ROC curve, cutoff values, AUC, Accuracy, 95% confidence interval (CI), Sensitivity and Specificity were recorded, >943.11pg/ml, 0.871, 87.1%, 0.765 to 0.941, 87.3% & 90.0% for NOA versus OA differentiation respectively which is nearly same as finding of other observation were reported that Sensitivity, Specificity and threshold value were equal to 100, 73 and > 2.3µg/ml.[28] Whereas, other study reported that AUC (0.99) with sensitivity equal to 94% and the ECM1 (<2.3µg/ml) suggest an OA, but high seminal plasma level of ECM1 (>2.3µg/ml) suggest NOA[10] which is approximately same the sensitivity in the current study.

CONCLUSION

Follicle stimulating hormone and luteinizing hormone were have moderate predictive value of differentiating OA versus NOA but they can be of diagnostic value if combined with SP ECM1 for diagnosis of OA.

AUTHORS CONTRIBUTION STATEMENT

This research was done by Dr.Hussain Khaleefa Kadhem Al Dulaimy with Prof. Dr. Ula Al Kawaz and Assist. Prof. Dr. Hayder A. L. Mossa (corresponding author) in male infertility clinics and IVF laboratory at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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