Seminal clusterin level as a predictor for spermatogenesis before testicular sperm extraction

Original Article

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ABSTRACT

Background: A number of factors have been proposed to be of predictive benefit for males with a good probability to retrieve sperms; as testicular volume, serum FSH, total testosterone and Inhibin B levels. However, a little information has been present regarding the use of seminal Clusterin (CLU) for that purpose.

Aim: We designed the study in order to estimate seminal CLU level for non-obstructive azoospermia (NOA) males undergoing Testicular sperm extraction (TESE), also to detect the possibility of its use as a predictor for spermatogenesis in those patients. Further, we aimed to analyze its relation to testicular size, semen volume, presence or absence of varicocele, hormonal profile and result of MD-TESE.

Patients and Methods: A cross-sectional hospital-based study on a total of 176 males (of them; 88 with NOA and 88 normal fertile). A semen sample was obtained from each patient before micro-dissection (MD)-TESE surgery and from normal fertile males. Seminal CLU was measured in all samples by Enzyme linked immunosorbent assay (ELISA).

Results: Seminal CLU level significantly differ between NOA patients and normal fertile males (*P*-value = 0.000*). A significant positive relation was obtained between seminal CLU level with both semen volume and testicular size (*P*-value < 0.05). A non-significant correlation was obtained between seminal CLU level and result of micro-TESE (*P*-value > 0.05).

Conclusion: CLU concentration in se minal plasma, which is proportional to the expression level of CLU in the testis differ significantly between normal fertile males and NOA patients. Seminal CLU level not significantly associated with whether or not spermatozoa can be successfully retrieved by MD-TESE. Thus, Seminal CLU level couldn't be used as a useful biomarker for the assessment of spermatogenic status in NOA patients before MD-TESE.

Key Words: Azoospermia, clusterin, spermatogenesis, testicular sperm extraction (TESE)

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INTRODUCTION

Clusterin (CLU) also known as (apolipoprotein J, sulfated glycoprotein-2, or testosterone-repressed prostate message-2) is a glycoprotien first validated in ram rete testis fluid in 1983. It was named for its ability to elicit clustering among Sertoli cells[1].

It plays crucial tasks in an abundance of pathophysiological processes as complement regulation, tissue remodelling, lipid transport, apoptotic cell death, and reproduction[2].

Expression of CLU is complex in humans, displaying diverse patterns in different tissues and cells[3].

In the testis, it is produced by Sertoli cells, then secreted into the fluid of the seminiferous epithelium, and deposited onto the membranes of elongating spermatids and mature spermatozo[4]. Its concentration in the semen and plasma is about 400 and 100 µg/ml, respectively[5].

Little knowledge is present regarding its functional role of the male reproductive tract under physiological conditions. Moreover, its role in spermatogenesis is debatable[6].

Impaired spermatogenesis is the character which discriminates males with nonobstructive azoospermia (NOA), although focal areas of spermatogenesis may still present in their testes. Such spermatogenic foci could possibly be obtained by the testicular sperm extraction (TESE) technique[7].

A number of factors have been proposed to be of predictive benefit for males with a good probability to retrieve sperms such as testicular volume, serum follicle-stimulating hormone (FSH), total testosterone, and inhibin B levels[8]. Our objectives of the study were to estimate seminal CLU level for NOA males undergoing

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microdissection (MD)-TESE and to detect the possibility of using seminal CLU level as a predictor for spermatogenesis in those patients. Further, we aimed to analyze its relation to testicular size, semen volume, presence or absence of varicocele, hormonal profile, and result of MD-TESE.

PATIENTS AND METHODS

A cross-sectional hospital-based study was conducted at the Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Assiut University Hospital. The study design was approved by the Institutional Ethics and Research Committee of Faculty of Medicine Assiut University (N:IRB 1710016). All participants signed a written informed consent.

Patients

The study included 176 males (88 males with NOA who underwent MD-TESE as a preliminary step for ICSI, the age of whom range from 20 to 50 years and 88 normal fertile control participants with the same age range), while we excluded patients with cryptorchidism, testicular agenesis, and testicular atrophy.

Methods

Each patient was subjected to detailed medical history (including personal history, marital history, sexual history, and history about factors affecting spermatogenesis such as dietary deficiencies (such as of vitamins B, E, and A), exposure to medical agents like hormone/steroid therapy, antibiotics, sulfasalazine, alpha-blockers, 5 alpha-reductase inhibitors, chemotherapeutic agents, pesticides, recreational drugs (marijuana, excessive alcohol), exposure to metals (cadmium and lead), heat or radiograph. Meticulous clinical examination was performed for each patient (complete general and genital examinations). A semen sample was obtained from each patient and control and subjected to seminal CLU level measurement by enzyme-linked immunosorbent assay according to the manufacturer (SinoGeneClon Biotech Co., Hangzhou, China). Q1

Statistical analysis

Analysis of the data was performed using the Statistical Program for the Social Sciences (SPSS.18.0, SPSS, Inc., Chicago, Illinois, USA) software program. Quantitative data were expressed as mean ±SD. Qualitative data were expressed as frequency and percentage. Independent-samples t test of significance was used when comparing between two means. Pearson's correlation coefficient (r) test was used for correlating data, and one-way analysis of variance when comparing between more than two means. A P value of less than 0.05 was considered statistically significant. P value less than 0.001 was considered as highly significant, while P value more than 0.05 was considered insignificant.

RESULTS

This study was conducted on 88 NOA and 88 normal fertile males as a control. A nonsignificant difference was present between patients and controls as regards clinical and demographic data (P <0.5) (Table 1). The majority of the patients had moderate-sized testes (51.1%) and normal semen volume (70.5%) (Table 2, Figs 1 and 2).

Table 1: Demographic and social data of all participants

Personal data	Nonobstructive azoospermia males (N=88) [n (%)]	Normal fertile males (control) (N=88) [n (%)]	*P* value
Age (years)			
Mean ±SD	33.48±6.56	33.64±5.48	0.862
Range	21.0-46.0	25.0-43.0	
Residence			
Rural	42 (47.7)	52 (59.1)	0.131
Urban	46 (52.3)	36 (40.9)	
Education			
Educated	61 (69.3)	62 (70.5)	0.869
Noneducated	27 (30.7)	26 (29.5)	
Smoking			
Smoker	29 (33.0)	34 (38.6)	0.432
Nonsmoker	59 (67.0)	54 (61.4)	
Marital status			
Married	87 (98.9)	88 (100.0)	1.000
Single	1 (1.1)	0	

Table 2: Examination of nonobstructive azoospermia males

Examination	N=88 [n (%)]
Testicular size	
Small	33 (37.5)
Normal	10 (11.4)
Moderate	45 (51.1)
Semen volume (ml)	
Small	26 (29.5)
Normal	62 (70.5)
Mean ±SD	2.97±1.61
Median (range)	3.0 (0.5–6.0)

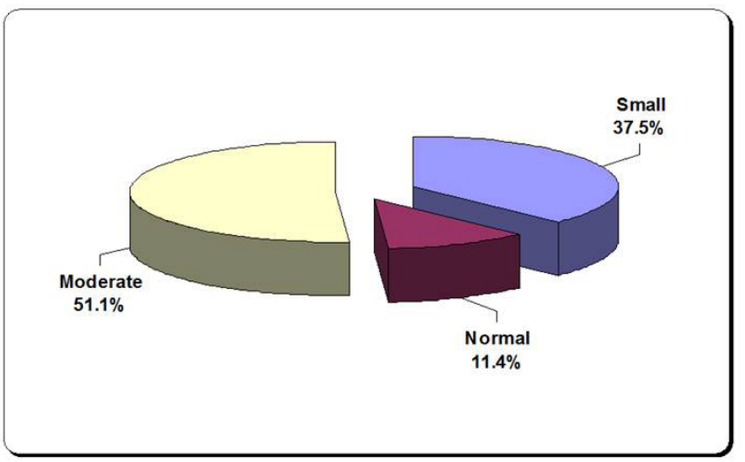

Fig. 1: Distribution of NOA males as regards testicular size. NOA, nonobstructive azoospermia.

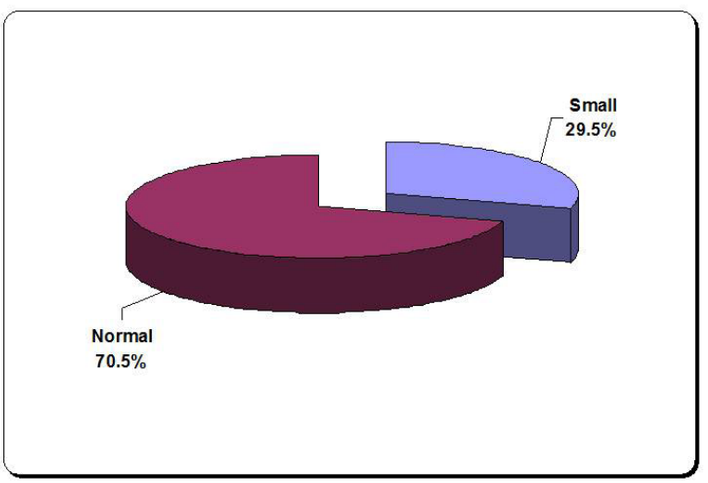

Fig. 2: Distribution of NOA males as regards semen volume. NOA, nonobstructive azoospermia.

Table 3 illustrates the hormonal profile in NOA males; 42 (47.7%) had normal FSH levels and 46 (52.3%) had abnormal levels (higher or lower than normal). Regarding total testosterone level; 66 (75%) had normal levels and 22 (25%) had abnormal levels (lower than normal). Seventy-seven (87.5%) males had normal estradiol (E2) levels and 11 (12.5%) had abnormal level (higher than normal) (Table 3, Fig. 3). As regards seminal CLU level, we found that it significantly differ between NOA patients and normal fertile males (*P*=0.000) (Table 4, Fig. 4).

Table 3: Hormonal profile of nonobstructive azoospermia males

Hormonal profile	N=88 [n (%)]
FSH level	
Normal	42 (47.7)
Abnormal	46 (52.3)
Mean ±SD	15.18±12.78
Median (range)	12.9 (2.5–48.4)
Total testosterone level	
Normal	66 (75.0)
Abnormal	22 (25.0)
Mean ±SD	4.57±2.35
Median (range)	4.9 (0.2–8.0)
Erstradiol (E2) level	
Normal	77 (87.5)
Abnormal	11 (12.5)
Mean ±SD	26.29±13.60
Median (range)	22.3 (5.3–60.2)

FSH, follicle-stimulating hormone.

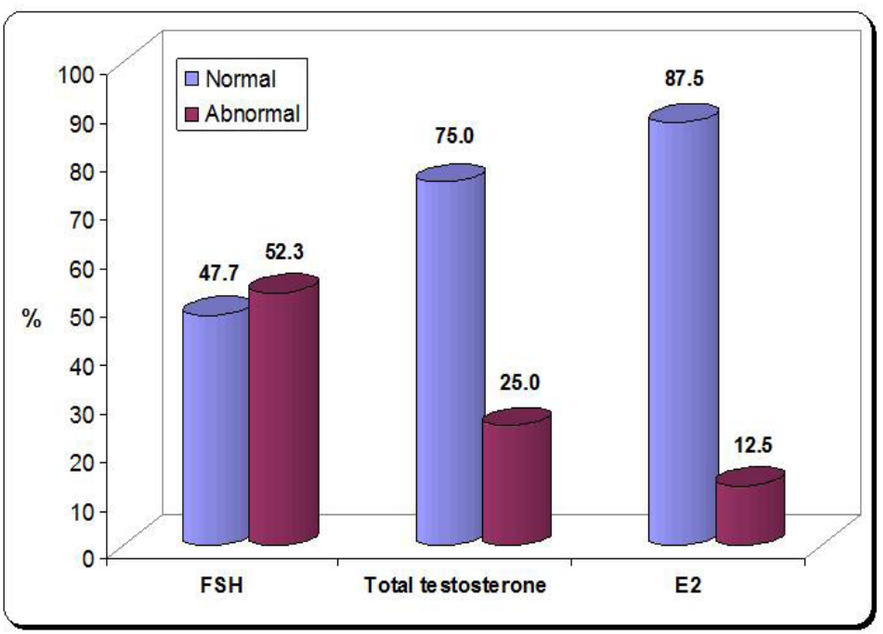

Fig. 3: Hormonal profile level in NOA males. NOA, nonobstructive azoospermia.

Table 4: Seminal clusterin level in nonobstructive azoospermia males and in normal fertile males (controls)

Seminal clusterin level	NOA males (N=88)	Control (N=88)	*P* value
Mean ±SD	78.13±59.24	128.83±66.23	0.000*
Median (range)	67.5 (2.0–244.0)	102.0 (60.0–280.0)	

*highly significant correlation between Seminal clusterin level in non-obstructive azoospermia males and in normal fertile males (controls).

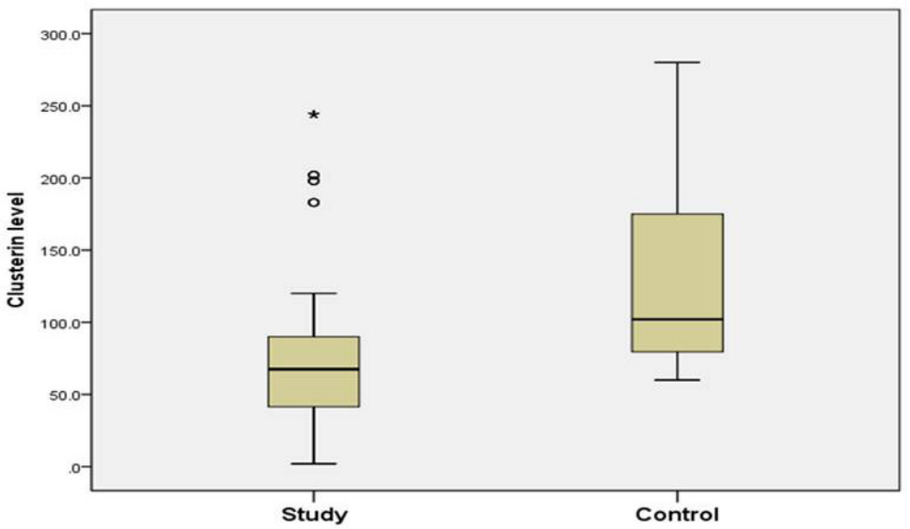

Fig. 4: Seminal CLU level in NOA males and normal fertile males. CLU, clusterin; NOA, nonobstructive azoospermia.

A significant positive relation was obtained between seminal CLU level with both semen volume and testicular size (P<0.05), while a nonsignificant relation was obtained between seminal CLU level with varicocele and hormonal profile (FSH, total testosterone, and estradiol) (*P*>0.05) (Table 5). Table 6 describes the correlation between CLU level and age, semen volume, and hormonal profile. We established a significant positive correlation between seminal CLU level and age and semen volume (*P*<0.05); nonetheless, a nonsignificant correlation was obtained with the hormonal profile (*P*>0.05).

Moreover, a nonsignificant relation was obtained between seminal CLU level and result of MD-TESE (*P*>0.05) (Table 7).

Table 5: Relation between seminal clusterin level with clinical data and hormonal profile of nonobstructive azoospermia males

	Clusterin level		
	Mean ±SD	Median (range)	*P* value
Testicular size			
Small	85.73±70.10	68.0 (4.0–244.0)	
Normal	113.60±53.38	107.0 (53.0–183.0)	0.036*
Moderate	64.67±47.63	63.0 (2.0–202.0)	
Semen volume			
Small	53.12±35.70	46.0 (2.0–120.0)	0.022*
Normal	88.61±64.07	68.0 (4.0–244.0)	

Varicocele			
Present	84.52±73.77	53.0 (2.0–202.0)	0.282
Not present	74.65±50.02	70.0 (4.0–244.0)	
Total testosterone level			
Normal	71.61±51.42	63.0 (2.0–202.0)	0.396
Abnormal	97.68±76.30	70.0 (27.0–244.0)	
Estradiol (E2) level			
Normal	80.27±61.86	67.0 (4.0–244.0)	0.990
Abnormal	63.09±34.14	87.0 (2.0–90.0)	

* Significant correlation between seminal Clusterin level and testiculas size.

Table 6: Correlation between clusterin level and (age, semen volume, and hormonal profile)

	Clusterin level	
	r value	*P* value
Age (years)	0.329	0.002*
Semen volume (ml)	0.282	0.008*
FSH	-0.163	0.129
Total testosterone	0.049	0.649
E2	-0.182	0.090

E2, estradiol; FSH, follicle-stimulating hormone.
*Significant correlation between seminal Clusterin level and age.
*Significant correlation between seminal Clusterin level and semen volume.

Table 7: Relation between seminal clusterin level and microdissection-testicular sperm extraction results

	Seminal clusterin level		
	Mean ±SD	Median (range)	*P* value
MD-TESE			
Positive	80.98±63.94	74.0 (4.0–244.0)	0.488
Negative	75.14±54.50	55.0 (2.0–202.0)	

MD-TESE, microdissection-testicular sperm extraction.

DISCUSSION

Although the CLU role in fertility has been suggested since decades, its exact role has not been determined yet[9].

In the current study, we searched seminal CLU level in 176 males (88 males with NOA underwent MD-TESE surgery and 88 normal fertile control) and analyzed its relation to testicular size, semen volume, presence or absence of varicocele, hormonal profile, and result of MD-TESE.

A closer study by Fukuda *et al.*[10] performed evaluation of the association of seminal CLU level and spermatogenesis. They conducted the study on 89 males, dividing them into 28 males with normozoospermia, 33 males with oligozoospermia, and 28 males with NOA who underwent MD-TESE.

In the current study, 31 (35.2%) males had varicocele, while it was absent in the remaining 57 (64.8%). Saleh *et al.*[11] explored CLU level in seminal plasma of infertile men with varicocele. They compared the level of seminal plasma CLU before and after varicocelectomy. They found a significant difference in seminal CLU level between infertile patients with varicocele and controls. There results were in agreement with Hosseinifar *et al.*[12], who proclaimed that CLU and other heat shock proteins were lower in amount in patients with varicocele, whereas in our study we obtained a nonsignificant relation between seminal CLU level and varicocele presence. In our study, we realize a significant

relation between seminal CLU level of all participants and testicular size. Seminal CLU level was higher in males with a larger testicular size than those with a smaller testicular size. Our result was in agreement with the study of Fukuda *et al*.[10], who declared that there was a significantly larger testicular volume in men with a high seminal CLU level than in men with a low level.

In the current study, the relation between seminal CLU level and hormonal profile were nonsignificant (P<0.05). Similarly, Fukuda *et al.*[10] found a nonsignificant relation between seminal CLU level and FSH and testosterone levels.

Regarding the level of seminal CLU in NOA males and fertile controls, we obtained a significant difference (P=0.000). Seminal CLU level was significantly elevated in fertile controls and diminished in NOA males. These findings were in agreement with Salehi *et al.*[6], who found that the seminal CLU level in fertile males was significantly higher than that of infertile males. Furthermore, Fukuda *et al.*[10] declared that the seminal CLU level in males with NOA was significantly decreased compared with those in the oligozoospermia or normozoospermia groups.

Zalata *et al.*[13] performed evaluation of the seminal CLU gene expression in fertile and infertile males. They conducted a study on 124 men who were divided into 26 healthy fertile men with normozoospermia, 32 with asthenozoospermia, 31 with asthenoteratozoospermia, and 35 with oligoasthenoteratozoospermia . They reported that CLU gene expression was significantly elevated in the semen samples in infertile males.

In the present study, spermatozoa were successfully retrieved from 44 (50%) patients of the 88 men who underwent micro-TESE. We obtained a nonsignificant relation between seminal CLU level and result of micro-TESE (P>0.05), whereas Fukuda *et al.*[10] advocated that seminal CLU level was significantly associated with whether or not spermatozoon was successfully retrieved by MD-TESE.

In conclusion, CLU concentration in seminal plasma, which is proportional to the expression level of CLU in the testis, differs significantly between normal fertile males and NOA patients. The relation between seminal CLU level and hormonal profile of NOA patients was not significant. However, the relation between seminal CLU level with both semen volume and testicular size was significant. Seminal CLU level is not significantly associated with whether or not spermatozoa can be successfully retrieved by MD-TESE. Thus, seminal CLU level could not be used as a useful biomarker for the assessment of spermatogenic status in NOA patients before MD-TESE.

We do recommend performing further studies on a larger sample size in order to authenticate or deny our postulation. In addition, we recommend studies correlating seminal CLU level with its serum level, and also to include more other hormones such as inhibin, LH, and anti-mullerian hormone.

CONFLICT OF INTEREST

There are no conflicts of interest.

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