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Evaluating the blood Serum Hormones, Cholesterol Levels in Seminiferous Plasma and Certain Semen Parameters in Males with Asthenozoospermia

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Abstract

Infertility is a medical condition that afflicts many men around the world and has many and complex causes. It is a malfunction in the male reproductive system. Its causes can be genetic, hormonal or a defect of semen components. This study was included two groups, the infertile (Asthenozoospermia) and the fertile or the control group (Normozoospermia), where each group consisted of 33 men average age (31.57 ± 1.11, 30.93 ± 1.04) respectively. Seminal fluid analysis (SFA) were conducted for them according to the recommendations of the World Health Organization (WHO), and the concentration level of cholesterol in seminal plasma was estimated using ELISA (Enzyme Linked Immuno-Sorbent Assay) and the concentration levels of blood serum hormones (FSH, LH, Prolactin, Testosterone) were estimated using the Chemiluminescence Immunoassay System (CL-900i) device. The study showed great convergence in the average age, BMI, semen volume and semen pH at Asthenozoospermia group and Normozoospermia group, without a statistical significant difference ($P>0.05$). However, there was a statistically significant difference between the Asthenozoospermia group and the Normozoospermia group in terms of sperm count, Progressive motility%, No-Progressive motility%, and Immotile% ($P<0.01$). The seminal plasma cholesterol levels was likewise significantly different between the two groups ($P<0.01$). The levels of serum FSH, LH,

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prolactin, and testosterone concentrations were comparable between the two groups (P<0.05), whereas there was no significant difference between them.

Keywords: Infertility, Asthenozoospermia, Normozoospermia, Cholesterol and seminal plasma.

تقييم هرمونات مصل الدم ومستويات الكوليسترول في البلازما المنوية وبعض معايير السائل المنوي لدى الذكور المصابين بوهن النطاف

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الخلاصة

العقم حالة طبية تصيب الكثير من الرجال حول العالم ولها أسباب عديدة ومعقدة. وهو خلل في الجهاز التناسلي الذكري. ويمكن أن تكون أسبابه هي أسباب وراثية أو هرمونية أو خلل في مكونات السائل المنوي. تضمنت هذه الدراسة مجموعتين ، مجموعة العقم (وهن النطاف) ومجموعة الخصوبة أو المجموعة الضابطة (السائل المنوي الطبيعي)، حيث تكونت كل مجموعة من 33 رجلاً متوسط العمر (31.57 ± 1.11 ، 30.93 ± 1.04) على التوالي ، تحليل السائل المنوي (SFA) أجريت لهم وفقًا لتوصيات منظمة الصحة العالمية (WHO) ، وتم تقدير مستوى تركيز الكوليسترول في بلازما السائل المنوي باستخدام مقايسة ماصة مناعية مرتبطة بالإنزيم (ELISA) ومستوى تركيز هرمونات مصل الدم (الهرمون المنبه للجريب ، الهرمون الملوتن ، البرولاكتين ، التستوستيرون) تمت باستخدام جهاز المقايسة المناعية الكيميائية (CL-900i). أظهرت الدراسة تقاربا كبيرا في متوسط العمر ومؤشر كتلة الجسم وحجم السائل المنوي ودرجة الحموضة في السائل المنوي في مجموعة وهن النطاف ومجموعة السائل المنوي الطبيعي، دون وجود فروق ذات دلالة إحصائية (P>0.05). ومع ذلك، كان هناك فرق ذو دلالة إحصائية بين مجموعة وهن النطاف ومجموعة السائل المنوي الطبيعي من حيث عدد الحيوانات المنوية، الحركة التقدمية٪، الحركة غير التقدمية٪، و الغير المتحركة٪ (P <0.01). كانت مستويات الكوليسترول في البلازما المنوية مختلفة بشكل كبير بين المجموعتين (P <0.01). كانت مستويات تركيز FSH، LH، البرولاكتين، والتستوستيرون في الدم مماثلة بين المجموعتين (P>0.05)، في حين لم يكن هناك فرق كبير بينهما.

الكلمات المفتاحية: العقم ، استنزاف النطاف ، السائل المنوي الطبيعي ، الكوليسترول ، البلازما المنوية.

Introduction

One of the primary reasons for male infertility is Asthenozoospermia (AZS), which is characterized by decreased or nonexistent sperm motility. Because a fully functioning flagellum is necessary for sperm motility, mutations in the genes responsible for flagella assembly and motile control may result in AZS [1]. In mammals, the ability of sperm to fertilize an egg depends on its ability to advance effectively via the female genital system. The flagellum, an organelle that has been preserved throughout evolution and provides the mechanical power for sperm propulsion and movement, supports this key characteristic. Nearly 80% of infertile men have AZS [2]. Evidence shows that over the last several decades, male fertility indices have been dropping globally. Negative lifestyle choices may, least partially, account for this. These include the use of cigarettes and alcohol, recreational drug usage, poor eating patterns, metabolic syndrome and obesity, genital heat stress, chemical exposure, and psychological stress [3]. Treatments for cancers and other non-malignant illnesses in men may have serious adverse effects, including infertility. This could be brought about by a decrease in spermatogonial stem cells or a change in the functioning of testicular tissue cells such Sertoli cells and Leydig cells [4]. Male reproductive function is reliant on cholesterol homeostasis. Cholesterol is necessary for mammalian cell activities and integrity, regulating the permeability and flexibility of the cell membrane [5]. Androgens play a crucial role in the development of male reproductive organs such as the epididymis, vas deferens, seminal vesicle, prostate and the penis. Androgens are needed for puberty, male fertility and male sexual function. High levels of intratesticular testosterone, secreted by the leydig cells, are necessary for spermatogenesis [6]. Spermatozoa are powered by mitochondria. They are also capable of generating reactive oxygen species (ROS). While tyrosine phosphorylation plays a role in cholesterol efflux, sperm-egg contact, and fertilization are all dependent on a modest concentration of ROS. In addition, mitochondria involve testosterone production [7]. LH is Luteinizing hormone, which the anterior pituitary gonadotropes release [8]. In adults, luteinizing hormone (LH) binding to Leydig cell LH receptors stimulates cAMP production, which in turn increases the quantity of cholesterol transferred into the mitochondria. The mitochondrial inner membrane is where cholesterol is converted to pregnenolone, and the

smooth endoplasmic reticulum enzymes and mitochondria are where pregnenolone is converted to testosterone [9]. The most crucial hormone for male health is testosterone. Erectile dysfunction and decreased sexual desire are both correlated with decreased testosterone levels in males [10]. Infertility results from hyperprolactinemia. A decrease in pulsatile secretion of LH in the pituitary and a suppression of pulsatile GnRH release from the hypothalamus are both effectively caused by high prolactin levels [11]. Prolactin's main function in animals is to control lactation. The anterior pituitary gland lactotroph cells primarily produce and emit the hormone prolactin [12]. A glycoprotein called follicle-stimulating hormone (FSH) operates on gonadal target cells, controlling gametogenesis [13]. Is released by the pituitary gland as part of a coordinated hypothalamic-pituitary-gonadal axis event. Absence or low FSH secretion is linked to a number of reproductive diseases [14]. Absolute or relative LH and FSH deficit results from the generation or activity of gonadotropins being impaired, which impairs gametogenesis in addition gonadal steroid synthesis and lowers fertility [15].

Material and Methods

The study was conducted in a clinic for infertility and IVF in Iraq, Diyala Governorate. The study included thirty-three men with Asthenozoospermia were compared against thirty-three healthy men as a control group (Normozoospermia). By collecting semen samples from the men (in a designated laboratory room), seminal fluid analysis (SFA) was carried out on the seminal fluids for the two groups. The 120 mL sample cups were warmed to body temperature (37 ^{0}C). Each sample was then placed in the incubator for 20 minutes at 37 ^{0}C to turn into a liquid before being examined. The examinations were carried out in accordance with the guidelines provided by the World Health Organization (WHO Lab Manual, 2010–2021) [16,17]. To gauge the volume of the ejaculate samples, a graduated cylinder with a conical base was employed. A light microscope is used to measure the characteristics of the semen, which includes

a. Sperm concentration: in which it is calculated the number of spermatozoa in each ejaculate as well as the concentration of spermatozoa per ml.

b. Sperm motility: divided into rapidly progressive spermatozoa of at least 25 µm/s, slowly progressive 5 to < 25 µm/s, non-progressive, < 5 µm/s and immotile, no active movements tail.

Then each sample was placed in a centrifuge at 4000 rpm for 20 minutes to separate the seminal plasma for the purpose of examining the pH by means a pH paper, as well as to examine the level of cholesterol by means the ELISA device.

For both groups, blood samples were drawn from veins and put in gel tubes to be used for hormone assays (FSH, LH, prolactin, and testosterone). then separating the serum for testing using the Chemiluminescence Immunoassay System (CL-900i) by centrifuging it at 3000 rpm for 10 minutes.

Statistical Analysis:

The data was gathered, compiled, summarized, analyzed, and presented using SPSS version 24 and Microsoft Office Excel 2010. ANOVA in one direction was used to assess how the means of numerical variables differed across two groups. P-values lower than 0.05 were used to determine the significance threshold. P values less than 0.001 were used to establish a level of high significance.

Results

Statistical analysis of the results from the inspection of semen and of anthropometrics are presented in Table (1), which included the (mean±SD) for Age, BMI, Semen Volume, sperm count, semen pH and motility. The average age, BMI, volume, and pH of the seminal samples from the Asthenozoospermia group are similar to those of the Normozoospermia group, as indicated in Table (1), and there are no statistically significant differences ($P>0.05$) between the two groups. The sperm characteristics of the Asthenozoospermia group did not fall within the WHO standard range, as was to be anticipated. In the Asthenozoospermia group, the No-progressive motility percentage and immotile percentage were significantly more than in the Normozoospermia group with a statistically significant difference ($P<0.01$). The Asthenozoospermia group had significantly lower values for sperm count and Progressive motility percentage compared to the Normozoospermia group with a statistically significant difference ($P<0.01$).

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Table 1: shows the mean±SD and the P value for Age, BMI, Semen Volume, sperm count, semen pH and motility in tow groups

Parameter	Group	No.	Mean ± SD	P value
Age (Year)	Normozoospermia	33	30.93 ± 1.04	0.698
	Asthenozoospermia	33	31.57 ± 1.11	
BMI	Normozoospermia	33	26.73 ± 1.00	0.140
	Asthenozoospermia	33	25.06 ± 0.68	
Semen Volume (ml)	Normozoospermia	33	3.53 ± 0.26	0.073
	Asthenozoospermia	33	2.90 ± 0.26	
Sperm Count (million/ml)	Normozoospermia	33	66.36 ± 1.81	<0.000
	Asthenozoospermia	33	35.93 ± 2.69	
Semen pH	Normozoospermia	33	7.63 ± 0.08	0.808
	Asthenozoospermia	33	7.66 ± 0.09	
Motility				
Progressive %	Normozoospermia	33	55.60 ± 0.91	<0.000
	Asthenozoospermia	33	23.93 ± 2.08	
No-progressive %	Normozoospermia	33	10.00 ± 0.00	<0.000
	Asthenozoospermia	33	12.42 ± 20.65	
Immotile %	Normozoospermia	33	34.54 ± 0.90	<0.000
	Asthenozoospermia	33	68.63 ± 1.49	

The results from the inspection of cholesterol in semen plasma and hormones in blood serum are offered in Table (2). As can be seen in Table 2, there were statistically significant variations in the levels of cholesterol between the Asthenozoospermia and Normozoospermia groups ($P<0.01$). FSH, LH, and testosterone levels were greater in the Asthenozoospermia group than in the Normozoospermia group, although there were no statistically significant differences ($P>0.05$). Asthenozoospermia had lower levels of prolactin than Normozoospermia, but there was no discernible difference ($P>0.05$).

Table 2: shows the mean±SD and the P value for Cholesterol, FSH, LH, Prolactin and Testosterone in tow groups

Parameter	Group	No.	Mean ± SD	P value
Seminal plasma Cholesterol	Normozoospermia	33	387.43 ± 5.54	<0.000
	Asthenozoospermia	33	191.94 ± 12.17	
Serum hormones				
FSH (m. IU / ml)	Normozoospermia	33	4.79 ± 0.42	0.993
	Asthenozoospermia	33	4.805 ± 0.35	
LH (m. IU / ml)	Normozoospermia	33	4.92 ± 0.37	0.225
	Asthenozoospermia	33	6.19 ± 0.43	
Prolactin (ng / ml)	Normozoospermia	33	15.82 ± 1.04	0.341
	Asthenozoospermia	33	14.25 ± 1.18	
Testosterone (ng / ml)	Normozoospermia	33	3.70 ± 0.14	0.296
	Asthenozoospermia	33	4.02 ± 0.21	

Discussion

The age, BMI, and the semen analyses of men who were registered in this study are shown in Table 1. The age of Asthenozoospermia patients (31.57±1. 11 years) and Normozoospermia control group (30.93±1.04years) was comparable and the differences were non-significant (p>0.05). The BMI of Asthenozoospermia group (25.06±0.68kg.m^{-2}) and Normozoospermia group (26.73±1.00kg.m^{-2}) was comparable and the differences were non-significant (p>0.05). Also, semen volume in Asthenozoospermia group(2.90±0.26ml) and Normozoospermia group(3.53±0.26ml) was comparable and showed non-significant differences (p>0.05). while Sperm Count, Progressive motility were non-comparable and showed high significant difference (p<0.001) between two groups. These results agree with of Elbashir et.al., study results [18]. Semen pH showed non-significant differences (p>0.05) Asthenozoospermia group (7.66±0.09) and Normozoospermia group (7.63±0.08) this results similar to study result of Hade et. al., [19]. In a previous study compared between Asthenozoospermia and Normozoospermia men, the semen cholesterol levels were significantly lower (p<0.05) in Asthenozoospermia men compared with Normozoospermia men [20]. This agrees with the findings of the study. Additionally, in a different study, the Asthenozoospermia group's cholesterol levels were considerably greater than those of the fertile control group when compared [21]. This finding conflicts with the study.

The blood serum analyses for FSH, LH, Prolactin and Testosterone hormones of men who were listed in this study are shown in Table 2. All analyzed hormones of Asthenozoospermia group and Normozoospermia group were comparable and the results of statistical analysis showed non-significant differences (p>0.05) between two groups. Previous study for Guo et al., in 2014 showed there was a significant difference between the two groups in Sperm concentration, Sperm progressive motility (%) (p=0.0001), did not find a significant difference in Age, BMI, Serum FSH, Serum LH, Serum Prolactin and Serum Testosterone [22]. These results are agree with our study results.

Conclusion

A significant decrease in the seminal plasma cholesterol concentration was found in Asthenozoospermia group compared to Normozoospermia and this difference can be used as

biomarker in predicting of infertility of men. However, there is no discernible difference between the Asthenozoospermia group and the Normozoospermia group in terms of the blood serum hormones FSH, LH, Prolactin, and Testosterone. According to the study's findings, the problem in the semen parameters (particularly sperm motility) of the group with Asthenozoospermia is caused by an aberration in the amount of semen plasma cholesterol. Therefore, we could improve their fertility by increasing the level of cholesterol in the seminal plasma.

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