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Research Article

ASSESSMENT OF SERUM AND SEMINAL AROMATASE ACTIVITY ON SPERM PARAMETERS FOR INFERTILE PATIENTS

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ABSTRACT

Aromatase is the enzyme that catalyzes the last step of estrogen biosynthesis. It is present in the various testicular cells including germ cells. The aromatase gene (Cyp19) is unique in humans and its expression is regulated in a tissue and more precisely, in a cell-specifi c manner via the alternative use of various promoters located in the first exon. Three groups of infertile patients (n=59): normozoospermia (n = 38), oligozoospermia (n = 8) and NOA (n = 13), referred to High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University,Baghdad, Iraq. levels of Serum and seminal aromatse were measured by ELISA. In addition to semen analyses and statistically analyzed. The study revealed Significant decrement ($P \leq 0.05$) was observed in the level serum aromatase for males with normozoospermia as compared to the other groups of the present study. Meanwhile, significant elevation ($P \leq 0.05$) was observed in the level of aromatase for male suffering from oligozoospermia as compared to normozoospermia and azoospermia. There were a significant decrement in the level of seminal aromatse for males complaining from normozoospermia as compared to the other groups of male infertility factors. Additionally, there was a significant difference ($P \leq 0.05$) for males with oligozoospermia in the level of seminal aromatase as compared to the others.The study concluded that excessive production of aromatase in both serum and seminal fluid associated with the infertility.

Keywords: male infertility; aromatse; semen quality; spermatogenesis.

INTRODUCTION

The cytochrome P450 enzyme complex called Aromatase. This enzyme was first reported in human placental tissues by K. J. Ryan in 1959.This enzyme converts androgens into estradiol (Timm, 2005). It is expressed in many tissues such as the adipose tissue, gonads and brain. The regulation of the level and activity of aromatase determines the levels of estrogens that have endocrine, paracrine and autocrine effects on tissue (Boon et al., 2010). Aromatase deficiency is a rare disorder and is usually caused by single base-pair changes resulting in amino acid substitution or premature stop codons (Simpson, 2000).

In most cases, the affected mother experiences virilization during third trimester of pregnancy. Affected female newborns have pseudohemaphrodism with clitoromegaly and hypospadias (Chen, 2002). The mechanism of action for estrogens in the male reproductive organs remain to be clarified in addition to the regulation of the aromatase gene expression, not yet fully understood especially according to the testicular development. In addition one should kept in mind that not only rodent spermatozoa. But ejaculated human spermatozoa express a functional aromatase and together with estrogen receptor (ER) .These data open new considerations about the role of estrogens all along the male genital tract(Carreau et al., 2012).

There is another study was reported by Denis who showed that immunocytochemical procedures using fluorescent probes connected with either confocal microscopy or flow cytometry

can be also useful to keep on with further investigations about the localization of proteins in the compartmentalized spermatozoa or the acrosome reaction. The dual location of aromatase both in the equatorial segment, the mid-piece and the tail could explain the double role of this enzyme in acrosome reaction and motility (Denis et al., 2009).

MATERIALS AND METHODS

The study population consisted of 38 normozoospermic, 8 ligozoospermic and azoospermic 13 men who were referred to High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, during the period from March, 2014 to December, 2014. The stratification of men into the normozoospermic and oligozoospermic and azoospermic groups was based on World Health Organization (WHO, 2010) criteria.

The ejaculates were collected after abstinence period of (3-5days). In a sterile, non-toxic, disposable Petri-dish by masturbation achieved in a private room near the laboratory prepared for this purpose in order to reduce the exposure of the semen to inconstancies in temperature and to control the time between collection and analysis, Specimen was labeled with patient's name and lab number. Containers were positioned in an incubator at 37°C permitted for liquefaction (Nafa and ESHRE, 2002). The liquefied semen was carefully mixed by glass Pasteur pipette for few seconds, and then the specimen was examined in detail by macroscopic and microscopic examination.

Seminal plasma preparation and storage

Centrifugation of semen samples for 15 minutes at 3000 rpm. Then recovering and positioning the supernatant of seminal plasma was quickly and carefully to freeze at -20° C for later measurements. Concentrations of aromatase were measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Blood Collection

Aspiration of five milliliters of peripheral venous blood was from each male. Then collecting blood samples in plain tubes let clotting and then centrifuged at 2500 rpm for 10 minutes. The specimens were categorized into two groups according to the results of sperm analysis. Concentrations of aromatase were measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Statistical analysis:

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago).

Sperm parameters, levels of plasma and serum aromatase were analyzed using complete randomized design (CRD) (one way ANOVA). Differences among means were computed using the Duncan multiple ranges test (Duncan, 1955).

RESULTS:

Table (1) represents semen parameters for normozoospermic, oligozoospermic and Azoospermic males took part in this study. The macroscopic examination of semen parameters showed that the semen volume, semen liquefaction time and semen pH were within normal values when compared with the criteria of WHO (2010). Also, the microscopic examination which include the sperm concentration, sperm grade motility, total progressive sperm, normal sperm morphology, sperm agglutination and round cells were within the normal values when compared with the criteria of WHO (2010).

In the same table, macroscopic examination of semen parameters for oligozoospermic males revealed normal values as compared with the criteria of WHO (2010).Besides, the sperm concentration of the microscopic examination was lesser than the normal values as compared with the criteria of WHO (2010). But the other sperm parameters of the microscopic examination were within normal values when compared with the criteria of WHO (2010). Semen parameters for azoospermic males participated in this study. Furthermore, the macroscopic examination of semen parameters for azoospermic males revealed that the semen volume, semen liquefaction and pH were within the normal values when compared with the criteria of WHO (2010). On the other hand, the sperm concentration was zero when compared with the standard criteria of WHO (2010), the round cell count was within the normal values.

In this study table (1) explains semen parameters for normozoospermic, oligospermic and azoospermic males participated in this study. The macroscopic examination of semen parameters revealed that the semen volume, semen liquefaction time and semen pH were within normal values

Table 1: Semen parameters for Normozoospermic, Oligozoospermic and azoospermic males participated in this study.

Semen parameters	Normozoospermia (no. 38)	Infertile patients		WHO(2010)criteria
		Oligozoospermia (no.8)	Azoospermia (no.13)	
Semen volume(mL)	2.589 ±0.17	2.775 ±0.53	2.162 ±0.22	1.5-5 mL
Semen liquefaction(min)	44.026 ±1.94	49.375 ±3.95	44.620 ±2.97	Within 60 Minutes
Semen viscosity	Norma	Normal	Normal	Drops/≤2cm thread
Semen pH	7.711 ±0.04	7.488 ±0.14	7.508 0.08	7.2-8.0
		Microscopic Examination		
Sperm Concentration	48.824 ±3.32	2.729 ±1.53	0.000	≥15millions/ml
Sperm motility(%)	73.324 ±1.09	67.625 ±2.24	0.000	Progressive motile sperm(32%) Within 60 minutes
Sperm grade activity (%) Progressive sperm motility	41.750 ±0.67	41.750 ±0.67	0.000	
Sperm grade activity (%) Non Progressive sperm motility	25.875 ±2.61	25.875 ±2.61	0.000	
Sperm grade activity (%) Immotile sperm	32.750 ±2.84	32.750 ±2.84	0.000	
Total Progressive sperm (millions/ejaculate)	48.969 ±4.31	3.588 ±2.63	0.000	≥8.2 millions/ejaculate
Normal sperm morphology (%)	37.921 ±0.51	36.250 ±1.75	0.00	≥30%
Sperm Agglutination (%)	3.079 ±1.03	0.000	0.00	≤10%
Round cells count (HPF)	5.500 ±0.55	5.375 ±1.73	0.00	≤5 cells/HPF

when compared with the criteria of WHO (2010). Also, the microscopic examination which include the sperm concentration, sperm grade motility, total progressive sperm, normal sperm morphology, sperm agglutination and round cells were within the normal values when compared with the criteria of WHO (2010).

In the same table, the macroscopic examination involving semen parameters for oligozoospermic males showed normal values as compared with the criteria of WHO (2010). Moreover, the sperm concentration of the microscopic examination was lower than the normal values when compared with the criteria of WHO (2010). But the other parameters of microscopic examination were within normal values when compared with the criteria of WHO (2010).

Semen parameters for azoospermic males participated in this study. Additionally, the macroscopic examination of semen parameters for azoospermic males revealed that the semen volume, semen liquefaction and pH were within the normal values when compared with the criteria of WHO (2010). On the other hand, the sperm concentration was zero when compared with the standard criteria of WHO (2010). However, the round cell count was within the normal values.

Figure (1) shows the level of serum aromatase activity or cytochrome 450 classified according to male infertility factors. Significant decrement ($P \leq 0.05$) was observed in the serum aromatase for males with normozoospermia as compared to the other groups of male infertility factors. Meanwhile, significant elevation ($P \leq 0.05$) was observed in

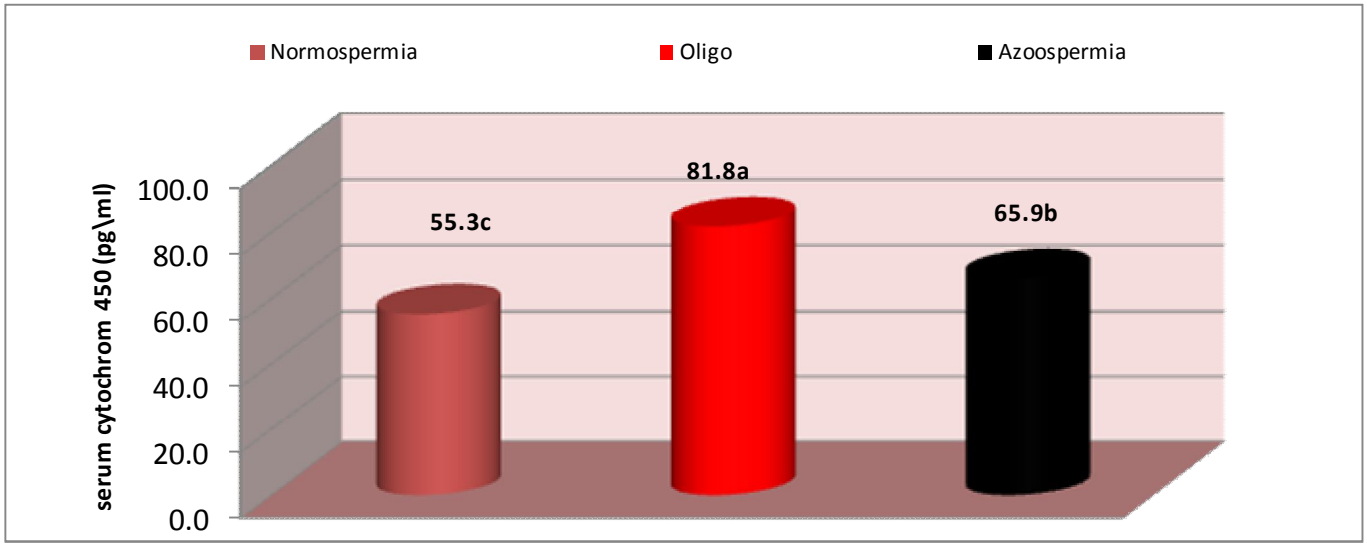

Figure 1: Level of serum Cytochrome 450classified according to male infertility factor.
Means with different superscripts within each column are significantly different (P<0.05).
Means with similar superscripts within each column are non significantly different (P>0.05).

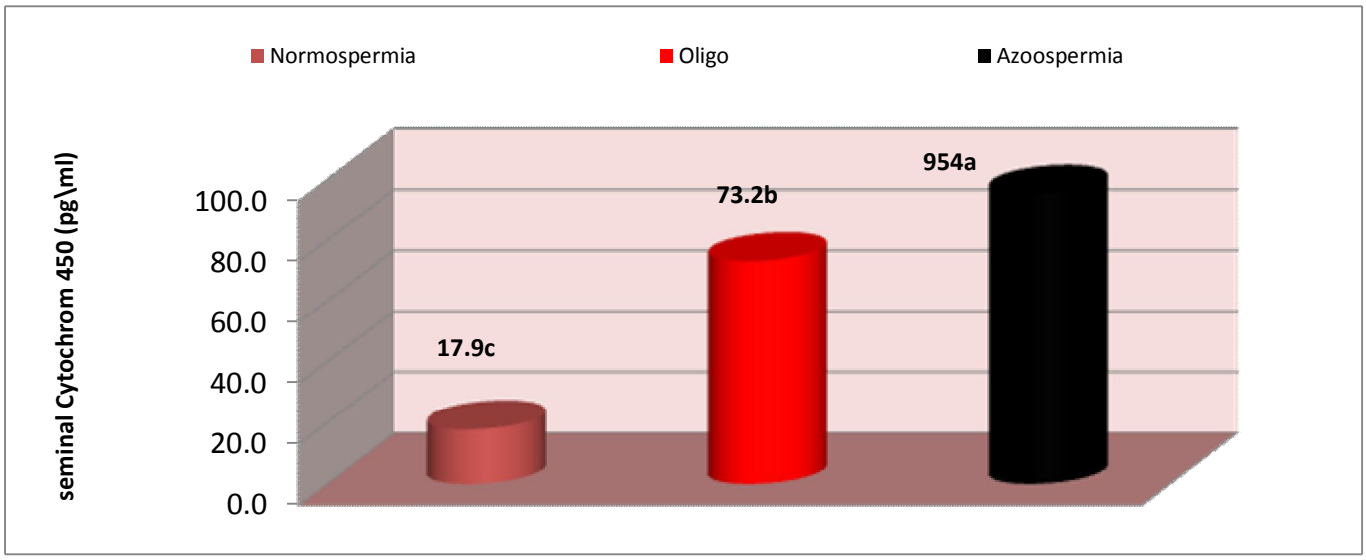

Figure 2: Level of seminal Cytochrome 450 classified according to male infertility factor.
Means with different superscripts within each column are significantly different (P<0.05). Means with similar superscripts within each column are non significantly different (P>0.05).

the level of aromatase for male suffering from oligozoospermia as compared to normozoospermia and azoospermia. Meanwhile, azoospermia revealed a significant difference (P≤0.05) in the level of serum aromatase as compared to the others.

Figure (2) shows the level of seminal aromatase activity or cytochrome 450 classified according to present study. There were significant decrements in the level of seminal aromatse for males complaining from normozoospermia as compared

DISCUSSION:

The current study showed significant decrement (P≤0.05) was observed in the serum aromatase for males with normozoospermia as compared to oligozoospermia and azoospermia. This may be due to Metabolic abnormalities involving slight truncal obesity, hyperinsulinemia, elevated serum triglyceride and low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol as well as some liver dysfunction are present in male aromatase

-deficient patients (Jones et al., 2007). Or it may be due to various mutations in the coding region of the CYP19A1 gene that lead to a decrease or loss of enzyme function, and as a result of oestrogen deficiency (Oraniec and Simpson, 2010). The current result in consistent with other study (Schlegel , 2012).They reported that some men with severely defective sperm production commonly have excess aromatase activity. The level of serum cytochrome 450 was at highest levels in males complaining from oligozoospermia. Also, it was at lowest level in males suffering from infertility with normozoospermia and azoospermia.This study in agreement with research revealed that the oligozoospermic men were found more frequently have short CYP19 (TTTA)n alleles compared to normozoospermic men. In addition, an association was observed between reduced sperm concentration and the CYP19 (TTTA)7 allele, both in normozoospermic men and in the total study population. This association supports the hypothesis that short CYP19 (TTTA)n alleles, and especially the CYP19 (TTTA)7 allele, may influence the transcription, mRNA stabilization or post translational expression regulation of aromatase, causing an alteration in estrogen levels that might lead to altered hormone levels in the testis and impaired spermatogenesis (Lazaros et al., 2011).

The expression of androgen receptors, P450arom and ERs (α and β) in testicular cells is related to the length of the photoperiod. More precisely, P450arom and ERβ are much more expressed in testes (especially in spermatocytes and elongated spermatids) of long photoperiod (Carreau et al., 2003). The study suggests that the actions of estrogen on male germ cell development are a consequence of paracrine and indeed intracrine interactions. Present results together with other observations about aromatase deficiency in men (Robertson et al., 1999). In obese men, the aromatization of C19 androgens like testosterone and androstenedione is a key step in estrogen biosynthesis and is catalyzed by the aromatase enzyme, a product of the CYP19 gene (Hammoud et al., 2006). It is believed that the elevation in estrogen levels in obese males is due to increased conversion of adrenal and testicular androgens owing to the increase in available aromatase enzyme in the fatty tissue (de Boer et a l., 2005). Estrogen production by adipose tissue is dependent on the availability of androgenic precursors in the circulation (Simpson et al., 1999).

The study concluded that excessive production of aromatase in both serum and seminal fluid associated with the infertility.

REFERENCES

1. Boon, W. C., Jenny, D., Chow, Y. and Evan, R. (2010). Multiple roles of estrogens and the enzyme Aromatase. Progress in Brain Research, Elsevier B.V. PP 233.
2. Carreau , S., Lambard ,S., Delalande ,C., Denis-Galeraud , I. and Bilinska , B .(2003). Aromatase expression and role of estrogens in male gonad: a review. Reprod Biol Endocrinol. 11;1:35 .Review.
3. Carreau, S., Bouraima-Lelong, H. and Delalande, C.(2012).Estrogen, a female hormone involved in spermatogenesis• Advances in Medical Sciences • 57(1) : 31-36 •
4. Chen, S. (2002). Modulation of aromatase activity and expression by environmental chemicals. [Frontiers in Bioscience 7, d1712-1719.
5. de Boer, H., Verschoor, L., Ruinemans-Koerts, J., Jansen, M. (2005). Letrozole, normalizes serum testosterone in severely obese men with hypogonadotropic hypogonadism. Diabetes Obes Metab. 7: 211–215.
6. Denis, G. C., Travert, C., de Vi enne, L., Said, A. and. Carreau, S. (2009). New insights about the evaluation of human sperm quality: the aromatase example. Folia Histochemica et CYytobiologica. 47(5): pp. S13-S17.
7. Hammoud, A. O., Gibson, M., peterson, M., Blake, D. H., ammilton, B., Hamilton, D, H. and Carrell, D. T.(2006) .Obesity and MaleReproductive Potential. Journal of Andrology, 27 (5):619-626.
8. Jones, M. E., Boon, W. C., Mclnnes, K., Maffei, L., Carani, C., and Simpson, E. R. (2007). Recognizing rare disorders: Aromatase deficiency. Nature Clinical Practice Endocrinology and Metabolism, 3, 414–421.
9. Lazaros, L., Xita , N., Kaponis, A., Hatizi, E., Plachouras, N., Sofikitis, N., Zikopoulos, K. and Georgiou, I. (2011).The association of aromatase (CYP19) gene variants with sperm concentration and motility, Asian J Androl, 13(2): 292–297
10. Oraniec, I. C. and Simpson, E.R.(2010). Aromatase research and its clinical Endokrynologia Polska/Polish Journal of Endocrinology Tom/Volume 61; numer/Numbersignificance 126-134.
11. Robertson, K. M., O'Donell, I., Jones, M. E., Meachem, S. J., Chinboon, W., Fisher, C. R., . Graves, K. H., Mclachlan, R . I. and Simpson, E.(1999). Impairment of spermatogenesis in mice lacking a functional aromatase (cyp 19) gene Proc. Natl. Acad. Sci. USA 96. 7986–7991.
12. Schlegel , P.N. (2012). Aromatase inhibitor for male infertility. Fertil Steril, 98:1359-1362.

13. Simpson, E. R. (2000). Genetic mutations resulting in loss of aromatase activity in humans and mice. J Soc Gynecol Investigation 7, S18-S21.
14. Timm, D. E. (2005). Final detailed review paper on aromatase EPA contract number 68-W-01-023 wor assignment 2-7 and 5-5, Task 2. Prepared for grey E. Timm work assignment manager U.S. environmental protection agency endocrine disruptor screening program Washington, D.C. prepared by By Battelle 505 King Avenue Columbus, OH 4320143201.pp93.
15. WHO. (2010). Reference values and semen nomenclature. In: WHO laboratory manual for the Examination and processing of human semen. 5th. Ed. Cambridge: Cambridge University Press.pp 162-224.

