

Estimation of Aromatase Enzyme in Obese Infertile Men in Correlation with Sex Hormones and BMI

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Abstract

Background:

Male obesity is associated with physiological changes that can negatively affect male fertility. Obese men have demonstrated a 3-fold increase in the prevalence of oligozoospermia when compared with men with normal Body Mass Index (BMI).

Objective:

To find the correlation between Aromatase enzyme with the following hormones (follicle stimulating hormone(FSH), Luteinizing hormone(LH), Estradiol(E₂), and testosterone) in the obese infertile male.

Subjects, Materials and Methods:

Seventy obese infertile male (patients) and twenty obese fertile male (control) are enrolled in this study. Aromatase enzyme, FSH, LH, E₂ and testosterone hormones were measured in their serum. All serum samples were measured by ELISA method.

Results:

Median human cytochrome enzyme level was significantly higher in patients than in control group, 87.55+ 12.48 pg./ml versus 56.95+9.88 pg./ml, P= 0.014. In patients group, a significant positive correlation was found between human cytochrome enzyme, FSH, and LH.

Conclusions:

The present study showed clearly that male obesity negatively affects fertility through changes in hormone levels. The results suggest that increased estrogen because of aromatization in the fatty tissue, reflected through the increase in human cytochrome enzyme (aromatase), may be an important mechanism for the hypoandrogenemia and altered sperm parameters.

Keywords: Aromatase enzyme, Male infertility, Obesity.

Introduction:

Male factor infertility is associated with a higher incidence of obesity and it is well recognized as a risk factor for female infertility. Male fertility both directly and indirectly has been proposed to be affected by obesity, which inducing variation in erectile dysfunction, hormonal profiles behaviour, sleep and scrotal temperatures ⁽¹⁾.

The relationship between infertility and male obesity can be attributed to more than just sexual dysfunction and other altered physical manifestations of obesity. Erectile dysfunction is significantly associated with overweight or obesity, and about 76% of men who reported with an erectile dysfunction or decrease in libido are overweight or obese ⁽²⁾. Regarding the hormonal profile, there is a complicated network of hormones negative and positive feedback mechanisms, which include testosterone, FSH, LH and E₂ ⁽³⁾.

Aromatase enzyme (called estrogen synthase or estrogen synthase) converts androgen hormones, which are involved in the sexual development of the male, to other forms of the female sex hormone estrogen. Aromatase alters testosterone to estradiol and androstenedione to estrone ⁽⁴⁾. Different tissues expressed this enzyme such as the brain, ovary, placenta, bone, skin, and adipose tissue. A single gene CYP 19A1 encoded the aromatase enzyme and its expression is controlled by tissue-specific promoters. Aromatase enzyme is a member of the cytochrome P450 enzyme family and a product of the CYP 19A1 gene ⁽⁵⁾.

The location of aromatase enzyme is in the endoplasmic reticulum of the cell,

where it is well regulated by tissue-specific promoters that are in turn controlled by cytokines, hormones, and other factors. The last steps of estrogens biosynthesis from androgens catalyzed by this enzyme, specifically it convert androstenedione (the common precursor of male and female sex hormones) to E₁ and testosterone to E₂ ⁽⁶⁾.

A decrease in the testosterone-estrogen ratio is consistently displayed in obese infertile men. These obese had 6% higher levels of estradiol and 25–32% lower levels of testosterone than normal-weight men ⁽⁷⁾. The severity of obesity determines the degree to which levels of estradiol are increased and testosterone decreased ⁽⁸⁾. On the other hand, the increased conversion of androgens into estrogens, which is characteristic of obesity, depresses the function of the pituitary gland by disturbing normal feedback in the testis. (BMI) negatively correlated with testosterone, FSH, and Inhibin B levels and positively correlated with E₂ levels ⁽³⁾.

Subjects, Materials, and Methods:

This study is carried out between 1st of July 2014 and the 15th of January 2015. A group of 70 infertile obese patients and 20 fertile obese males were recruited in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, AL-Nahrain University, Baghdad-Iraq. The subjects were included in this study according to the following criteria:

Ensuring male factor (exclusion of female cause and proven male cause by seminal fluid analysis), stopping hormonal treatment to patients for at least two months prior to sample

collection and no other possible secondary cause for male infertility (by clinical examination and U/S examination) done by specialists Urologist.

Statistical Analysis: Data were summarized, presented and analyzed using Statistical Package for Social Sciences (SPSS) software program version 16. Chi-square test was conducted to study the association between nominal variables such as the association between groups and BMI classes. Independent samples t-test was used to compare the difference in mean numeric variables between two groups. Mann Whitney U test was used to compare median human cytochrome enzyme (aromatase) difference between patient and control groups. P-value of ≤ 0.05 was considered significant.

Results:

Mean of (BMI) in patients and in control groups is shown in table 1. There was no significant difference in mean of (BMI) between control subjects and patients, $P= 0.705$.

Table (1): Comparison of mean (BMI) between the control group and patient group.

Group	N	Mean(BMI)	SE	Minimum	Maximum	P-value
Control	20	35.81	0.70	31.04	41.52	
Patients	70	35.45	0.47	30.01	46.32	0.705
Total	90	35.51	0.40	30.01	46.32	

Comparison of mean and median human cytochrome enzyme level between patients and control groups is shown in table 3. Mann Whitney U test showed that median human cytochrome enzyme level was significantly higher in-patient than in control group and $P= 0.014$.

Table (2): Comparison of mean and median human cytochrome enzyme level between patients and control groups.

Groups	Median	Mean	SE	Minimum	Maximum	P-value
Control	56.95	76.46	9.88	32.12	176.76	
Patients	87.55	137.40	12.48	23.37	470.37	0.014
Total	76.61	123.86	10.29	23.37	470.37	

Presence of a wide significant difference in enzyme level in both groups, suggests a possibility of a value that predicts patient group. For this purpose, a receiver operator characteristic curve (ROC) analysis was performed. This test showed that a value of ≥ 87.554 is a perfect cut-off to segregate groups into patients and controls as shown in table 3.

Table (3): Coordinates of ROC curve

Positive if more than or equal to	Sensitivity	1 - Specificity
....
....
79.003	0.543	0.300
79.634	0.543	0.250
82.148	0.529	0.250
85.636	0.514	0.250
87.554	0.500	0.250
88.649	0.486	0.250
91.439	0.471	0.250
96.436	0.471	0.200
99.276	0.457	0.200
104.661	0.443	0.200
....
....

The graph of sensitivity against (1-specificity) is shown in figure 1.

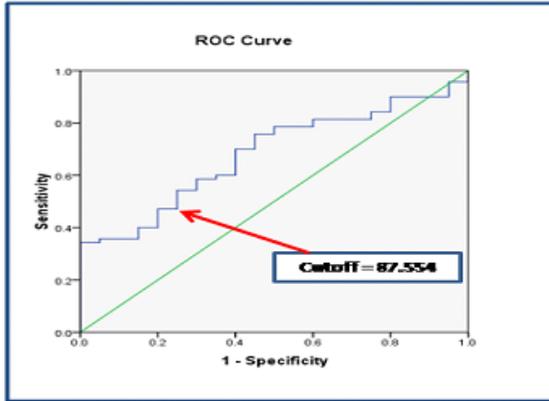


Figure (1): ROC curve showing the best human cytochrome enzyme cut-off value.

The specificity of the test was 75 %, sensitivity was 50%, positive predictive value (PPV) was 87.5 %, and negative predictive value (NPV) was 30%. The accuracy of the test was 68.1 % (it is very close to 0.7). The test was significant with a P-value of 0.014. These results are shown in table 4.

Table (4): Parameters of ROC analysis

Parameter	Value	Interpretation
Cutoff	≥87.608	Considered positive
Sensitivity	50 %	Poor
Specificity	75 %	Good
PPV	87.5 %	Good
NPV	30 %	Poor
Accuracy (AUC)	68.1% (0.681)	Although being Poor, it is very close to 0.7
P-value	0.014	Significant

Correlation of human cytochrome enzyme with other variables in control group, the BMI, FSH, LH, estradiol, and testosterone is shown in table 5. A significant positive correlation was found between human cytochrome

enzyme and LH, whereas, no significant correlation was found between human cytochrome enzyme and the rest of other variables.

Table (5): Correlation between human cytochrome enzyme and other variables in control group

Variables	R-value	P-value
Age(yr.)	-0.007	0.977
BMI	0.068	0.777
FSH (IU/L)	-0.006	0.980
LH (IU/L)	0.623	0.003
Estradiol (pg/ml)	-0.176	0.458
Testosterone(ng/ml)	-0.188	0.427

A significant positive correlation was found between human cytochrome enzyme, FSH, and LH. No significant correlation was found between other variables, age, BMI, estradiol, and testosterone. These results are shown in table 6.

Table (6): Correlation between human cytochrome enzyme and other variables in patients group

Variables	R-value	P-value
Age(yr.)	-0.111	0.359
BMI	-0.003	0.977
FSH (IU/L)	0.660	<0.001
LH (IU/L)	0.582	<0.001
Estradiol (pg/ml)	-0.095	0.432
Testosterone(ng/ml)	-0.063	0.602

A comparison was made between patients and control groups as shown in table 7, taking into consideration mean hormone levels.

Table (7): Mean serum hormone levels in patients and control group

Hormone	Groups	Mean	SE	Minimum	Maximum	P-value
FSH (IU/L)	Control	3.63	0.39	1.29	7.50	0.042
	Patients	6.76	0.80	0.77	40.40	
	Total	6.06	0.64	0.77	40.40	
LH (IU/L)	Control	3.21	0.24	2.00	5.73	0.094
	Patients	4.48	0.39	1.37	22.51	
	Total	4.19	0.32	1.37	22.51	
Estradiol (Pg./ml)	Control	14.75	3.35	0.50	71.07	0.066
	Patients	19.64	1.03	5.06	42.42	
	Total	18.55	1.11	0.50	71.07	
Testosterone (ng/ml)	Control	3.53	0.32	1.56	6.35	0.832
	Patients	3.46	0.14	0.51	5.82	
	Total	3.47	0.13	0.51	6.35	

Discussion:

Aromatase enzyme, which converts testosterone to E2, is highly expressed in peripheral fat tissue and any increase in aromatase activity is thought to result in increased E2 production, which inhibits secretion of FSH and LH from the pituitary gland⁽⁹⁾.

The extra-glandular aromatization of circulating androgen precursors is the major source of estrogen in all men. Importantly in men, the testes account at most for 15% of circulating estrogens, while the remaining 85% is due to peripheral aromatization of circulating androgen precursors in different tissues⁽¹⁰⁾, these are from adipose tissue, brain, skin, and the endothelium. In addition, it has been demonstrated that testicular androgen precursors contribute more to the total amount of circulating estradiol than adrenal androgens⁽¹¹⁾. These extra gonadal sites of estrogen biosynthesis lack the ability to synthesize C19 precursors from cholesterol so; their estrogen-producing activity totally depends on the availability of these circulating C19 androgenic steroids⁽¹²⁾. On the other hand, the estrogen synthesized within these extra gonadal

compartments may be also locally active in a paracrine or intracranial fashion⁽¹³⁾. It is clear that with more adipose tissue, the associated increase in adipose aromatase activity dominates any effect of the polymorphisms on intrinsic aromatase activity⁽¹⁴⁾. It is known, that aromatase is a specific marker of the undifferentiated adipose mesenchymal cell phenotype, but it is less expressed in mature adipocytes. Thus, factors that stimulate adipocyte differentiation, such as Peroxisome proliferator-activated receptor gamma (PPAR γ) agonists could also lead to the down-regulation of aromatase gene and a reduction in aromatase activity⁽¹⁵⁾. It is well known, if there are more adipocytes, there could be more aromatase activity even with reduced production of estrogen per fat cell.

The results of the present study demonstrated clearly that obesity might contribute to the decline in fertility rate among the studied patient group. As far as the hormones level are concerned, the FSH was significantly higher in the patient group than in control group, this result does not agree with results reported by other investigators⁽¹⁶⁾, who showed no statistically significant relationships between (BMI) and FSH hormone. Also reported by another study a lower FSH level in obese patients⁽¹⁾.

The mean LH was not significantly different in the patient group from in control group, but it seems to be slightly higher than in the controls. (MacDonald et al.) demonstrated that LH does not seem to be affected by (BMI) in men⁽¹⁶⁾. A study showed that there was no significant correlation explored between (BMI) and LH level⁽¹⁷⁾, whereas another study found that (BMI) significantly influenced LH

value⁽¹⁸⁾. Qin D. D. *et al.* claimed that obesity could reduce the level of LH⁽¹⁹⁾.

Mean estradiol was higher in the patient group than in control group, although the difference was not significant, it gives an indication that there is an association between (BMI) and an increase in this hormone. In males, E2 is driven mainly from intratesticular and peripheral aromatization of the C19 androgens (androstenedione, testosterone) by aromatase, a product of the CYP19A1 gene. CYP 19A1 is a single copy gene which is situated on the long (q) arm of chromosome 15 at position 21.1⁽²⁰⁾

It is believed that any increase in estrogens in obese males is owing to increased conversion of testicular and adrenal androgens due to the increase in available aromatase enzyme in the fatty tissue⁽²¹⁾. MacDonald *et al.* stated that the levels of estradiol generally appear unaffected by (BMI), but a few studies⁽²²⁾ have suggested slightly higher levels with increasing (BMI).

The reduced pituitary function or hypogonadotropic hypogonadism in obese men is likely multifactorial. (Schneider G, *et al.*)⁽²²⁾ stated that in obese men both estrone and estradiol are increased due to increased peripheral aromatization of androgens. Estrogens have a negative effect on the hypothalamus that alters the gonadotropin-releasing hormone GnRH pulses and suppresses gonadotropin, FSH and LH secretion⁽²³⁾. Such a role for estradiol is also confirmed, showing an increase in gonadotropins and sex steroid production after the administration of aromatase inhibitors to obese men⁽²⁰⁾.

A statistically significant association was observed between (BMI) and estradiol^(17, 24). (Egwurugwu *et al.*)⁽²⁵⁾, demonstrated significant and positive correlations between (BMI) and serum concentrations of progesterone and estradiol. Increase in (BMI) was associated with increase in serum levels of E2 and progesterone. Roth, M. Y. clearly expressed that high levels of estrogens in obese males result from the increased conversion of androgens into estrogens due to the high bioavailability of the aromatase enzyme⁽²⁶⁾.

The present study exhibited that the mean testosterone was not significantly different in the patient group than in control group, a negative significant correlation was found between human cytochrome enzyme and this hormone. This result seems to be different from other studies in which obese tissue leads to the lowering of the male hormone, testosterone, which directly affects fertility. Majority of studies that investigate the effect of obesity on testosterone reported decreased levels. This reduction is may be at least in part caused by the decreasing in the sex hormone binding globulin (SHBG) levels that will influence the half-life of testosterone⁽²⁷⁾. Whether the reduction in testosterone levels is adequate to exert a biological effect, influencing spermatogenesis, is a subject of debate.

The increase in the number and size of adipocytes is associated with obesity. Many proposed mechanisms to explain the effect of obesity on male fertility focus on the influence of abnormal levels of adipose-derived hormones and adipokines related to reproductive organs and hormones on fertility. Subcutaneous fat, intra-abdominal fat and total body fat may be associated with low levels of testosterone in males,

and most obese males looking for infertility treatment present with a decreased in the testosterone ratio to estrogen^(28, 29). This decrease is explained by over activity of the human cytochrome enzyme, which is exist at high levels in the white adipose tissue and, therefore, is responsible for a key step in the biosynthesis of estrogens.

Receiver operating characteristic (ROC) curve analysis was used to calculate cut off values of variables because of the presence of wide significant differences in enzyme level in both groups.

Conclusions:

1. The results suggest that increased estrogen because of aromatization in the fatty tissue, reflected through the increase in cytochrome enzyme (aromatase), may be an important mechanism for the hypoandrogenemia and altered sperm parameters.
2. The difference in the effect of weight on hormonal profile among men participated in this study may explain some of the divergence of the obtained results with respect to fluctuations in the value of some hormones.
3. A significant positive correlation was found between human cytochrome enzyme, (FSH) and (LH). No significant correlation was found with other variables, age, (BMI), duration of marriage, estradiol and testosterone.

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