Estimation of Aromatase Enzyme in Obese Infertile Men

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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 estimate the role of aromatase enzyme in decreasing sperm parameters in obese infertile men.

Subjects, Materials and Methods:

Seventy obese infertile male (patients) and twenty obese fertile male (control) are enrolled in this study. Seminal fluid analysis done according to criteria of WHO (2010).

Results:

A significant negative correlation was found between human cytochrome enzyme and sperm concentration, progressively motile sperm, non-progressively motile sperm, immotile sperm and normal morphology.

Conclusion:

The present study showed clearly that male obesity negatively impacts fertility through direct association between increased (BMI) and semen parameters. The results suggest that increased estrogen as a result 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.

Key words: **Aromatase enzyme, Male infertility, Obesity, Sperm parameters.**

Introduction:

Male factor infertility is associated with a higher incidence of obesityand 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 semen parameters,erectile dysfunction, hormonal profiles behaviour, scrotal temperatures and sleep [(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)]. There have been several studies which reported an inverse correlation in the relation between the seminal fluid parameters and obesity by affecting both sperm concentration, sperm motility and morphology [(1)].

Aromataseenzyme(called estrogen synthetize or estrogen synthase) converts androgen hormones, which are involved in the sexual development of the male, to another forms of the female sex hormone estrogen [(3)]**.** Aromatase alter, testosterone to estradiol and androstenedione to estrone. Different tissues expressed the enzyme such as 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 is a member of the cytochrome P450 enzyme family and a product of the CYP 19A1 gene [(4)].

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 E_1 and testosterone to E_2 [(5)].

Obese malesare three times more probable than healthy men of normal weight to have a sperm count of less than 20 million /ml, also known as oligozoospermia [(6)]. Males with a Body mass index (*BMI)*of more than (25 kg/m^2)had a lower total sperm concentration than males of normal weight.With increasing of (*BMI*), for any reason, the volume of ejaculate decreased steadily.Several reports exist of a considerable negative

relationship between *(BMI)* and sperm concentration, in some contradictions which have been noted; in these studies a correlation between sperm concentration in obese males compared to controls was explained, but wasn't considered significant [7].

Some unanimity on the effects of obesity on sperm motilityhas been established, however there is no completeagreement.Researchers found that sperm motility correlatednegatively with (BMI), and concluded that the incidence of low progressively motilesperm count increased with increasing (BMI)[8].

Some studies concluded that the total motile sperm count as well as the rapid progressively sperm motility correlated negatively with the waist and hip circumference of men. Despite this evidence, not all studies have included sperm motility within their measurement parameters, and other studies have found no effect of (BMI) or obesity on sperm motility[9].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.

Subjects, Materials and Methods:

This study was carried out between 1st of July 2014 and the 15th of January 2015. A group of 70 infertile obese patients and 20 fertile obese male was recruited in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, AL-Nahrain University, Baghdad-Iraq. The patients 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 US examination) done by specialists Urologist.

(BMI) was calculated by dividing the subject's mass by the square of his or her height, typically expressed in metric (kg/m^2), this shown as in table 1.

Table (1): The BMI classification

BMI	<18.5 (Kg/m^2)	18.5-24.9 (Kg/m^2)	25.0-29.9 (Kg/m^2)	30.0-34.9 (Kg/m^2)	35.0-39.9(Kg/m^2)	≥40.0 (Kg/m^2)
Classification	Underweight	Normal weight	overweight	Class I obesity	Class II obesity	Class III obesity

Freshly ejaculated semen samples were obtained by masturbation after (3-5) days of sexual abstinence. The specimens were placed in an incubator at 37°C for (15-30) minutes, in healthy case, to allow the time for liquefaction and then evaluate the seminal fluid parameters by macroscopic and microscopic examination. Seminal fluid analysis was done according to criteria of WHO (2010).

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 association between nominal variables such as the association between groups and BMI classes. Independent samples t-test was used to compare 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 2. There was no significant difference in mean of (BMI) between control subjects and patients and P= 0.705.

Table (2): Comparison of mean (BMI) between control group and patient group.

Group	N	Mean(BMI)	SE	Minimum	Maximum	P-value
Control	20	35.81	0.70	31.04	41.52	0.705
Patients	70	35.45	0.47	30.01	46.32	
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 (3): 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	

Correlation of human cytochrome enzyme in control group with sperm parameters is shown in table 4.No significant correlation was found between them.

Table (4): Correlation between human cytochrome enzyme and Sperm parameters in control group

Variables	*R-value*	*P-value*
Concentration (10^6/ml)	0.291	0.213
Progressive motile sperm (%)	0.103	0.665
Non -Progressive motile sperm (%)	-0.018	0.941
Immotile sperm (%)	-0.039	0.869
Normal morphology (%)	-0.034	0.886

A significant negative correlation was found between human cytochrome enzyme and sperm parameters.This result shown in table 5.

Table (5): Correlation between human cytochrome enzyme and sperm parameters in patients group

Variables	R-value	P-value
Concentration (10^6/ml)	-0.327	0.006
Progressive motile sperm (%)	-0.263	0.028
Non -Progressive motile sperm (%)	-0.284	0.017
Immotile sperm (%)	-0.317	0.008
Normal morphology (%)	-0.260	0.030

Sperm parameters (concentration, motility and morphology) in patient and control groups are shown in table 6. The mean sperm concentration $p < 0.001$, mean progressively motile sperm $p=0.027$ and mean normal morphology $p=0.002$ were significantly lower in patients group than in control group. Mean of non- progressively motile sperm $p=0.239$ and mean of immotile sperm $p=0.582$ are not significantly different.

Table (6): Sperm parameters in patients and control groups

Parameters	Groups	Mean	SE	Minimum	Maximum	P-value
Concentration ($*10^6$/ml)	**Control**	53.00	6.66	9.00	145.00	<0.001
	Patients	26.94	2.28	0.00	78.00	
	Total	32.73	2.56	0.00	145.00	
Progressive motile sperm (%)	**Control**	28.60	3.33	2.00	50.00	0.027
	Patients	19.66	1.90	0.00	46.00	
	Total	21.64	1.69	0.00	50.00	
Non –Progressive motile sperm (%)	**Control**	24.85	1.60	9.00	39.00	0.239
	Patients	20.90	1.72	0.00	44.00	
	Total	21.78	1.39	0.00	44.00	
Immotile sperm (%)	**Control**	46.05	4.67	19.00	88.00	0.582
	Patients	42.23	3.44	0.00	100.00	
	Total	43.08	2.86	0.00	100.00	
Normal morphology (%)	**Control**	32.05	2.19	8.00	48.00	0.002
	Patients	20.86	1.78	0.00	45.00	
	Total	23.34	1.54	0.00	48.00	

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[10].

The extraglandular 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 [11], these are from adipose tissue, brain, skin, and the endothelium. Also it has been demonstrated that testicular androgen precursors contribute more to the total amount of circulating estradiol than adrenal androgens[12]. 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[13].

On the other hand, the estrogen synthesized within these extragonadal compartments may be also locally active in a paracrine or intracranial fashion [14]. It is clearly that with more adipose tissue, the associated increase in adipose aromatase activity dominates any effect of the polymorphisms on intrinsic aromatase activity [15]. 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 (PPARγ) agonists could also lead to the down regulation of aromatase gene and a reduction in aromatase activity [16]. 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 rising level of estrogen and the low level of testosterone affect sperm making and production. In a study by(Hofny *et al.*) [17], obese men had 6% higher levels of estradiol and 25–32% lower levels of testosterone than normal men. The severity of obesity determines the degree to which levels of estradiol are increased and testosterone decreased[1]. 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[18].

The mean progressively motile sperm was found to be significantly lower in patients than in control group. Some unanimity on the effects of obesity on sperm motility has been founded, but there is no complete agreement. (Hofny *et al*.) [17], found that there is a negative correlation between the (BMI) and sperm motility, and (Hammoud, A. O., et al.)[19] concluded that the incidence of low progressively motile sperm count increased with increasing (BMI).

The presence of a negative correlation between semen parameters and overweight / obesity was confirmed by (Kort *et al*.) [20], which reporting the presence of a significantly decreased percentage of normal motile sperm in both overweight and obese men. Other studies on the relationship between male obesity and sperm motility have shown conflicting results. In astudy by (Jensen, T.K.) [21], there was no relation between increasing male (BMI) and percent of motile sperm. (FejesI,*et al*.) [22], found a negative correlation between body weight and total motile sperm count.

The present study revealed that the mean normal morphology was significantly lower in patient group than in control group, and that there was no relationship between increasing male (BMI) and abnormal sperm morphology. In a study, by (Kort *et al*.) [20], sperm morphology was taken into account when they calculated a composite marker of male fertility, the number of normal motile sperm, such that the interpretation of an effect of (BMI) on sperm morphology alone is impossible. However, most studies have shown no correlation between obesity and abnormal sperm morphology [7, 19, 21, 23].

Conclusion:

This study clearly showed that there is an evidence that the male obesity can affect fertility through the direct link between increased body mass index(BMI) and the sperm parameters via the mechanism of increased estrogen as a result of aromatization in the fatty tissue, which reflected through the increase in human cytochrome enzyme (aromatase), that may be an important mechanism for the hypoandrogenemia and altered sperm parameters.

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