Original Article

Effect of Body mass index on interleukin2, 6 and soluble fibroblast associated surface antigen in infertile men

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Abstract:

Background: obesity is an important cause of adverse health problems, including male infertility. Testosterone is essential for spermatogenesis and permits the release of mature sperms. In the absence of testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage.

Aromatase expression is directly related to the degree of adiposity; it is dependent on cytokine stimulation and requires the presence of glucocorticoids, 17β-estradiol in the plasma of adult men is formed by aromatization convert testosterone and androstinedione to 17β-estradiol

Apoptosis is an important process in the context of germ cells since they undergo both mitosis and meiosis, and this process is affected by interleukin 6and 2 (IL6 and IL2).

Objectives: To assess the effect of body mass index on serum sex steroidal hormones, seminal antiapoptotic factor soluble fibroblast associated surface antigen (sFas) and inflammatory markers (IL6, IL-2), with conventional semen parameters in infertile men.

Patients and methods: One hundred and six male partners of infertile couples were involved in the study. Height and weight of them were measured to calculate the body mass index (BMI). Serum testosterone and estradiol 2.were measured for all.Semen sample was taken after 2-7 days of abstinence. Conventional semen analysis was done for each sample according to the protocol of (WHO) 2010, after incubation and liquefaction period (30-60 min). Semen plasma was collected for analysis of interleukins (2 and 6) and sFAS .by specific kits Patients with severe oligospermia (below million sperm/ml) were excluded from the study. The rest (77) subjects were divided into three groups according to BMI (WHO, 2010) as follows: Normal (BMI < 25 kg/m2) (n=24): Overweight (BMI ≥ 25 and < 30 kg/m2) (n=30): Obese (BMI > 30 kg/m2) (n=23).

Results: The results showed a significant decrease in Testosterone level and Testosterone/E2 ratio in overweigh and obese subjects as compared to normal. The results of conventional semen analysis showed significant difference in pH and no. of round cell between the two groups. There was positive correlation between IL2 with sFAS in the two groups, and negative correlation of IL2 with total progressive cell and motility in both overweigh and obese groups. There was positive correlation of IL6 with no. of round cells in both studied groups.

Conclusion: BMI affects Testosterone level and Testosterone/E2 ratio (they were significantly decreased in overweigh and obese patients). A positive correlation between IL2 and sFAS in obese patients as well as positive correlation of IL6 with round cells in obese patients indicate the role of apoptosis which is essential for normal fertility to remove abnormal and unwanted cells in ejaculate, in infertile overweight and obese patients.

Key words: Infertility, BMI, IL2, IL6,sFAS.

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Introduction:

Obesity is a well-recognized risk factor for many health problems including infertility, however, its relation to decreased sperm count was not documented until 2004 by Jensen et al and Magnusdottir et al, 2005. (1)(2). Infertility can be defined as the inability to fulfill pregnancy after a reasonable time of sexual intercourse with no contraceptive measures. Male factors alone constitute 25%–30% of all cases of infertility in the form of defective sperm quality, and they contribute To another 30% in combination with female factors. (3)Testosterone is a steroid hormone produced by the Leydig cells under control of Luteinizing hormone (LH) from anterior pituitary gland. (4). It is essential to maintain spermatogenesis and male fertility. (5). Over 80% of the 17β-estradiol in the plasma of adult men is formed by extragonadal and extraadrenal aromatization of circulating testosterone and androstinedione by the enzyme aromatase particularly in the adipose tissue. The remainder (20%) comes from the Leydig cells. (6); some 17β-estradiol is also produced by aromatization of androgens in testicular germ cells, sperms and Sertoli cells (7).Aromatase expression is directly related to

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the degree of adiposity; it is dependent on cytokine stimulation and requires the presence of glucocorticoids (4) An interesting observation is that the estrogen content of the fluid in the rete testis is high, and the walls of the rete contain numerous ERα estrogen receptors. In this region, fluid is reabsorbed and the spermatozoa are concentrated. If this does not occur, the sperm entering the epididymis are diluted in a large volume of fluid, and infertility results (8) . In addition, spermatogonial stem cell renewal is promoted by estradiol implantation so estrogen is considered as an indispensable "male hormone" in the early spermatogenetic cycle (9).Cytokines function as up- and down-regulators of immunological, inflammatory and reparative host responses to injury. Interleukin 2 (IL-2) (a glycoprotein with a molecular weight of 15,400) is one of the major cytokines and exerts numerous immunological effects by stimulating the proliferation and growth of T, B and natural killer (NK) cells. Moreover, almost any cell possessing IL-2R will be stimulated to grow by IL-2 (10) . IL-6 is expected to cooperate with IL-1alpha to strengthen the local inflammatory reaction directly or through affecting some immune cells response in testis during early infection.(11) .IL-6 is a 26 Dka protein produced by a number of cell types, including Sertoli cells (12). Several evidences indicates that cytokines are involved in male infertility; they are secreted by different parts of the male genetic tract and may exert effects on the steroidogenesis, spermatogenesis and sperm function (13,14) .In response to inflammation of the testis, Sertoli cells play their regulatory role via secretion of a series of cytokines (15). Apoptosis is an important process in the context of germ cells because the cells are undergoing both mitosis and meiosis, errors during this process is corrected by apoptotic cell death to eliminate cells with genetic defects (16).This process is initiated by the cell surface protein Fas. sFas is a marker of overall apoptosis triggering, at the same time regulating apoptosis by competing with the cell surface receptor. Previous reports have suggested that the Fas mediated system is involved in the elimination of defective spermatozoa from the ejaculate and shows possible irregularities that could account for certain forms of male infertility (17).50%–70% of germ cells in the testis undergo apoptosis at different stages of spermatogenesis. Testicular apoptosis can be initiated through the Fas/FasL pathway. Fas, is believed to be a key initiator of apoptosis in the testis. (1819,) It is possible that both androgens and estrogens participate in the regulation and maintenance of spermatogenesis, and that estrogen deficiency or abundance has an impact on the gonad's output of sperm. Clinical trials with aromatase inhibitors have resulted in a tendency to improved seminal parameters. It is thought that this improvement is dependent on suppression of estrogen-to testosterone ratio, with an associated increase of FSH (20). Also, estradiol and testosterone inhibit LH in a negative feedback loop. Estradiol is formed from testosterone by the enzyme aromatase, which is present in a number of cell types (21).

Subjects and methods

One hundred and six male partners of infertile couples attending to High Institute for Diagnosis of Infertility and Assisted Technologies for Reproduction were involved in this study. All of them had no history of pregnancy or abortion for more than one year of marriage with regular unprotected sex and had no history of current or previous medical history of chronic disease. Height and weight of them were measured to calculate the body mass index (BMI).Two ml of venous blood were drawn for the assay of serum testosterone and estradiol 2 using the following kits. VIDAS® testosterone kit (Ref. 30 418, BioMérieux® SA, France) and VIDAS® estradiol II kit (Ref. 30 431, BioMérieux® SA, France), using the ELFA technique (Enzyme Linked Fluorescent Assay). Semen sample was taken from each of them by masturbation after 2-7 days of abstinence. Conventional semen analysis was done for each sample according to the protocol of World Health Organization (WHO) 2010, after incubation and liquefaction period (30-60 min). Azoospermic and severe oligospermia (less than 5 million sperm/ml) were excluded from the study. The rest (77) subjects were divided into three groups according to the results of BMI (WHO, 2010) as following:Normal (BMI < 25 kg/m^2) (n=24), Overweight (BMI ≥ 25 and < 30 kg/m^2) (n=30), Obese (BMI > 30 kg/m^2) (n=23) Semen plasma was collected after centrifugation of semen for 15 min. at 3000 rpm using Eppendorf centrifuge; and then frozen at -20 ˚C, till analysis of interleukins (2 and 6) and sFAS was done.

- Seminal plasma IL-2 was measured using (IL-2) bioAssayTM ELISA kit (USbiological, Catalog no. 17663-28E).
- Seminal plasma IL-6 was measured by (IL-6) bioAssayTM ELISA kit (USbiological, Catalog no. 18428-04). The kit was stored in refrigerator at 4 ˚C until time of use.
- While seminal plasma sFas was measured by using the kit of IBL International GMBH sAPO-1/FAS ELISA Enzyme immunoassay for the quantitative determination of human sAPO-1/Fas in human cell culture supernatants, serum, plasma or other body fluids ref. BE51901 (Germany).

Statistical analysis:

Data were analyzed using Microsoft Excel 2010 and SPSS Version 18. The results were presented as mean ± SD. Normally distributed data were analyzed using unpaired student t-test while abnormally distributed data (skewed) were statistically analyzed using Mann-Whitney U test. Pearson correlation coefficient was used for correlations. A value of $p < 0.05$ was considered to be significant.

Results:

The results of this study showed a significant decrease in Testosterone level and Testosterone/E2 ratio between normal and overweigh subjects as shown in table 1.

Table (1): Comparison of parameters between normal and overweight subjects by t test

Parameters	Normal(n=24) mean±SD	Overweight (n=30) mean±SD	P value
Age (year)	30.13+7.24	33.93+8.52	0.0817
Testosterone (ng/ml)	7.09+3.28	4.97+2.71	0.0145
Estradiol (E2) (pg/ml)	34.69+17.44	41.48+23.15	0.2248
Testosterone/E2 ratio	216.76+72.13	126.39+55.17	0.0000

While there was no significant difference between normal and overweight subjects regarding IL-2, IL-6 and s FAS. In regards to conventional semen analysis there was only significant difference in pH and no. of round cell between the two groups(table 2).

Table (2): Comparison of semen parameters between normal and overweight subjects by t test

Parameters	Normal (n=24) mean±SD	Overweight(n=30) mean±SD	P value
Volume (ml)	2.14+0.79	2.1+0.75	0.8723
Liquefaction duration (min.)	41.04+16.61	43.67+15.7	0.5572
pH	8.09+0.2	7.94+0.2	0.0088
Concentration (mill.)	51.54+32.16	39.8+28.41	0.1672
Progressive motility %	31.38+16.02	30.87+18.7	0.9148
Non-progressive motility %	22.42+11.77	20.57+10.25	0.5464
Immotile %	46.21+23.47	48.6+23.17	0.7098
Total progressive motile /ejaculate (mill.)	36.74+29.18	35.45+46.99	0.9016
Normal morphology %	21.25+13.97	24.5+13.51	0.3929
Agglutination %	2.08+4.74	1.13+3.5	0.4171
Round cell	14.08+10.0	7.23+4.19	0.0038
Epithelial cells	0.46+1.28	0.1+0.55	0.2116

The comparison between normal and obese there was a significant difference in Testosterone and Testosterone/E2 ratio (table3), no significant difference between normal and obese subjects regarding IL-2,IL-6 and s FAS.

Table (3): Comparison of parameters between normal and obese subjects by ttest

Parameters	Normal (n=24) mean±SD	Obese (n=23) mean±SD	P value
Age (year)	30.13+7.24	32.87+6.66	0.1827
Testosterone (ng/ml)	7.09+1.28	5.52+1.09	0.0585
Estradiol (E2) (pg/ml)	34.69+17.44	48.86+34.51	0.0871
Testosterone/E2 ratio	216.76+72.13	131.41+67.26	0.0001

There was significant difference in pH and round cells regarding conventional semen analysis (table 4) in obese subjects .

Table (4): Comparison of semen parameters between normal and obese subjects by t test

Parameters	Normal (n=24) mean±SD	Obese (n=23) mean±SD	P value
Volume (ml)	2.14+0.79	2.29+0.85	0.5257
Liquefaction duration (min.)	41.04+16.61	40.22+15.7	0.862
pH	8.09+0.2	7.98+0.13	0.0305
Concentration (mill.)	51.54+32.16	40.52+24.75	0.1939
Progressive motility %	31.38+16.02	34.65+15.03	0.4732
Non-progressive motility %	22.42+11.77	20.09+9.67	0.4614
Immotile %	46.21+23.47	45.26+16.44	0.873
Total progressive motile /ejaculate (mill.)	36.74+29.18	38.31+46.12	0.8905
Normal morphology %	21.25+13.97	24.22+14.55	0.4797
Agglutination %	2.08+4.74	0.3+1.11	0.0853
Round cell	14.08+10.0	6.35+2.84	0.0018
Epithelial cells	0.46+1.28	0.0+0.0	0.0938

Table 5 shows a positive correlation between IL2 and sFAS in normal and obese subjects also it shows a negative correlation between IL2 with total progressive motility and morphology.

Table (5): Correlation of IL-2 with different parameters in the three BMI groups

Parameter	Normal		Overweight		Obese	
	r	P	R	P	r	P
IL-6	0.236	0.316	0.357	0.103	0.479	0.044
sFas	0.637	0.003	0.079	0.726	0.706	0.001
volume	-0.517	0.02	0.244	0.274	-0.296	0.233
pH	0.288	0.218	-0.058	0.797	-0.341	0.166
Conc.	-0.327	0.159	-0.422	0.051	-0.386	0.113
PR %	-0.428	0.06	-0.321	0.145	-0.559	0.016
NP %	-0.431	0.058	0.141	0.531	0.1	0.692
IM %	0.46	0.041	0.181	0.42	0.437	0.07
Total progressive/ejaculate	-0.65	0.002	-0.466	0.029	-0.491	0.038
Morphology %	-0.764	0.000	-0.319	0.148	-0.537	0.022
Agglutination %	-0.053	0.824	-0.312	0.157	-0.348	0.157
Round cells %	0.218	0.356	-0.067	0.766	0.463	0.053

IL6 shows positive correlation with round cell in overweight and obese patients table (6)

Table (6): Correlation of IL-6 with different parameters in the three BMI groups

Parameter	Normal		Overweight		Obese	
	R	P	r	P	r	p
sFas	0.269	0.252	-0.104	0.646	0.314	0.205
volume	-0.243	0.302	0.425	0.048	-0.234	0.349
pH	-0.013	0.958	0.355	0.105	-0.132	0.602
Conc.	-0.195	0.41	-0.19	0.398	-0.06	0.814
PR %	-0.091	0.702	0.035	0.878	-0.081	0.75
NP %	-0.091	0.701	0.49	0.021	0.178	0.479
IM %	0.098	0.681	-0.248	0.265	-0.02	0.938
Total progressive/ejaculate	-0.243	0.301	-0.111	0.624	-0.221	0.378
Morphology %	-0.261	0.267	0.134	0.551	-0.07	0.783
Agglutination %	0.015	0.949	-0.106	0.639	-0.22	0.381
Round cells %	0.028	0.907	0.59	0.051	0.479	0.044

Discussion:

The parallel change in obesity and sperm count suggests a potential link between obesity and male fertility.(22)

Obesity is associated with altered spermatogenesis and erectile dysfunction. The altered spermatogenesis is mainly due to hypoandrogenism and the deleterious effect of increased levels of estrogens. All these factors can affect the ability of a male fertility. (23).(24) In this study there was decrease in no of progressive motility of sperms in obese and overweight these results are similar to those found by Fernandez et al 2011 (25) There was an increase in estrogen level in obese people, estrogen is well known as growth factor so it affects the level of IL2, this interleukin is a growth factor for WBCs, and this explain the increase in no. of round cells in semen of obese male, which affects fertility. Also IL6 is affected by estrogen as there was increase in estrogen and decrease Testosterone/ E2 ratio so we expect to find increased level of IL6 in obese male although the increase was not statistically significant which may need larger no. of patients in future work. But there was a positive correlation between IL2and sFAS in obese patients; IL2 shows negative correlation with total progressive motility and morphology so as IL2 increased there is decrease in progressive motility and morphology because IL2 is well known pro inflammatory and growth factor for lymphocyte this support the idea of inflammation in infertility. also IL6 shows positive correlation with round cell in obese and overweigh subjects These interleukins are known as proinflammatory as its effect on sFas seen in table (7,8) ,so they increase inflammatory process and reduce the whole apoptotic process which is necessary to remove cells that represent a threat to the integrity of the organism, such as infected cells, immunocompetent cells when the immune response decreases or DNA damaged cells as abnormal sperms (26) . Although the role of apoptosis during spermatogenesis and in somatic cells is well established, there is disagreement regarding the importance of apoptotic processes in ejaculated spermatozoa. It is not clear whether the apoptotic markers detected in ejaculated mammalian spermatozoa are residues of an abortive apoptotic process started before ejaculation or whether they result from apoptosis initiated in the post-ejaculation period (27) (28) .Furthermore, independently of the origin of the apoptotic changes that are detected in sperm, the exact relation of these changes with the motility, capacitation, acrosome reaction and other relevant parameters, which globally will determine the fertility ability of these cells, is still a matter of debate. Finally, a correlation among apoptotic changes and impaired sperm function has been found by Barroso et al. 2006 (29).(30)The fact that a large proportion of ejaculated spermatozoa display caspase activity may be indicative of an apoptotic mechanism involved in sperm selection within the genital tract (31).

Conclusion:

BMI affects male fertility testosterone level and Testosterone/ E2 ratio. Inflammatory cytokines affect fertility as there is a positive correlation between IL2 and sFAS in obese patients, IL2 shows negative correlation with total progressive motility and morphology. IL6 shows positive correlation with round cell in obese and overweigh subjects. All these affect apoptosis which is essential for normal fertility to remove abnormal and unwanted cells in ejaculate, so it affects fertility in obese patients.

Authors' contribution:

Israa Jaaffar design the study and its aims, Anam R alsalihi who provides the place of research and financial support and. Majid H Ahmed have participated in the collection of data and processing of them. Review of the article by all authers.

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