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"Comparative Study of Serum Testosterone/Estradiol Ratio in Normal, Overweight and Obese Male Subjects in Tertiary Care Centre"

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Key Words:

Obesity, Adipose Tissue, Testosterone, Estradiol, Central Obesity

Abstract:

BACKGROUND

In this study we evaluated the serum testosterone/estradiol ratio in normal, overweight and obese male subjects in tertiary care centre.

MATERIALS AND METHODS

This was a hospital based cross sectional study conducted among 186 male patients who presented to the General Medicine OPD of JSS Hospital, Mysuru over a period of 18 months. The study was conducted after obtaining clearance from Institutional Ethics Committee and written informed consent from the study participants.

RESULTS

The mean testosterone levels (215.2 ± 157.0 ng/dl) were found to be significantly low in obese males. Mean estradiol levels (39.7 ± 24.2pg/ml) were found to be high in obese males. Mean T/E2 ratio (64.4 ± 54.7) was found to be significantly low in obese males. No significant difference was observed in mean testosterone levels and estradiol levels with association between the age groups, however T/E2 ratio was found to be significantly low in the age group of 51 - 60 years.

CONCLUSION

Testosterone and T/E2 ratio showed a negative correlation with respect to BMI and waist circumference of more than 90cms (central obesity) without co-morbidities and thus low levels of testosterone can contribute to central obesity. Estradiol showed a positive correlation with respect to BMI and waist circumference of more than 90cms (central obesity) without co-morbidities. No significant difference was observed in mean testosterone levels and estradiol levels with association between the age groups, however T/E2 ratio was found to be significantly low in the age group of 51 - 60 years.

1. Introduction

The most significant androgen in males is testosterone. In mature Leydig cells, testosterone is biosynthesized. Normal level of testosterone secretion in adults is 4 to 9 ng/day. Since testosterone is the main androgen found in circulation in adult testicles, it has a negative feedback effect on the pituitary's ability to secrete gonadotropins. The free form of testosterone, which makes up just 2% of the total circulating form and is bound to SHBG (60%) and albumin (38%), is the portion that is physiologically active. Numerous diseases result in lower testosterone levels. Different pathways affect the illness state in different ways. In addition to the damage done by the underlying illness, this state of hypogonadism in males results in additional quality of life deterioration. Studies have shown that low testosterone levels in those with

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chronic conditions have a separate impact on mortality. In these circumstances, testosterone replacement treatment trials have been conducted with positive outcomes.[1] Contrary to visceral (omental) fat, estradiol levels have a strong positive relationship with body fat percentage and, in particular, subcutaneous belly fat. Omental fat's aromatase activity is, in fact, just one-tenth that of gluteal fats. Estrogens have a vital role in defining a men's sex desire in addition to being essential for the regulation of the gonadotrophin feedback, numerous brain functions, bone maturation, control of bone resorption, lipid metabolism, and skin metabolism in males.[2]

Measures of total testosterone (free, bio-available and bound to sex hormone binding globulin) are consistently inversely correlated with obesity, according to various epidemiological studies.[3] The degree of obesity and total testosterone have been found to be significantly inversely correlated in other studies,[4] that are unrelated to the metabolic syndrome (MetS).[5] In type 2 diabetic males, the relationship between waist circumference and plasma total and free testosterone levels, as well as SHBG is inverse, and there is a link between low testosterone levels and body mass index (BMI) and waist circumference.[6] Obese persons frequently have dyslipidaemia, which involves high TG levels, very low VLDL levels, and low levels of HDL cholesterol (HDL-C). The improper fat metabolism caused by this unbalanced lipid profile eventually leads to an increase in the accumulation of adipose tissue and ultimately leads to obesity and higher risk of cardiovascular issues. In certain studies, testosterone deprivation is linked to higher LDL and triglyceride levels, lower HDL in men with and without T2DM, or all of these are signs of atherogenic lipid profiles.[7] It is indisputable that there is an inverse relationship, mediated by a bidirectional mechanism, between testosterone levels and male body mass index (BMI). Initially, homeostatic processes enhance the rate of testosterone release. The conversion of testosterone to estradiol occurs in adipose tissue, particularly visceral fat. Once a certain level of fat mass is achieved, adipocytokines and insulin resistance start to limit testosterone synthesis, causing levels to drop. Low testosterone levels promote fat storage, especially visceral fat in the belly, and may harm other organs including the liver, muscles, and arteries by triggering the development of early stages of lipid streaks in atherogenesis. Estrogen and testosterone have historically been regarded as the female and male sex hormones respectively. However, the main type of estrogen in males is estradiol, also has a significant impact on male sexual function. Males who are obese have higher levels of both estrone and estradiol. Therefore, elevated circulating estrogen levels among obese people due to elevated peripheral conversion may unreasonably amplify the negative feedback on gonadotrophin secretion.

2. Materials and Methods

This was a hospital based cross sectional study conducted among 186 male patients who presented to the general medicine OPD of JSS Hospital, Mysore over a period of 18 months. The study was conducted after obtaining clearance from Institutional Ethics Committee and written informed consent from the study participants.

Inclusion Criteria

- Male subjects who are between age group of 20 to 60 years
- Male subjects are categorised into
- Normal group - 18.5 to 22.9 kg/m^2
- Overweight group - 23.0 to 24.9 kg/m^2
- Obese group - >24.9 kg/m^2 according to Indian consensus guideline.

Exclusion Criteria

- Diabetes mellitus
- Hypertension
- Thyroid disorders
- Chronic renal disease
- Chronic liver disease
- Secondary obesity
- Post bariatric surgery

Study Procedure

General physical examination and anthropometric assessment were taken and that includes Weight, Height, BMI, Hip circumference, Waist circumference, waist to hip ratio, blood pressure, pulse rate.

Biochemical assessment were done after obtaining the valid consent from the study subjects and then blood samples were collected for estimation of serum testosterone, serum estradiol, FBS, TSH, RFT and LFT. Testosterone and Estradiol levels was estimated by

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ECLIA (electro chemiluminescence binding assay) method using Cobas e 411 analyzer.

Statistical Methods

- MS Excel 2010 is used to enter the data and Statistical Package for Social Sciences (SPSS) software version 2020 is used to analyse it.
- The mean, standard deviation, frequency, and percentages are used in the descriptive statistical analysis.
- The Pearson product moment correlation, chi-square test, and one-way ANOVA give the inferential statistical analysis.
- Data is given as tables and statistical significance is considered when a p-value is < 0.0

3. Results

Table 1: Comparison of Mean BMI and Mean hip circumference between the study groups

BMI (kg/m^2)	Normal males	Overweight males	Obese males	F value	P value, Sig
Mean ± SD	20.4 ± 1.4	24.0 ± 0.6	28.5 ± 3.0	268.68	0.000, Sig
HC (cm)	**Normal males**	**Overweight males**	**Obese males**	**F value**	**P value, Sig**
Mean ± SD	81.9 ± 9.0	89.8 ± 10.0	95.5 ± 14.5	22.218	0.000, Sig

Table 1 shows the mean BMI when compared to normal males, overweight males and obese males was found to be statistically significant. Mean BMI (28.5 ± 3.0 kg/m^2) was significantly high in obese males. The mean hip circumference when compared to normal males, overweight males and obese males was found to be statistically significant. Mean hip circumference (95.5 ± 14.5 cm) was found be significantly high in obese male subjects.

Table 2: Comparison of Mean Testosterone levels, Mean Estradiol levels and Mean T/E2 ratio between the study groups

Testosterone (ng/dl)	Normal males	Overweight males	Obese males	F value	P value, Sig
Mean ± SD	382.85 ± 201.8	277.6 ± 157.5	215.2 ± 157.0	14.71	0.000, Sig
Estradiol (pg/ml)	**Normal males**	**Overweight males**	**Obese males**	**F value**	**P value, Sig**
Mean ± SD	30.1 ± 16.8	33.4 ± 24.4	39.7 ± 24.2	3.084	0.048, Sig
T/E2 ratio	**Normal males**	**Overweight males**	**Obese males**	**F value**	**P value, Sig**
Mean ± SD	171.6 ± 160.6	97.78 ± 58.1	64.4 ± 54.7	17.390	0.000, Sig

Table 2 shows the mean Testosterone levels, when compared to normal males, overweight males and obese males was found to be statistically significant. Mean Testosterone levels (215.2 ± 157.0 ng/dl) were found to be significantly low in obese males. The mean Estradiol levels when compared to normal males, overweight males and obese males was found to be statistically significant. Mean Estradiol levels (39.7 ± 24.2 pg/ml) were found to be high in obese males.

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The mean T/E2 ratio in the above mentioned table was shown to be statistically significant when compared to normal, overweight and obese males. Males who are obese were found to have a considerably low mean T/E2 ratio (64.4 ± 54.7)

Table 3a. Distribution of study group according to waist circumference			
Waist circumference	**Normal males** **n (%)**	**Overweight males** **n (%)**	**Obese males** **n (%)**
Less than 90cms	56 (90.3)	39 (62.9)	13 (21.0)
More than 90cms (Central obesity)	6 (9.7)	23 (37.1)	49 (79.0)
Total	62 (100)	62 (100)	62 (100)
χ^2 value=62.132	**df=2**	**p value=0.000, Sig**	

Table 3a shows, the waist circumference of more than 90cm (central obesity) and less than 90 cm when compared to normal males, overweight males and obese males was found to be statistically significant. Waist circumference of more than 90cm (central obesity) was found to be significantly high in obese males (79%) and waist circumference of less than 90cm was found to be more in normal males (90.3%).

Table 3b. Distribution of study group according to waist circumference and Testosterone levels, Estradiol levels and T/E2 ratio				
Mean ± SD	**Waist circumference**		**T value**	**P value, Sig**
	Less than 90cms	**More than 90cms (Central obesity)**		
Testosterone (ng/dl)	315.34 ± 181.78	259.34 ± 189.1	2.039	0.043, Sig
Estradiol (pg/ml)	31.0 ± 21.77	39.03 ± 22.38	2.455	0.015, Sig
T/E2 ratio	132.98 ± 128.76	81.1 ± 75.55	3.115	0.002, Sig

Table 3b shows, there was a statistically significant difference were observed in the mean testosterone levels, mean estradiol levels and mean T/E2 ratio between the waist circumference of more than 90cms (central obesity) and less than 90cms. Mean testosterone levels (259.34 ± 189.1 ng/dl) and T/E2 ratio (81.1 ± 75.55) were found to be decreased and Mean Estradiol levels (39.03 ± 22.38 pg/ml) were found to be increased with respect to waist circumference of more than 90cms (central obesity) when compared with waist circumference of less than 90cms

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Table 4. Correlation between age group and Testosterone, Estradiol and T/E2 ratio

Age group	Testosterone (ng/dl) Mean ± SD	Estradiol (pg/ml) Mean ± SD	T/E2 ratio Mean ± SD
20 – 30 years	345.74 ± 210.2	30.08 ± 16.37	153.34 ± 148.23
31- 40 years	291.08 ± 174.70	34.21 ± 17.25	106.95 ± 130.36
41 – 50 years	298.44 ± 190.59	31.47 ± 19.87	106.53 ± 76.82
51 – 60 years	247.17 ± 164.24	39.33 ± 29.12	84.12 ± 66.27
F value	2.603	1.823	3.641
P value, Sig	0.053, NS	0.145, NS	0.014, Sig

Table 4 shows, there was found to be statistically no significant difference in mean testosterone levels and estradiol levels between age groups, however T/E2 ratio was found to be statistically significant association between the age groups. Mean T/E2 ratio (84.12 ± 66.27) was shown to be substantially lower in the 51–60 age group

Table 5a. Correlation of BMI with Testosterone, Estradiol and T/E2 ratio

		BMI (kg/m2)
Testosterone (ng/dl)	Pearson Correlation	-.348**
	Sig. (2-tailed)	.000
	N	186
Estradiol (pg/ml)	Pearson Correlation	.196**
	Sig. (2-tailed)	.007
	N	186
T/E2 Ratio	Pearson Correlation	-.347**
	Sig. (2-tailed)	.000
	N	186

The above Pearson product moment correlation had shown that, the Testosterone levels and T/E2 ratio was found to be decreased with increase in BMI and the estradiol levels was found to be increased with increase in BMI. There is a negative correlation of testosterone levels (-.348) and T/E2 ratio (-.347) with respect to BMI and positive correlation of estradiol levels (.196) with respect to BMI.

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Table 5b. Correlation between waist circumference and Testosterone, Estradiol and T/E2 ratio

		WC (cm)
Testosterone (ng/dl)	Pearson Correlation	-.203**
	Sig. (2-tailed)	.005
	N	186
Estradiol (pg/ml)	Pearson Correlation	.193**
	Sig. (2-tailed)	.008
	N	186
T/E2 Ratio	Pearson Correlation	-.274**
	Sig. (2-tailed)	.000
	N	186

In the above Pearson product moment correlation table, there is a negative correlation of testosterone levels (-.203) and T/E2 ratio (-.274) with respect to waist circumference and positive correlation of estradiol levels (.193) with respect to waist circumference.

4. Discussion

Body Mass Index

The obese group had the highest mean BMI of (28.5 ± 3.0 kg/m^2) followed by the overweight group with a mean BMI of (24.0 ± 0.6 kg/m^2).Waist circumference of more than 90cm (central obesity) without co-morbidities was found to be significantly high in obese males (79%), as well as a higher mean hip circumference (95.5 ± 14.5 cm). All the above parameters were found to be statistically significant.

Testosterone

Mean testosterone levels were significantly higher in normal males (382.85 ± 201.8 ng/dl) as opposed to obese males (215.2 ± 157 ng/dl) which were the least amongst the three study groups. The age group of 20-30 years had the highest mean testosterone (345.74 ± 210.2 ng/dl). Male testosterone levels peak in their early adult years. However, testosterone levels start to steadily decrease around the age of 30. This change is due, in part to ageing men increased propensity for testosterone to be converted to estrogen by aromatase. Additionally, once the ratio of testosterone to estrogen starts to change, there is a greater possibility that estrogen will attach to androgen receptors, which are typically used by testosterone. In response, the body produces less testosterone, which further tips the scales.

Testosterone showed negative correlation with BMI (Pearson's correlation -0.348). This was in concordance in a study which was done by **Shamim et al.**[8]

Osuna et al.[9] who conducted a cross-sectional analysis, examined the relationship between BMI and TT in 77 men between the ages of 20 and 60, they discovered a strong negative correlation between TT and BMI suggesting a mechanistic connection between the two. In a research of 57 males between the ages of 70 and 80, **Vermeulen et al**.[10] also discovered that there is a drop in testosterone levels when compared with insulin levels, abdominal fat, and body fat percentage. A negative association was found in the current study of mean testosterone levels (259.34 ± 189.1 ng/dl and Pearson's correlation coefficient -0.203) with respect to waist circumference of more than 90cms (central obesity). Similar results were obtained in a study by **Svartberg et al**.[11] who conducted a survey-based study, which included 1548 men aged 25

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to 84, found a substantial ($p<0.001$) inverse association between waist circumference and testosterone.

Estradiol

In obese males (39.7 ± 24.2 pg/ml), the mean estradiol levels were found to be considerably higher as compared to overweight (33.4 ± 24.4 pg/ml) and normal BMI males (30.1 ± 16.8 pg/ml). The mean estradiol levels (39.03 ± 22.38 pg/ml) were found to be significantly increased with respect to waist circumference of more than 90cms (central obesity). This was in agreement with a research carried out by **Wang et al**.(12)

Though not statistically significant, a higher level of estradiol (39.33± 29.12 pg/ml) was observed between the age group of 51 to 60 years. An inverse relationship between age and free estradiol levels was noticed by **Orwoll et al.**(13) High levels of estradiol can decrease fertility and cause hypogonadotropic hypogonadism in overweight and obese people by affecting the release of GnRH, LH, and FSH.

Given that obese men have somewhat have higher estrogen levels than men with a normal BMI, it has been hypothesized that lower testosterone levels in obese men are brought on by increased estrogen synthesis and its effects on the hypothalamic-pituitary-testicular axis.[9] Estradiol is thought to be linked to males overall obesity and fat distribution indices. Males who are obese typically exhibit a "hyperestrogenic hypogonadotropic hypogonadism" hormonal profile. Low levels of total and free testosterone in obese men have been related to the increased activity of the aromatase cytochrome P450 enzyme, which is abundantly expressed in white adipose tissue. The aromatization of C19 androgens, such as testosterone and androstenedione, is an essential step in the synthesis of estrogens. Particularly in the central region, obesity appears to be linked to a proportionate drop in circulating androgen levels.

Estradiol concentrations were 6% higher and total testosterone serum concentrations were 25 – 32% lower in obese guys compared to normal-weight men, according to research by **Aggerholmet al**.(14) Males with high adulthood BMI had 14% lower testosterone, 9% lower inhibin B, and 20% more estradiol than men with low adulthood BMI, according to **Ramlau-Hansen et al**.(15)

There was a positive correlation of estradiol levels (Pearson's correlation coefficient196 and Pearson's correlation coefficient193) with respect to BMI as well as waist circumference observed in the present study which was in contrast to studies by **Pasquali et al**.(16) and **Dhindsa et al.**(17) who did not find any correlation between BMI and estradiol levels. **Ahmad et al.**(18) demonstrated a favourable correlation between waist hip ratio and serum estrogen levels.

T/E2 Ratio

Spermatogenesis is influenced by the local hormonal balance of testicular testosterone and estradiol ratio. By adversely influencing normal spermatogenesis, the loss of this balance may cause infertility. Therefore, assessing the T/E2 ratios change may provide useful information. The mean T/E2 ratio in the current study was found to be significantly low in obese (64.4 ± 54.7) males as compared to normal BMI males (171.6 ± 160.6). This could be attributed to the fact that a vicious cycle of obesity related estrogenic hypogonadism is caused by the accumulation of adipose tissue in central obesity, which increases the rate at which total testosterone is converted to estradiol by aromatase activation and suppresses the hypothalamic-pituitary unit.

The mean T/E2 ratio (84.12 ± 66.27) was found to be significantly low in the age group of 51 - 60 years. Mean T/E2 ratio (81.1 ± 75.55) had significantly decreased with respect to waist circumference of more than 90cms (central obesity). There was a negative correlation of T/E2 ratio with respect to BMI as well as waist circumference with a Pearson's correlation coefficient of (-0.347) and (–0.274) respectively. **Oztekin et al.**(19) also had similar outcomes. The T/E2 ratio and a rise in BMI did not significantly correlate in a study by **Keskinet al**.(20) encompassing 454 individuals, however BMI and total testosterone, however, correlated inversely. The T/E2 ratio and waist circumference were found to be negatively correlated in studies by **Ali Hamza et al.**(21) **Alajeely et al**,(22) and **Rohrmann et al.**(23) The findings of our study and previous studies indicate that obesity reduces testosterone, which has a detrimental effect on male reproductive capacity.

The drop in testosterone levels in overweight and obese patients has been accounted for in a number of ways. Because SHBG levels are lower, more testosterone can be converted into estradiol in adipose tissue. In a few instances, this has resulted in a minor rise in plasma estradiol levels, but even in those instances when estradiol levels are not elevated, the T/E2 ratio may fall as a result of the lowered testosterone levels. It has been demonstrated that infertility is linked to a decline in the T/E2 ratio in humans.

According to **Pan et al.**[26] there is an antagonistic association between T/E2 and erectile dysfunction. Raised scrotal temperature is a consequence of adipose tissue accumulation in the supra-pubic and thigh areas of obese people, this might have a detrimental effect on spermatogenesis and fertility in obese males. Obese patients may have certain scrotal lipid tissue build-up patterns that are not seen in infertile non-obese individuals.

Larger randomized and prospective studies are necessary to completely understand the effects of obesity on sperm quality, the hormonal axis and male infertility, despite the fact that greater BMI has been shown to negatively influence reproductive capacity through a number of modes of action. Weight loss and testosterone treatment can both break this vicious cycle, as evidenced by the bidirectional inverse relationship between high fat mass and testosterone levels. Although HPT axis reactivation is achievable with weight reduction, many obese men may find it difficult to shed the required amount of weight and keep it off with typical lifestyle changes. However, achieving a healthy weight loss should be the first priority as it offers so many other advantages.

5. Conclusion

Testosterone and T/E2 ratio showed a negative correlation with respect to BMI and waist circumference of more than 90cms (central obesity) without co-morbidities and thus low levels of testosterone can contribute to central obesity. Estradiol showed a positive correlation with respect to BMI and waist circumference of more than 90cms (central obesity) without co-morbidities. No significant difference was observed in mean testosterone levels and estradiol levels with association between the age groups, however T/E2 ratio was found to be significantly low in the age group of 51 - 60 years.

Ethics Approval and Consent to Participate

Study was conducted after obtaining clearance from Institutional Ethics Committee – JSS Medical college, Mysuru, Karnataka and after obtaining written informed consent from the study participants.

Conflict of Interest
The authors declare that , there is no conflict of interest
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