ORIGINAL ARTICLE

RELATIONSHIP OF SERUM TESTOSTERONE LEVELS WITH ANTHROPOMETRIC PARAMETERS OF OBESITY IN YOUNG MALES OF SOUTH PUNJAB

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Background: Obesity is the epidemic of current century. It deranges the delicate metabolic and endocrine balance essential for well being of an individual. Men are specifically affected by circumferential obesity which reduces their testosterone level and deprives them of its metabolic, immune and anti inflammatory benefits resulting in emergence of glycemic, atherosclerotic and inflammatory disorders. **Methods:** It was a cross sectional comparative study carried out at Department of Physiology, Post Graduate Medical Institute (PGMI) Lahore in collaboration with medical OPD of Lahore General Hospital. A total of 40 subjects, between 20 to 40 years of age, were categorised into non obese and obese groups and each group consisted of 20 subjects. Serum testosterone levels of subjects were measured through Enzyme Linked Immunosorbent Assay (ELISA). **Results:** Testosterone levels, represented as median (IQR), for groups A and B were found to be 680 (575.0–78.5) and 412.5 (338.0–542.5) ng/dl respectively. Serum Testosterone levels of obese subjects were significantly lower as compared to non-obese subjects (p=0.003) and they had an inverse correlation with both Waist Hip Ratio (WHR) (p=0.004) and Waist Circumference (WC) (p=0.002) but not with Body Mass Index (BMI) (p=0.058). **Conclusion:** Circumferentially obese men have lower testosterone levels as compared to age and ethnicity matched non obese men. WHR and WC have an inverse correlation with serum testosterone levels and they are better indicators for predicting testosterone decline in obese men as compared to BMI.

Keywords: Obesity, testosterone, Waist Hip Ratio (WHR), Waist Circumference (WC), Body Mass Index (BMI)

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INTRODUCTION

Obesity is currently known as the epidemic of 21st century.[1] It is being predicted, by WHO, that by the mid of this decade one in every three individuals will be overweight and one in every ten people will be obese. Nutrition either directly (through energy imbalance) or indirectly (via hormonal influences) results in emergence of obesity through epigenetic processes. Lack of physical activity and diet composed of dense foods rich in simple sugars, combined together, result in development of generalized as well as circumferential obesity.[2] The cut-off values at which individuals are termed as obese, according to various anthropometric parameters, vary with ethnicity.[3] According to WHO standards South Asians with a Body Mass Index (BMI) of >25 or a Waist Hip Ratio (WHR) of >0.9 are considered obese.

In Pakistan the prevalence of obesity, projected by national health survey of Pakistan 1990–94 in terms of BMI, was put at 25%. However certain other surveys found its incidence to be around 41% in their localities. Recent surveys which project obesity in circumferential terms, calculated through Waist Circumference (WC) or WHR instead of BMI, are putting its prevalence at 57%. It indicates that circumferential obesity is rather more prevalent in Pakistan.[4]

BMI gives only a crude idea of total body fat. Therefore more precise anthropometric parameters, of WC and WHR, have been devised which give actual idea of body fat composition and predict the risk for the development of obesity associated glycemic and atherogenic disorders more precisely. Current studies are employing both WC and WHR as relatively more reliable tools, for representation of obesity and for projection of obesity associated risk factors, than BMI.[5]

Testosterone is the major androgen in men and it regulates muscle, bone and fat metabolisms, sexual development and function, spermatogenesis and erythropoiesis.[6] It reduces both overall fat mass and central adipose store which is most closely associated with the development of insulin resistance and atherogenic disorders.[7] It affects the myocytic stem cell in a positive fashion and has a negative effect on the lipoid stem cell.[8]

Testosterone increases insulin receptor expression and phosphorylation, which enhances cell's responsiveness to insulin by enhancing expression of Glucose Transport Receptor-4 (GLUT4), Hexo-Kinase 2 (HK2), Phosphofructokinase (PFK) and Glucose-6-Phosphate Dehydrogenase (G6P-dehydrogenase) and by

an increased glycogen synthase and reduced glycogen phosphorylase activity.[9] Declining testosterone levels, in men with obesity, reduce insulin sensitizing effects of testosterone and put them at an increased risk of systemic diseases through emergence of insulin resistance.[10]

Testosterone through the activation of hormone sensitive lipase promotes lipolysis and reduces triglyceride (TG) uptake by the adipose tissue. It enhances lipid oxidation and affects the TG and Very Low Density Lipoprotein (VLDL) kinetics. It increases Scavenger Receptor-B1 (SR-B1) mediated uptake of High Density Lipoprotein (HDL) by liver through Hepatic Lipase (HL) and Apo-A1 modulation. The process is known as reverse cholesterol transport. If these processes are disturbed, as happens in testosterone deficient subjects, they result in the development of dyslipidemia and atherosclerosis.[11] This brings about changes within the vascular wall and results in disorders like coronary artery disease.[12]

Testosterone causes both aromatase receptor (ARm) and androgen receptor (AR) activation in vascular smooth muscle and vascular endothelial cells through a Nicotinamide Adenine Dinucleotide Phosphate (NADPH) system resulting in generation of nitric oxide (NO) which causes vasodilatation. This effect is masked in men with low testosterone levels (obese men), where testosterone decline results in generation of Reactive Oxygen Species (ROS) instead of NO. This along with deranged endothelin levels, in obese men, produces overall vasoconstrictive effect putting them at an increased risk of vascular diseases.[13]

Normal testosterone levels in men range from 300 ηg/dl to 1,000 ηg/dl.[14] According to European standards a day time level of 300 ηg/dl or less is considered to be an indicator of testosterone decline in men.[15]

Development of circumferential obesity and the resultant changes in BMI, WC and WHR lead to decreased serum testosterone levels.[16] This helps in emergence of insulin resistance, creating a compensatory hyperinsulinemia, which results in dysregulated insulin like factor 3 (INSL-3), Luteinizing Hormone (LH) and Sex Hormone Binding Globulin (SHBG) levels and a deranged LH/FSH ratio which alters Hypothalamo-Pituitary Axis (HPT) axis and reduces testosterone levels. Obese men also have an enhanced production of estradiol (oestrogen) as a result of excessive aromatization within the fat tissue. These raised oestrogen levels negatively affect the HPT axis which results in decreased production of gonadotropins and hence testosterone.[17]

In present study we proposed that testosterone levels decline with emergence of circumferential obesity and that they are inversely related to WC and WHR in young males.

METHODOLOGY

It was a cross-sectional comparative study conducted on young male population of Southern Punjab. Approval of research protocols was obtained from the Ethical Review Committee of PGMI, Lahore. Data were collected after obtaining fully informed, understood, and voluntary consent of the subjects.

The sample size for each group was calculated with a power of 90% and an alpha level of 5% through WHO extended software 'Sample size determination in health studies: a Practical Manual' version 2.0. It was a study based on convenience sampling. Study population consisted of forty male subjects between 20 and 40 years of age and it was equally divided into non obese (Group A) and obese (Group B) groups with 20 individuals falling in each group.

Since testosterone levels see an age related decline from the fourth decade of life onwards[18], individuals till 40 years of age were included in the study to exclude the effect of age related testosterone decline in study population. Healthy males fulfilling the criteria of obese and non-obese subjects were included in this study. According to WHO 2000 Guidelines (for South Asians), a BMI of ≥25 and a WHR of >0.9 was taken as a cut-off value to differentiate between non obese and obese subjects which were age and ethnicity matched. Males who had developed morbid obesity having a BMI of ≥30 (WHO, 2000), who were taking exogenous testosterone and those with genetic obesity were excluded from this study.

To calculate BMI, height (meter) and weight (Kg) were measured in subjects wearing usual clothing, and without shoes, with height and weight wall mounted stadiometer (SMIC health scale). Body Mass Index of the subjects was calculated by using the formula: BMI= Body weight in Kg/(Height in m)^2. To Calculate WHR, subjects were made to stand straight with arms by their sides, approximated feet and with weight evenly distributed on them. After proper exposure of abdomen with minimal clothing, waist circumference (Cm) was measured in the horizontal plane, midway between costal margin and the iliac crest. Measurements were taken at the end of normal expiration with non stretchable plastic tape. Hip circumference (Cm) was taken at the widest portion of the gluteal region, with minimal clothing and in standing position. Waist to Hip ratio was calculated by the formula: WHR= WC/HC

To collect the blood sample, selected subjects were instructed to hold an overnight fast of 12 hours before the day of sampling. Three ml of venous blood was drawn between 8 and 10 AM and was added to the yellow top vacutainers. It was allowed to stand for 45 minutes before centrifugation so that it could develop a stable clot. Following centrifugation at a speed of 3,000 rpm for 3 minutes, the derived serum samples were

aliquoted identification numbers and stored immediately at -20 °C for a later analysis.

Serum testosterone levels were measured by enzyme linked radio immunosorbent assay (ELISA) by using the ASTRA BIOTECH Testosterone ELISA Kit Ref: 21-02A (Germany). The tests were run on QMlab Plate Reader (Reid Well Plate). The values were derived in nmol/L initially and were converted to ηg/dl figures by dividing them with the conversion factor of 0.0347 (1 ηmol/L=28.8 ηg/dl). Assay Range was 0.2–50 ηmol/L (6–1154 ηg/dl), Assay Sensitivity was 0.2 ηmol/L (6 ηg/dl), Assay specificity was 100% for human serum testosterone, Intra-assay precision was 3.77% and Inter-assay precision was 7.39%.

The data was analysed on SPSS-18. Data was analysed first for normality distribution by Shapiro-Wilk's and Kolmogorov Smirnov's tests. Mean±SD for the normally distributed while Median and IQR for the non-normally anthropometric and biochemical parameters of the study were calculated. Mann-Whitney-U test was applied to compare [Median (IQR)] of testosterone levels. Spearman's rho correlation was applied to correlate the quantitative variables and $p \leq 0.05$ was considered statistically significant.

RESULTS

Age and ethnicity matched, young non-obese and obese, male subjects had a significant difference of BMI, WC and WHR between them and were expected to show significant difference in their testosterone levels too.

For the subjects, of groups A and B respectively, Age in years [25 (22–25.5) and 28.5 (25–31) respectively], Height in meters [1.74 (1.55–1.74) and 1.70 (1.68–1.74) respectively], Waist circumference in cm [81.28 (76.2–83.82) and 91.44 (86.36–93.98) respectively], Hip circumference in cm [97.79 (91.44–101.6) and 99.06 (96.98–101.6) respectively] and WHR [0.83 (0.81–0.85) and 0.92 (0.92–0.925) were calculated as median (IQR) while Weight in Kg [66.08±7.56 and 79.91±7.47 respectively], and BMI [23.24±1.87 and 27.07±1.65] were calculated as Mean±SD. Serum testosterone levels, for subjects of Group A and B calculated as median (IQR), were found to be [680.0 (575.0–778.5) ηg/dl] and [412.5 (338–542.5) ηg/dl] respectively.

Testosterone levels of the non obese subjects of group A [680 (575–778.5) ηg/dl] were compared to obese subjects in group B [412.5 (338–542.5) ηg/dl] by application of Mann-Whitney U test. Difference was statistically significant (p=0.003) (Table-1).

No significant correlation was found between testosterone levels, BMI, WHR and WC in individual groups (n=20). It was found however, that the subjects of groups A and B combined together regardless of their obesity status (n=40), depicted an inverse correlation of testosterone levels with WHR (r= -0.451, p=0.004) and with WC (r= -0.472, p=0.002). No similar correlation could be derived between serum testosterone levels and BMI (r= -0.308, p=0.058). (Table-2, Figure-1, 2).

Table-1: Comparison of serum testosterone levels by Mann-Whitney U test

Variable	Groups In Comparison (n=20)		*P*
	Group A	Group B	
Testosterone (ng/dl) [Median (IQR)]	680 (575.0–778.5)	412.5 (338.0–542.5)	0.003*

Table-2: Correlations between testosterone and anthropometric parameters of obesity by using spearman's correlation

Parameter (n = 40)	BMI		WHR		WC	
	r	*p*	*r*	*p*	*r*	*p*
Testosterone	-0.302	0.058	-0.451	0.004*	-0.472	0.002*

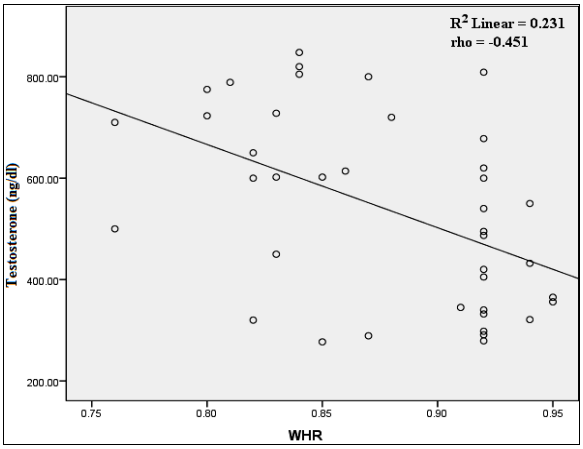

Figure-1: Scatter plot showing significant inverse correlation between serum testosterone levels and WHR in younger subjects (group A and B), using spearman's rho correlation (p=0.004)

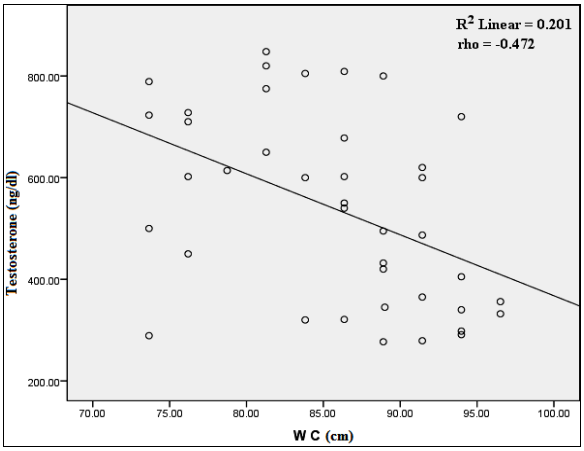

Figure-2: Scatter plot showing significant inverse correlation between serum testosterone levels and WC in younger subjects (group A and B), using spearman's rho correlation (p=0.002)

DISCUSSION

Testosterone levels decline in men as they develop circumferential obesity and they have an inverse correlation with anthropometric parameters which represent obesity in circumferential terms, i.e., WC and WHR.

Testosterone levels of the non-obese subjects of groups A were found to be higher than their obese counterparts in groups B, suggesting that testosterone levels decline in circumferentially obese men. This finding is in line with the results being projected in contemporary literature, which suggest that obesity lowers testosterone levels in men as it negatively regulates testosterone levels.[19]

It has been proposed previously that aromatization of testosterone within the adipose tissue is responsible for testosterone decline in obese men.[20] Obesity related enhancement of adipose tissue is also associated with hyperinsulinemia, as a result of development of insulin resistance. This hyperinsulinemia in obese men suppresses the secretion of LH and hence testosterone, resulting in low testosterone levels.[21] Moreover, obesity in men is associated with low levels of Sex Hormone Binding Globulin (SHBG), which also account for low testosterone levels in them too.[22]

The subjects of groups A and B, regardless of their obesity status, showed an inverse correlation between testosterone levels and WHR (p=0.004). These findings are comparable to a study where it has been suggested that testosterone levels are inversely correlated with WHR.[23] The increase in WHR has been associated with deranged insulin levels and insulin resistance. The insulin levels have been proposed to affect the negative feedback control over hypothalamo-pituitary-adrenal axis. Therefore deranged insulin sensitivity disrupts gonadotropic axis and in turn results in decreased testosterone levels by disrupting one or more of the control mechanisms that are involved in its synthesis.[24]

Testosterone levels were correlated with waist circumference (WC), and it was found that testosterone had a much stronger inverse relationship with WC as compared to WHR. This finding is in accordance with another study which suggests that serum testosterone levels are strongly correlated to WC as compared to WHR.[25] It is also supported by the proposal, that WC is a much better indicator of predicting testosterone decline in men as compared to both WHR and BMI.[26]

Testosterone levels did not have a significant correlation with BMI, although a negative correlation of testosterone with BMI has been reported in literature. It is, however, suggested that extreme changes in BMI, especially if it crosses the limit of 40, will result in significant decline in testosterone levels. This decline may be attributed to raised leptin and low LH and SHBG levels which suppress testosterone secretion.[27] It is also supported by another contemporary work, where it was suggested that testosterone levels had an inverse relation with BMI in men, if their BMI was ≥35.[28] We had excluded morbidly obese men (BMI ≥30) and this had probably resulted in an insignificant correlation between testosterone levels and BMI in our study.

CONCLUSION

Serum Testosterone levels decline significantly in circumferentially obese men and have an inverse correlation with both WHR and WC, but not with BMI. Waist-hip ratio and WC are better parameters to predict obesity associated endocrine abnormalities in obese men compared to BMI.

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