ORIGINAL ARTICLE

COMBINED EFFECTS OF AGING AND OBESITY ON SERUM TESTOSTERONE LEVELS OF OTHERWISE HEALTHY MALES OF SOUTH PUNJAB

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Background: Aging men experience gradual decline in serum testosterone levels (andropause) which is accentuated if aging is coupled with obesity. This study aimed to note the age and obesity related testosterone decline in men. **Methods:** It was a cross-sectional study comprising of 80 healthy male subjects categorized into younger (20–40 years) and elder (41–60 years) groups which were equally divided into non-obese and obese subgroups. Serum testosterone levels were measured using ELISA. **Results:** Serum testosterone levels (ng/dl) of young non-obese subjects (Group A) were significantly higher [680 (575.0–778.5)] as compared to their elder (Group B) [286.0 (263.5–370.0)] counterparts (p=0.000), and so was true for comparison between (Group C) younger [412.5 (338.0–542.5)] and (Group D) elder obese subjects [258.0 (220.0–287.5)] (p=0.000). Serum testosterone levels of obese elder subjects (group D), though lower than their age and ethnicity matched non-obese (Group B) counterparts, were not statistically significant (p=0.114). Moreover, serum testosterone levels of non-obese (Group A+B) subjects were negatively correlated to Waist Circumference (WC) and Waist Hip Ratio (WHR) [(rho= -0.374, p=0.018) and (rho= -0.355, p=0.025) respectively] while within obese subjects (Group C+D) serum testosterone levels were negatively correlated to waist circumference only [(rho= -0.643, p=0.000)]. **Conclusion:** Circumferential obesity coupled with aging results in a steeper decline in serum testosterone levels which can puts obese aging men at high risk of systemic disorders.

Keywords: Obesity, testosterone, aging men, Waist Hip Ratio, WHR, Waist Circumference, WC, Body Mass Index, BMI

Pak J Physiol 2021;17(3):27–31

INTRODUCTION

Testosterone, the key androgen, is essential for the endo-metabolic, immunological and psychological well being of men and is of absolute importance for maintenance of their muscle mass and bone composition.[1] Its levels tend to decrease in men, from fourth decade onwards, a phenomenon currently termed as andropause.[2]

Aging, in men is associated with elevated levels of cytokines, such as Interleuin-1 (IL-1) and Tumour Necrosis Factor-α (TNFα), which cause insulin resistance within hypothalamus and pituitary. This reduces the release of gonadotropin releasing hormone (GnRH) which in turn decreases testosterone levels in aging men.[3] Declining testosterone levels result in a reduced fat free mass in elderly men due to reduced stimulation of the cells of myocytic lineage, which leads to high estrogen levels (via conversion of testosterone to estrogen) that further affect gonadotropin secretion and hence testosterone levels negatively.[4] An enhanced fat mass, due to reduction in viable muscle mass, also increases the levels of adipocytoines (such as leptin) which also suppress gonadotropin release and thus further reduce testosterone levels. Moreover, sex hormone binding globulin (SHBG) levels tend to rise within aging men too which also affects bio-available testosterone levels in a negative fashion.[5]

Obesity is being recognized as the epidemic of 21st century ravaging the societies across globe. It's prevalence amongst various South Asian populations varies. The prevalence of obesity within Pakistani population was around 25% as per statistics of national health survey of Pakistan (1990–94). Certain recent studies are putting its prevalence within Pakistani population to up to 57%.[6,7] Since men do harbor circumferential obesity specifically, rather than general adiposity, hence WC and/or WHR are better indicators of adiposity/obesity in men as compared to the general parameter of Body Mass Index (BMI). According to World Health Organization (WHO), South Asian men with a BMI of ≥25 and/or WHR of ≥0.9 are termed as obese.[8]

The dysregulated metabolism within obese people leads to dysregulation of endocrine function (insulin resistance as well as deranged cholesterol/HDL ratio)[9], immune environment (dysregulation of cytokines[10], and adipocytokines like adiponectin and omentin[11]) along with that of anti-inflammatory substances (Vit D)[12] which create a fertile ground for emergence of metabolic syndromes and multi system failures.

This study on otherwise healthy men of South Punjab was aimed to note the age and obesity related testosterone decline in men so that the clinicians could be provided with scientific data that can help them monitor the systemic environment of aging and obese men in a better way since due to the loss of beneficial effects of testosterone on their internal harmony, they are likely to be at an increased risk of development of a myriad of systemic disorders.

SUBJECTS AND METHODS

It was a cross-sectional comparative study conducted on healthy adult male population of South Punjab. The sample size for each group was calculated with a power (1-β) of 90% and a significance (α) level of 5% through WHO (Geneva) extended software, 'Sample size determination in health studies: a Practical Manual' version 2.0. Study population consisted of 80 healthy subjects which were equally divided into younger (20–40 years) and elder (41–60 years) groups, each of which was further subdivided into non-obese and obese subgroups. Since testosterone levels tend to decline from fourth decade of life onwards hence the 40^{th} year of life[13] of study subjects was considered as the cutoff beyond which the subjects were termed as elder. Thus, Group A and Group B consisted of 20 younger non-obese and 20 elder non-obese subjects respectively while Group C and Group D had 20 younger obese and 20 elder obese subjects respectively.

According to WHO (2000 and 2008) guidelines South Asians with a BMI of ≥25 and/or a WHR of >0.9 are termed as obese, hence these were the cutoffs adopted for our study. The BMI and WHR of the subjects were measured as per criteria set by WHO. Three ml of venous blood of subjects was drawn in early hours of the morning before which subjects were advised to have an overnight fast of 10 hours. The collected blood samples were immediately centrifuged at a speed of 3,000 rpm for three minutes after which the drawn serum samples were immediately stored at -20 ºC for a later analysis.

Serum testosterone levels were calculated through competitive solid phase enzyme linked radioimmunosorbent assay (ELISA) by using ASTRA BIOTECH Testosterone ELISA Kit Ref: 21-02A (German Make). This assay had an Assay Range of 0.2–50 nmol/L (6–1154 ng/dl), an Assay Sensitivity of 0.2 nmol/L (6 ng/dl), an Assay specificity of 100% for human serum testosterone, an Intra-assay precision of 3.77% and an Inter-assay precision of 7.39%.

Genetically and morbidly obese men (BMI of ≥30 as per WHO 2000 guidelines) along with those taking exogenous testosterone were excluded from this study. Subjects were screened, twice in the week preceding sample collection, for deranged fasting blood glucose levels. After screening, subjects who had a fasting blood glucose level of ≥126 mg/dl (WHO 2008 guidelines for hyperglycemia), a systolic blood pressure of ≥140 and/or diastolic blood pressure of ≥90 (WHO 2013 guidelines for hypertension) on first and/or second screening occasion were excluded from study.

The data were entered on SPSS-22. Data were analysed for normality distribution via Shapiro-Wilk's and Kolmogorov Smirnov's tests and Mean±SD of normally distributed, while Median (IQR) of non-normally distributed variables were calculated. Mann-Whitney-U test was applied to compare [Median (IQR)] of serum testosterone levels between various groups. Spearman's rho correlation was applied to determine correlation between various quantitative variables, and $p \leq 0.05$ was considered to be statistically significant.

RESULTS

The Mean±SD of normally distributed and Median (IQR) of non-normally distributed parameters of study subjects (of all four groups) have been represented in Table-1. Comparisons in Table-2 show that serum testosterone levels of younger non-obese subjects (Group A) were significantly higher than those of their elder non-obese counterparts (Group B) and so was true for the comparison between obese younger (Group C) and obese elder (Group D) groups indicating that testosterone levels decline significantly with age both with or without obesity. Table-2 also shows that serum testosterone levels of elder obese subjects of study (Group D) were lower as compared to their age matched non-obese counterparts (Group C), though insignificantly on statistical grounds, indicating that when aging in men is coupled with obesity the testosterone decline in men is rather more prominent.

No significant negative correlation was found between testosterone levels, WHR and WC in individual groups. It was, however, found that serum testosterone levels had a negative correlation with waist circumference in both non-obese (Group A+B) and obese (Group C+D) subjects (p=0.018 and p=0.000 respectively) when combined together regardless of their age. Serum testosterone levels were negatively correlated to WHR in non-obese subjects (p=0.025) but a statistically significant result could not be derived for obese subjects in this instance (p=0.397). This indicates that serum testosterone levels have a more significantly negative correlation with WC as compared to WHR. These findings have been represented in Table-3.

A correlation of serum testosterone levels, of whole of the study population, with indicators of obesity (such as WC and WHR) has been extended in Figure-1. However, serum testosterone levels (of whole population) had non-significant negative correlation with BMI (rho= -0.200, p=0.076).

Table-1: Median (IQR) and (Mean±SD) of anthropometric parameters of the study population

Parameter	Group A (n=20)	Group B (n=20)	Group C (n=20)	Group D (n=20)
Age (Year)* [Median (IQR)]	25.0 (22.0–25.5)	45.0 (42.0–51.2)	28.5 (25.0–31.0)	49.5 (45.0–50.0)
Height (m)* [Median (IQR)]	1.74 (1.55–1.74)	1.77 (1.74–1.80)	1.70 (1.68–1.74)	1.74 (1.71–1.80)
Waist Circumference (Cm)* [Median (IQR)]	81.28 (76.20–83.82)	83.82 (83.82–86.99)	91.44 (86.36–93.98)	96.52 (93.98–96.52)
Hip Circumference (Cm)* [Median (IQR)]	97.79 (91.44–101.60)	99.06 (96.52–101.60)	99.06 (93.98–101.60)	104.14 (101.6–104.14)
Waist Hip Ratio* [Median (IQR)]	0.83 (0.81–0.85)	0.85 (0.84–0.87)	0.92 (0.92–0.92)	0.92 (0.92–0.93)
Weight (Kg)** (Mean±SD)	66.08±7.56	73.35±3.0	79.91±7.47	84.25±5.25
Body Mass Index** (Mean±SD)	23.24±1.87	23.5±0.93	27.07±1.65	27.46±1.4

*Non normally and **Normally distributed anthropometric parameters of study population

Table-2: Comparison of serum testosterone levels within study groups

Groups in Comparison (n=20)		*p*
Group A 680 (575.0–778.5)	Group B 286 (263.5–370.0)	0.000*
Group C 412.5 (338.0–542.5)	Group D 258 (220.0–287.5)	0.000*
Group A 680 (575–778.5)	Group C 412.5 (338–542.5)	0.003*
Group B 286 (263.5–370.0)	Group D 258 (220.0–287.5)	0.114

The comparison has been drawn via Mann-Whitney U test.
*Significant

Table-3: Correlations of serum testosterone levels with WC and WHR derived via Spearman's Correlation

Variable	A+B (Non-obese) n=40		C+D (Obese) n=40	
	rho	*p*	*rho*	*p*
WC	-0.374	0.018*	-0.643	0.000*
WHR	-0.355	0.025*	0.138	0.397

*Statistically significant

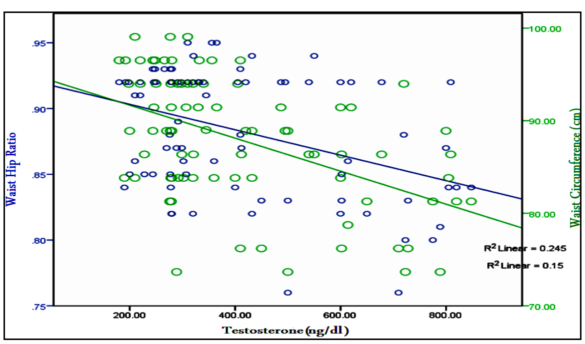

Figure-1: Correlations between testosterone and anthropometric parameters of obesity derived via Spearman's correlation in total study population

DISCUSSION

Testosterone concentrations of the subjects of groups A, B, C and D were compared and it was found that testosterone levels of the young non-obese subjects in group A were the highest, while those of elder obese subjects, in group D were the lowest. Testosterone levels of younger subjects of group A and C were higher than their elder counterparts in groups B and D regardless of their obesity status indicating an age related decline of testosterone levels in men.

Such findings have also been reported in contemporary literature where it is reported that testosterone levels decline at a rate of up to 2% per year, after third decade of life, because of several changes within the hypothalamo-pituitary axis of aging men. These changes emerge in the background of an age associated testicular impairment which begins after late 30s in men.[14]

Testosterone levels of the non-obese subjects of groups A and B were found to be higher than their obese counterparts in groups C and D, regardless of their age, as is projected by other studies too suggesting that testosterone levels decline in circumferentially obese men.[15]

It has been proposed that aromatization of testosterone within an enhanced adipose tissue is responsible for testosterone decline in obese men.[16] Obesity related enhancement of adipose tissue is also associated with hyperinsulinemia (as a result of development of insulin resistance) which suppresses the secretion of LH, thus resulting in low testosterone levels.[17] Obesity in men is associated with low levels of SHBG, which also account for low testosterone levels in them.[18]

Testosterone levels of non-obese elder subjects of group C, though lower, were not significantly different from their age matched obese counterparts in group D. This is in contrast with the results of other studies which show that testosterone levels decline with obesity.[19]

It has been suggested that a single time sampling may reveal inconsistent results regarding testosterone levels as compared to those deduced by serial samplings.[20] Since our study was a single time cross-sectional study, this could have led to our results being inconsistent with those being projected by available literature. This contradiction might have appeared as a result of small sample size too. This supposition is supported by the fact that non-obese subjects of groups A and B combined together, regardless of their age, had higher testosterone levels as compared to their obese counterparts in groups C and D.

Serum testosterone levels of non-obese subjects of groups A and B, regardless of their age showed an inverse correlation with WHR. Testosterone levels in whole of study population were inversely related to WHR. These findings are comparable to studies which suggest that testosterone levels are negatively correlated to WHR.[21] Increase in WHR is associated with deranged insulin levels and insulin resistance which affects the negative feedback control over hypothalamo-pituitary-adrenal axis[22] and results in decreased testosterone levels by disrupting one or more of the control mechanisms involved in its synthesis[23].

Testosterone levels were correlated with WC and had a more significantly negative relationship with WC as compared to WHR (in groups A+B, groups C+D, and in total study population). This finding is in accordance with another study, recently conducted in Pakistan[24], which suggests that serum testosterone levels show strong negative correlation with WC as compared to WHR. It is also supported by another research which states that WC is a much better indicator of predicting testosterone decline in men as compared to WHR and BMI.[25]

Testosterone levels did not have a significant correlation with BMI (neither within groups nor in whole of study population put together). Though a negative correlation of testosterone with BMI has been reported in literature[24], it is suggested that only extreme changes in BMI, especially if it crosses the limit of 40, can result in significant decline in testosterone levels. This decline may be attributed to raised leptin levels which suppress testosterone secretion.[26] This contradictory result of ours could also be supported by an earlier work where it was suggested that testosterone levels had an inverse relation with BMI in men (if their BMI was ≥35) because low LH and SHBG at a BMI of ≥35 lead to low testosterone levels.[27] Since BMI of our subjects was <30 hence it's most likely to have led to this finding that stands against popular belief.

CONCLUSION

This study in healthy males of South Punjab revealed that serum testosterone levels decline with enhancing age and adiposity and that this decline is sharpest with the increase of waist circumference in aging men. This extends valuable information to clinicians who, while treating aging men with increased WC, should consider the lack of testosterone's beneficial effects on systemic environment of their patients and consider testosterone replacement therapy for possibly better results.

LIMITATIONS & RECOMMENDATIONS

This was a cross-sectional study with a limited sample size. Further cohort studies with enhanced sample size are recommended.

REFERENCES

1. Corriger A, Duclos M, Corcuff JB, Lambert C, Marceau G, Sapin V, *et al*. Hormonal status and cognitivo-emotional profile in real-life patients with neuropathic pain: A case control study. Pain Pract 2019;19(7):703–14.
2. Samipoor F, Pakseresht S, Rezasoltani P, Kazemnajad Leili E. Awareness and experience of andropause symptoms in men referring to health centers: a cross-sectional study in Iran. Aging Male 2017;20(3):153–60.
3. Veldhuis J, Yang R, Roelfsema F, Takahashi P. Proinflammatory cytokine infusion attenuates LH's feedforward on testosterone secretion: modulation by age. J Clin Endocrinol Metab 2016;101(2):539–49.
4. Ayaz O, Howlett SE. Testosterone modulates cardiac contraction and calcium homeostasis: cellular and molecular mechanisms. Biol Sex Differ 2015;6:9.
5. Amjad S, Baig M, Zahid N, Tariq S, Rehman R. Association between leptin, obesity, hormonal interplay and male infertility. Andrologia 2019;51(1):e13147.
6. Amin F, Fatima SS, Islam N, Gilani AH. Prevalence of obesity and overweight, its clinical markers and associated factors in a high risk South-Asian population. BMC Obes 2015;2:16.
7. Siddiqui M, Ayub H, Hameed R, Nadeem MI, Mohammad TA, Simbak N, *et al*. Obesity in Pakistan: Current and future perceptions. Curr Trends Biomed Eng Biosci 2018;17(2):555958.
8. Gadekar T, Dudeja P, Basu I, Vashisht S, Mukherji S. Correlation of visceral body fat with waist-hip ratio, waist circumference and body mass index in healthy adults: A cross sectional study. Med J Armed Forces India 2020;76(1):41–6.
9. Morgan A, Mooney K, Mc Auley M. Obesity and the dysregulation of fatty acid metabolism: implications for healthy aging. Expert Rev Endocrinol Metab 2016;11(6):501–10.
10. Rodrigues KF, Pietrani NT, Bosco AA, Campos FMF, Sandrim VC, Gomes KB. IL-6, TNF-α, and IL-10 levels/polymorphisms and their association with type 2 diabetes mellitus and obesity in Brazilian individuals. Arch Endocrinol Metab 2017;61(5):438–46.
11. Landecho MF, Tuero C, Valentí V, Bilbao I, de la Higuera M, Frühbeck G. Relevance of leptin and other adipokines in obesity-associated cardiovascular risk. Nutrients 2019;11(11):2664.
12. Zakharova I, Klimov L, Kuryaninova V, Nikitina I, Malyavskaya S, Dolbnya S, *et al*. Vitamin D insufficiency in overweight and obese children and adolescents. Front Endocrinol (Lausanne) 2019;10:103.
13. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. J Clin Endocrinol Metab 2001;86(2):724–31.
14. Cohen J, Nassau DE, Patel P, Ramasamy R. Low testosterone in adolescents & young adults. Front Endocrinol (Lausanne) 2019;10:916.
15. Fui MN, Dupuis P, Grossmann M. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J Androl 2014;16(2):223–31.
16. Lee HK, Lee JK, Cho B. The role of androgen in the adipose tissue of males. World J Mens Health 2013;31(2):136–40.
17. Bekaert M, Van Nieuwenhove Y, Calders P, Cuvelier CA, Batens AH, Kaufman JM, *et al*. Determinants of testosterone levels in human male obesity. Endocrine 2015;50(1):202–11.
18. Cooper LA, Page ST, Amory JK, Anawalt BD, Matsumoto AM. The association of obesity with sex hormone-binding globulin is stronger than the association with aging –implications for the interpretation of total testosterone measurements. Clin Endocrinol (Oxf) 2015;83(6):828–33.

19. Van de Velde F, Reyns T, Toye K, Fiers T, Kaufman JM, T'Sjoen G, *et al*. The effects of age and obesity on postprandial dynamics of serum testosterone levels in men. Clin Endocrinol (Oxf) 2020;92(3):214–21.
20. Sartorius G, Spasevska S, Idan A, Turner L, Forbes E, Zamojska A, *et al*. Serum testosterone, dihydrotestosterone and estradiol concentrations in older men self-reporting very good health: The healthy man study. Clin Endocrinol (Oxf) 2012;77(5):755–63.
21. Zeller T, Appelbaum S, Kuulasmaa K, Palosaari T, Blankenberg S, Jousilahti P, *et al*. Predictive value of low testosterone concentrations regarding coronary heart disease and mortality in men and women –evidence from the FINRISK 97 study. J Intern Med 2019;286(3):317–25.
22. DeMorrow S. Role of the Hypothalamic-Pituitary-Adrenal Axis in Health and Disease. Int J Mol Sci 2018;19(4):986.
23. Kurniawan LB, Adnan E, Windarwati, Mulyono B. Insulin resistance and testosterone level in Indonesian young adult males. Rom J Intern Med 2020;58(2):93–8.
24. Shamim MO, Khan FM, Arshad R. Association between serum total testosterone and Body Mass Index in middle aged healthy men. Pak J Med Sci 2015;31(2):355–9.
25. Yassin AA, Nettleship JE, Salman M, Almehmadi Y. Waist circumference is superior to weight and BMI in predicting sexual symptoms, voiding symptoms and psychosomatic symptoms in men with hypogonadism and erectile dysfunction. Andrologia 2017;49(4):e12634.
26. Zitzmann M, Nieschlag E. Testosterone levels in healthy men and the relation to behavioural and physical characteristics: facts and constructs. Eur J Endocrinol 2001;144(3):183–97.
27. Lima N, Cavaliere H, Knobel M, Halpern A, Medeiros-Neto G. Decreased androgen levels in massively obese men may be associated with impaired function of the gonadostat. Int J Obes Relat Metab Disord 2000;24(11):1433–7.

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Received: 10 Jul 2020 **Reviewed: 30 May 2021** **Accepted: 30 Aug 2021**

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MZ: Fieldwork