ORIGINAL ARTICLE

RELATIONSHIP OF SERUM INSULIN WITH SERUM TESTOSTERONE LEVELS IN NON-OBESE AND OBESE DIABETIC MALES

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Background: Obesity and diabetes whenever merge within males, brew hyperinsulinemia that accentuates their diabetic status by withdrawing crucial benefits of testosterone from over their glycaemic homeostasis. This study aimed to access and compare testosterone and insulin levels among diabetic males. **Methods:** It was a cross-sectional study, devised on comparative and correlational grounds, conducted in 2023 at medical OPDs associated with Multan Medical and Dental College, and Nishtar Medical University, Multan. Diabetic males aged 30–40 years were divided into non-obese diabetic males (NODMs, Group A) and obese diabetic male (ODMs, Group B) with 67 individuals falling into each group. Serum testosterone and serum insulin levels of the subjects were measured via ELISA. **Results:** Serum testosterone levels (ng/dL) of NODMs [455.0, 310–920] were significantly higher (p=0.00) than those of ODMs [241, 150–380]. Conversely, Serum insulin levels (pmol/L) of ODMs (243.1±25) were significantly higher (p=0.00) than their non-obese diabetic counterparts (133.2±22.7). Also, serum insulin levels had an inverse correlation with serum testosterone levels in both NODMs [(rho=-0.45, p=0.00)] and ODMs [(rho=-0.52, p=0.00)]. Serum testosterone levels showed a negative correlation with BMI and WHR while serum insulin levels showed a positive correlation with them in NODMs [{(rho=-0.23, p=0.05), (rho=-0.28, p=0.02)}, {(rho=-0.27, p=0.02), (rho=-0.24, p=0.04)}] and ODMs [{(rho=0.29, p=0.01), (rho=0.40, p=0.00)}, {(rho=0.21, p=0.08), (rho=0.27, p=0.02)}]. **Conclusion:** ODMs, in contrast to NODMs of South Asian origin, harbour a significant degree of hyperinsulinemia which in turn suppresses their serum testosterone levels.

Keywords: Obesity, testosterone, insulin, diabetes mellitus, Waist Hip Ratio, Waist Circumference, Body Mass Index

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INTRODUCTION

Diabetes Mellitus Type II, a state marked by fasting blood glucose levels ≥126 mg/dL on at least two consecutive occasions[1] is a menace that alters homeostasis of glucose as well as lipid metabolism[2] by dysregulating endocrine harmony associated with the physiology of insulin. This in turn profoundly affects its intricate relationship with other key players of glucose homeostasis, testosterone for instance.

According to Second National Health Survey of Pakistan[3], the prevalence of diabetes within Pakistani diaspora is 26.4% of which 7.1% stays undiagnosed and this percentage is increasing with every passing year making Pakistan the epicentre of the global diabetic pandemic. Though several factors lead to emergence of diabetes however in current era obesity, specifically circumferential obesity, has started to overshadow rest of the causes. It, through endocrine imbalance of key hormones of glucose metabolism, i.e., insulin and testosterone, not only sets the ground for emergence of diabetes but also for the development of diabetes associated metabolic syndromes.[4]

Insulin, the key hormone of glucose metabolism, is known to keep production of both testosterone and Sex Hormone Binding Globulin (SHBG) under check by negatively affecting the hypothalamo-pituitary-gonadotropin axis and by inhibiting the hepatocytic function respectively.[5] Hence it is plausible to deduce that obesity and diabetes, which are associated with relatively higher insulin levels, are likely to coexist with low testosterone levels.

Testosterone, is known to increase the lean body mass and decrease central fat mass by significantly affecting carbohydrate and lipid metabolisms as well as insulin receptor sensitivity.[6] Its levels decline with the development of circumferential obesity in men which causes insulin resistance and hyperinsulinemia that in turn support a diabetic status.[7]

Though both obesity (via testosterone decline) and diabetes (via insulin receptor resistance/ dysfunction) are said to co-exist with a hyperinsulinemic state[8], compensatory hyperinsulinemia specifically observed in obese diabetic males is projected to lead to a secondarily established hypo-gonadal status.[9] This highlights the scientific view that obesity and diabetes whenever combined unleash a two-way havoc on the endo-metabolic environment, first by depriving the body of the supportive role of testosterone on insulin receptor sensitivity and secondly by withdrawing the direct effect of insulin on all the stems

of metabolism through an exponential reduction in the sensitivity of insulin receptor to insulin.[10]

Studies have also indicated that testosterone administration in hypogonadal states, leads to normalization of insulin levels.[11] This, by supporting gonadotropic axis directly and by normalization of SHBG levels[12] indirectly, lets the body have the beneficial effects of testosterone on several intricate metabolic pathways associated with carbohydrate and lipid metabolism and eventually leads to reduction of risk of complex endo-metabolic syndromes.

This study was conducted to gather scientific data regarding hypogonadal state that co-exists with obesity and diabetes so that through evolution of smarter regimens for obese diabetic males the burden of diabetes and its associated syndromes could be reigned in.

MATERIAL AND METHODS

It was a cross-sectional, comparative, correlational study, conducted at the Medical OPDs of Ibn-e-Siena Research Institute, and Nishtar Hospital, Multan after approval from Institutional Review Board via letter No. 01/MMDC/ERC, dated 24 Jan 2022. Sample size (n=67 for each group) was calculated by utilizing the formula:

$$'n=\sigma^2(Z_{1-\alpha/2}+Z_{1-\beta})^2/(\mu_0-\mu_1)^2$$

derived from WHO extended software 'Sample Size Determination in Health Studies: a Practical Manual' version 2.0 by putting in the mean levels of testosterone of obese non-diabetic and diabetic individuals as projected by a contemporary research.[13] Total study population, consisted of 134 newly diagnosed 30–40 years old diabetic males, divided equally into non-obese diabetic male (NODMs) and obese diabetic male (ODMs) groups.

The subjects were termed obese and non-obese per WHO 2000 and 2008 guidelines[14]. Three mL venous blood from the subjects was drawn during early morning hours after a 10-hour fasting. Collected blood samples were immediately centrifuged at 3,000 rpm for 3 minutes and the serum samples were immediately stored at -20 °C for a later analysis.

Serum testosterone levels were measured by incorporating ASTRA BIOTECH Testosterone ELISA Kit Ref: 21-02 (Germany). It had an assay range of 0.2–50 nmol/L (6–1,154 ng/dL), sensitivity of 0.2 nmol/L (6 ng/dL), specificity of 100% for human serum testosterone, intra-assay precision of 3.77% and an inter-assay precision of 7.39%.

Serum insulin levels were analyzed via Abcam Human Insulin ELISA Kit, Ref: Ab278123 (USA). It had an assay range of 26.56–425 pmol/L, sensitivity of <7.13 pmol/L, specificity of 100% for human serum insulin, intra-assay precision of ≤9.4% and inter-assay precision ≤12%. Tests were run on ELIZA Reader.

Subjects with ages beyond the 40th year of life[15] were excluded from the study. Genetically obese men, those with morbid obesity (BMI of ≥30) and those on testosterone and/or insulin or oral hypoglycaemic treatment were also excluded.

Data was entered and analysed on SPSS-25 for normality distribution through utilization of Shapiro-Wilk and Kolmogorov Smirnov tests after which Mean±SD of normally distributed while Median (IQR) of non-normally distributed variables was derived. Mann-Whitney U-test was applied to compare serum testosterone levels [Median (IQR)] of NODMs and ODMs while student's *t*-test was utilized to draw the same comparison for serum insulin levels (Mean±SD) of both groups. Spearman's *rho* correlation was applied to determine a correlation between various quantitative variables. Value of $p \leq 0.05$ was considered as statistically significant.

RESULTS

The Mean±SD of normally distributed and Median (IQR) of non-normally distributed anthropometric as well as biochemical parameters of the study have been presented in Table-1. Serum testosterone levels of NODMs (Group A) were significantly higher than their obese counterparts (Group B) indicating that ODMs experience testosterone deficiency. Serum insulin levels of NODMs of Group A were significantly lower as compared to ODMs of Group B indicating that endocrine status of obese diabetics is characterized by marked hyperinsulinemia. (Table-2).

Serum testosterone levels showed a negative correlation with BMI as well as WHR in NODMs while insulin levels showed a positive correlation with both the indices of obesity used here. Moreover, within ODMs, serum testosterone levels showed a negative correlation with both BMI and WHR, similar to the one seen in NODMs, while insulin levels showed a positive correlation with WHR only in this group. These findings have been portrayed in Table-3.

Serum testosterone as well as serum insulin levels showed an independent negative correlation with each other in both NODMs and ODMs. These findings have been scripted in Table-4.

Table-1: Anthropometric and biochemical variables, within NODMS and ODMs [Median (IQR), and/or Mean±SD]

Anthropometrics	Group A (n=67)	Group B (n=67)
Age (Years)	39 (37–40)	39 (36–40)
BMI	24.1 (20–24.9)	28.1 (25.3–29.1)
WHR	0.87 (0.86–0.89)	0.91 (0.90–0.93)
Serum Testosterone (ng/dL)	455 (310–920)	241 (150–380)
Serum Insulin (pmol/L)	133.2±22.7	243.1±25

Table-2: Comparison of serum testosterone and serum insulin levels between NODMs and ODMs (Mann-Whitney U-test and Student's *t*-test)

Biochemical Variables	Group A (NOD) (n=67)	Group B (OD) (n=67)	*p*
Serum Testosterone (ng/dL) [Median (IQR)]	455 (310–920)	241 (150–380)	0.00
Serum Insulin (pmol/L) [Mean±SD]	133.2±22.7	243.1±25	0.00

Table-3: Correlation between biochemical and anthropometric variables within NODMs and ODMs

		Serum Testosterone (ng/dL)	Serum Insulin (pmol/L)
Group A (NODMs) (n=67)			
BMI	***rho***	-0.23	0.29
	p	0.05	0.01
WHR	***rho***	-0.28	0.40
	p	0.02	0.00
Group B (ODMs) (n=67)			
BMI	***rho***	-0.27	0.21
	p	0.02	0.08
WHR	***rho***	-0.24	0.27
	p	0.04	0.02

Table-4: Correlation of serum testosterone levels with serum insulin levels within NODMs and ODMs

	Serum Insulin (pmol/L)			
	Group A (NODMs) (n=67)		Group B (ODMs) (n=67)	
Biochemical Variables	***rho***	***p***	***rho***	***p***
Serum Testosterone (ng/dL)	-0.45	0.00	-0.52	0.00

DISCUSSION

In the wake of the above quest we, after applying standard methodologies, noted that serum testosterone levels of NODMs were significantly higher as compared to ODMs indicating that ODMs, in comparison to NODMs, were more likely to experience the absence of metabolic benefits of testosterone over theirs glycaemic homeostasis in addition to failure of insulin physiology.[16] This finding is supported by the studies which suggest that obesity in diabetics not only leads to the development of insulin receptor resistance via lowering testosterone levels, but is also more likely to make them respond poorly to anti-glycaemic regimens.[17]

In addition, the serum insulin levels of the NODMs were found to be within the normal physiological range while those of ODMs were well beyond the physiological range. Similar findings are echoed within the results of recent studies which do suggest that the disturbed glycaemic status of NODMs (without an obvious hyperinsulinemia) might rather be due to the disruption of insulin receptor signalling acquired potentially through genetic causes[18], while within ODMs it is more likely to be secondary to insulin receptor resistance and its associated hyperinsulinemia[19] which via disturbing the key interplay of testosterone with glycaemic homeostasis accentuates the glycaemic status harboured by obese diabetics.

We also observed that in both NODMs and ODMs serum testosterone had a negative correlation with the indices of obesity, i.e., BMI and WHR, while serum insulin levels had a positive correlation with BMI and WHR in NODs and with WHR in ODMs. These findings are comparable to those in contemporary literature, which suggest that increased adiposity leads to low levels of bioavailable testosterone.[20] This, merged with adiposity associated pro-inflammatory status, down-regulates insulin receptor signalling which dysregulates testosterone as well as insulin productivity.[21] All of these intricate physiological disruptions within ODMs thus set up a vicious cycle where low testosterone and altered insulin signalling, marked by considerable hyperinsulinemia, keep complementing each other and prevent glucose homeostasis from achieving normalcy again.

The more significant correlation of biochemical variables in ODMs with WHR as compared to BMI supports the view of the researchers from South Asia which advocates that for South Asian population, specifically for South Asian males, BMI is not a useful indicator for assessment of obesity associated endocrine disruption and that South Asian males can develop endocrine dysregulation with a normal BMI coexisting with an enhanced WHR too. This highlights the importance of the viewpoint that it is better to stick to WHR or waist circumference for assessing obesity and its associated endo-metabolic risks within obese South Asian men as compared to BMI.[22]

It was observed that within diabetic men both obesity and insulin resistance, characterized by hyperinsulinemia, had an independent negative correlation with serum testosterone levels. This finding also supports the views of researchers who suggest that both obesity and diabetes are independent factors for testosterone decline[23] which, through considerable increase in the productivity of insulin[24] directly and through SHBG mediated derangements[25] indirectly, establishes a significant hypogonadal status within ODMs. Hence it could be deduced that ODMs are more prone to develop chronic systemic disorders[26] because obesity and diabetes associated hypogonadal status sets a background over which complex endometabolic syndromes can thrive.

CONCLUSION

Diabetic males experience testosterone decline related to hyperinsulinemia which is more marked in ODMs. Within newly diagnosed diabetic males, testosterone levels depict an inverse correlation with BMI and WHR while insulin levels as positively correlated with anthropometric indices of obesity.

RECOMMENDATIONS & LIMITATIONS

Endocrinologists may consider hormone replacement therapy in their obese and/or diabetic charges who are not responding well to the endometabolic treatments. This will not only help the endocrinologists evolve smarter regimens for the obese diabetic subjects but can also prevent the emergence of systemic as well as metabolic syndromes within their patients. It was a cross-sectional study based on single time observation. Longitudinal/cohort studies that do incorporate larger sample populations are recommended for future researches in this very important domain.

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