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Original Article

Relationship between testosterone and cortisol levels and body mass index in men with type 2 diabetes mellitus

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Abstract

Stress can lead to type 2 diabetes mellitus (T2DM), as it is one of the agents responsible for increasing blood glucose levels. In turn, T2DM may be one of the most common causes of hypogonadism that negatively affects testosterone production in the testes. Emerging evidence links insulin resistance, a key feature of T2DM, with decreased testosterone secretion by the Leydig cell. Low gonadal steroids have been associated with metabolic abnormalities such as hyperglycemia. We sought to compare testosterone and cortisol levels and their relationship between Pakistani older/younger men's age groups with T2DM and age-matched controls. We enrolled 87 men with T2DM and 90 controls aged 21–60. Anthropometric measurements, serum testosterone and cortisol levels were assessed using the RIA system. We assessed the correlation between serum testosterone and cortisol levels and BMI. Testosterone levels were lower in T2DM patients than in controls (2.612±0.3524 *vs.* 5.143±0.4334 nmol/L, p=0.0001), and cortisol levels were higher in patients than in controls (599.8±35.69 *vs.* 441.7±18.33 nmol/L, p=0.0001). Testosterone levels in T2DM patients were positively correlated with cortisol levels and BMI (r=0.1582, 0.08621). In conclusion, most of the T2DM patients had low testosterone levels, whether they had high or low cortisol levels.

Keywords: T2DM, cortisol, testosterone, BMI.

Introduction

Type 2 diabetes mellitus (T2DM) is a group of metabolic complications caused by a lack of insulin secretion or reduced tissue susceptibility to insulin [1] and affects any group of age-linked with high illness and mortality rate [2]. The incidence of T2DM in the human population has reached epidemic proportions globally and is increasing rapidly. In T2DM, hypogonadism, a lack of function in the testes, adversely affects testosterone production [3]. In men, testosterone plays a key role in the development of male reproductive tissues as well as promoting secondary sexual characteristics [4]. Recently, the concepts of hypogonadism and testosterone deficiency have developed and these are currently seen as factors that contribute to insulin resistance and diabetes mellitus development in men. These suggestions stem from preliminary results that T shortage is associated with T2D in young men. Nevertheless, it is still in doubt if this is a link between male hypogonadism and the development of DT2 or indications in the hypertrophy of hypogonadism or if they together support an age-related form like a high level of fat mass. In the past, ideas emerged about whether male hypogonadism

and T shortage could lead to the progression of insulin resistance and T2DM [5]. Cortisol is a stress hormone, suggesting that there would be a connection between serum cortisol levels and diabetes [6]. Chronic stress has been shown to produce a physiological response mediated by stimulation of the hypothalamic-pituitary-adrenal axis (HPA) [7]. The HPA stimulation is caused by the release of corticotrophin-releasing factor (CRF) from the hypothalamus to the pituitary portal system of the median eminence, which directs the secretion of adrenocorticotropic hormone (ACTH) from the pituitary. Eventually, the ACTH stimulates the adrenal cortex to release the stress hormone cortisol. The peripheral release of cortisol and the major release of CRF in various areas of the brain initiates a cascade of biological responses to modify the homeostatic balance of organisms in response to stress [8]. In T2DM patients, an increase in the HPA axis activity has been noticed [9] and causes elevated cortisol levels in T2DM patients [10]. Testosterone and cortisol are markers of general health and decreased testosterone levels are linked to hypogonadism, i.e., erectile dysfunction and decreased physical stamina in advanced life. Earlier research has shown that high cortisol levels in circulation will result in reduced blood testosterone levels [11]. Thus, this study aimed to compare the serum testosterone and cortisol levels in elderly and young men with T2DM with those in elderly and young men without T2DM. We also aimed to determine the relationship between testosterone and cortisol levels and BMI in T2DM patients and healthy Pakistani men.

Material and methods

Study population

This cross-sectional study was conducted in Dera Ismail Khan divisional hospital in Khyber Pakhtunkhwa, Pakistan, from January to May 2017. A total number of 177 male subjects, aged 21 to 60, were included in this study. Among them, 90 non-diabetic, apparently healthy subjects were included in the control group. All subjects were socio-economical condition matched. Weight and height were taken in each subject for BMI calculation [12]. BMI was categorized according to World Health Organization (WHO) Asian criteria [13]. Informed written consent was taken from patients/guardians for participation in the study. The current research was approved by the Research Ethical Committee of PMAS Arid Agriculture University Rawalpindi. Inclusion criteria were male individuals diagnosed with diabetes. Excluded from the study were patients with neurological and cerebrovascular disorders such as chronic renal impairment, asthma, alcoholics, renal insufficiency, and advanced hepatic and suffering from any other endocrinological disorder in any of the participants included in the study.

Clinical assessment

Blood samples (~1.5 mL) were collected from every participant with minimal trauma from an antecubital vein visiting DHQ-DIK. Samples were centrifuged for 5 minutes at 3000 rpm following serum collection and separation. The serum samples were immediately kept frozen at -20° until they were analyzed. The blood samples were collected between 9:00 am and 12:00 pm to reduce the impact of the diurnal variation in the circulating cortisol and testosterone levels. Testosterone and cortisol were assessed by estimating serum testosterone and cortisol, measured by the radioimmunoassay (RIA) method using commercial kits. (Immunotech s. r. o. – Radiova 1 102 27 Prague 10 – Czech Republic). The detection range of the assay was from 5 nmol/l to 2000 nmol/l for cortisol and 0.02ng/ml to 20 ng/ml for testosterone.

Statistical analysis

Data were expressed as mean±SD and were analyzed by unpaired "t" test, Pearson's Correlation Coefficient "r" and analysis of variance (ANOVA) where applicable. The p-value≤0.05 was adjusted as a level of significance.

Results

The baseline characteristics, including age, weight, BMI and socio-economic status stratified by the study and control groups, are presented in Figure 1. The mean±SD level of age, weight, height and BMI in T2D diagnosed patients and the non-diabetic group was shown in Table 1. The mean±SD level of weight in T2D diagnosed patients *vs*. healthy subjects was statistically significant (p≤0.0010) than that of age (P<0.1119) and BMI (P<0.8943) (Figure 1 A, B and C). Additionally, in our study, the majority of the patients were from the middle socioeconomic status class (Figure 1 D).

In T2D diagnosed subjects, the serum level of cortisol was significantly higher (P<0.0001) than that of

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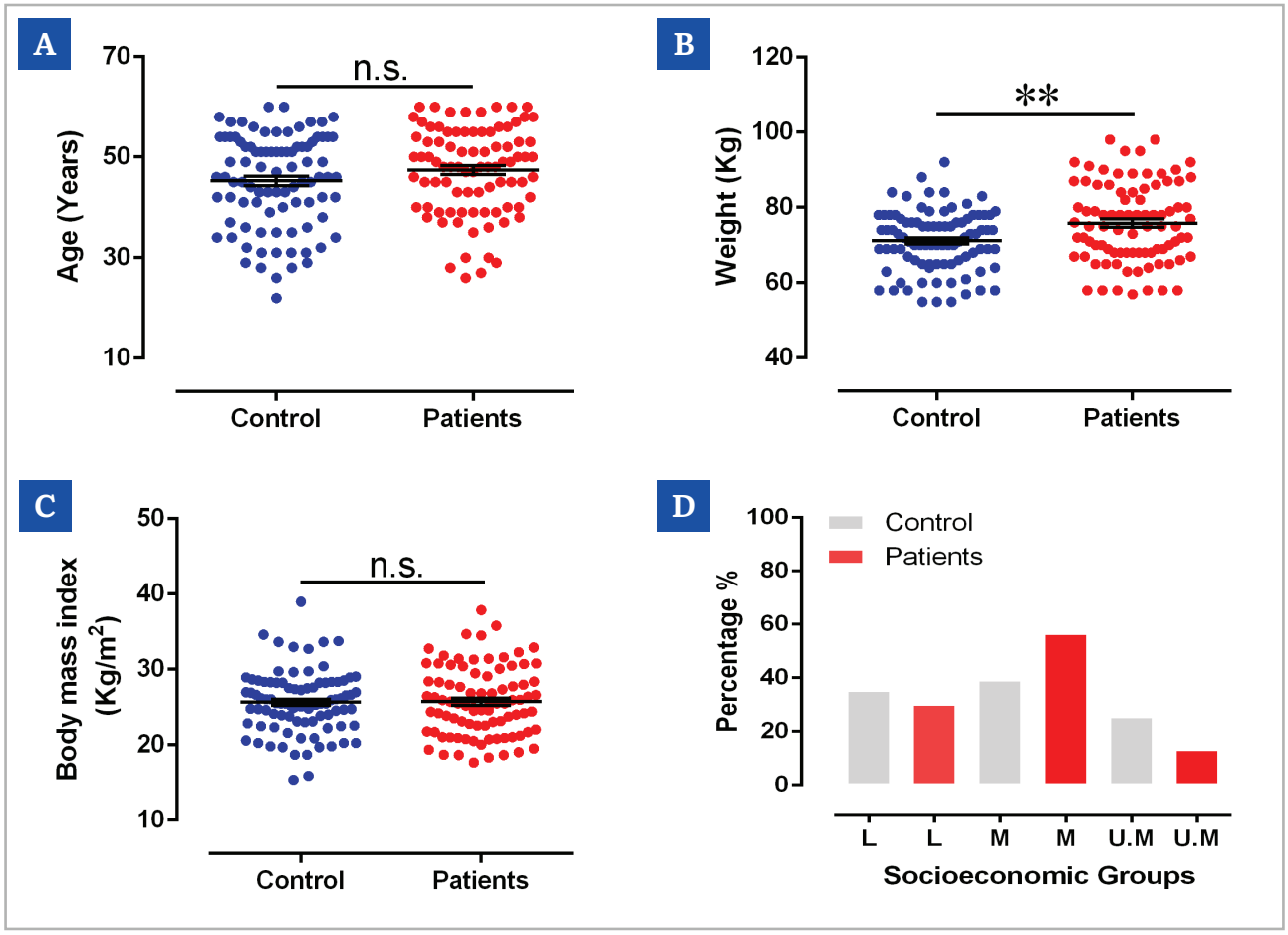

Figure 1: Baseline characteristics of healthy control (blue dots/silver column) and patients (red dots/red column). A – Graph showing patients *vs*. healthy control age-wise differences. B – Weight analysis across study participants. C – Graph showing BMI in both control *vs*. patients. D – Socioeconomic status of the study participants presented in percentage. P-values were calculated by Student t-test with recommended option. Error bars indicate S.E.M., **p b 0.05 for the indicated comparison.

the non-diabetic control group (Figure 2 A). Likewise, there was a significant difference between mean plasma concentrations of cortisol of controls and the patients in age groups of 21–30 (p=0.0215), 41–50 (p=0.0128) and 51–60 (p=0.00). On the other hand, the age group of 31–40 (p=0.1822) and group-wise comparison across T2D patients was a non-significant observation (Figure 2 A, B and C). The mean serum testosterone level in T2D subjects was significantly lower (p<0.0001) than that of the control group. There was a significant difference in

Table 1: Mean (±SD) of age, height, weight and BMI of non-diabetic healthy subjects *vs*. T2D patients.

Group (C/P)	Age (yrs), C/P	Height (cm), C/P	Weight (kg), C/P	BMI (Kg/m²), C/P
21–30 (n=06/06)	27.00±1.095/ 28.33±0.6667 ^{NS}	168.0±3.066/ 163.2±2.774 ^{NS}	70.83±2.971/ 69.67±5.194 ^{NS}	25.09±0.8133/ 28.47±2.530 ^{NS}
31–40 (n=17/17)	34.18±0.6197/ 38.35±0.3632 ****	168.6±1.993/ 168.3±1.707 ^{NS}	74.12±1.987/ 75.41±2.090 ^{NS}	26.21±0.8856/ 25.69±1.091 ^{NS}
41–50 (n=31/30)	44.55±0.4948/ 46.87±0.4542 **	165.2±1.708/ 166.5±2.291 ^{NS}	72.81±1.358/ 77.45±1.942 ^{NS}	26.80±0.6865/ 25.79±0.8570 ^{NS}
50–51 (n=36/34)	54.08±0.4407/ 55.71±0.5058 *	168.6±2.370/ 167.3±1.966 ^{NS}	68.33±1.285/ 75.55±1.836 **	24.44±0.7489/ 25.18±0.7086 ^{NS}

Note: Data were expressed as mean±SD. An unpaired "t" test was done. n – number of subjects; C/P – Control/Patients; **** – p≤0.05; ^{NS} – Non-significant. Normal range of BMI is 18.5–24.9 kg/m² (International) and for Asian >23 is overweight and >25 is obese.

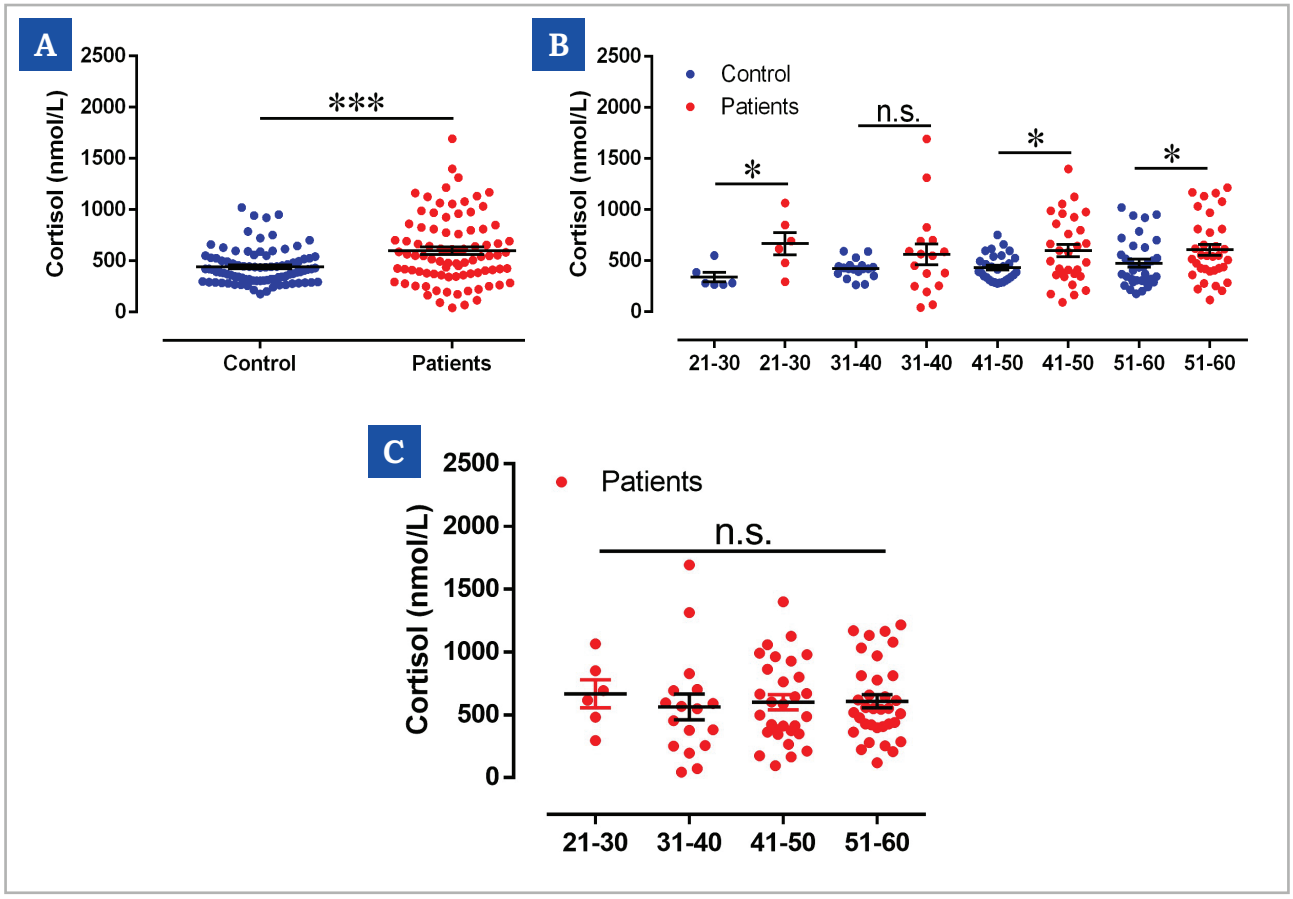

Figure 2: Cortisol rise in patients with diabetes. A – Left, representative graph showing healthy control *vs.* patient's cortisol levels. B – Graph showing cortisol readings across different age groups. Red dots representing the patients, while the blue ones referring the control. C – Graph representing group-wise comparison of cortisol in patients. P-values were calculated by Student t-test or ANOVA multiple comparison test with recommended option tukey. Error bars indicate S.E.M. ****, p b 0.05.

the age group of 21–30 (p=0.0002), 41–50 (p=0.0005) and 51–60 (p=0.0149), while non-significant difference was observed in the age group of 31–40 (p=0.2646) and group-wise comparison across T2D patients (Figure 3 A, B and C). As depicted in Figure 4 A, the T2D subjects serum level of cortisol was positively correlated with testosterone (r=0.1582; p<0.05). In the non-diabetic control group, the serum level of cortisol was positively correlated with testosterone level, and the relationship was statistically significant (r=0.3428; p<0.05) (Figure 4 B. On the other hand, a positive correlation (r=0.08621) was found between the BMI and testosterone of T2DM patients, while a negative (r=-0.02777) correlation between cortisol and BMI of T2DM patients was observed in the current investigation (Figure 4 C and D).

Discussion

In the present study, the total serum testosterone level was significantly decreased, and the serum cortisol level was significantly higher in diagnosed T2DM patients than that in non-diabetic subjects. BMI was positively correlated with serum testosterone levels. This finding is consistent with that of some other investigators [14–18]. It has been reported that high cortisol levels in blood induces hyperglycemia. The value found in this study may be a contributor to T2D development. High cortisol is associated with increased liver gluconeogenesis and glycogenolysis and, therefore, hyperglycemia [19]. The increased cortisol concentrations in our study may agree with a finding that individuals with T2DM may be under minor stress [20].

The patients with T2DM exhibited elevated cortisol levels and lower concentrations of testosterone in our study, indicating that these patients were experiencing some stress, which caused a reduction in the circulating concentrations of testosterone. An investigation into the suppression of testosterone concentrations by increasing cortisol concentrations revealed that chronic stress is associated with a decline in serum levels of testosterone.

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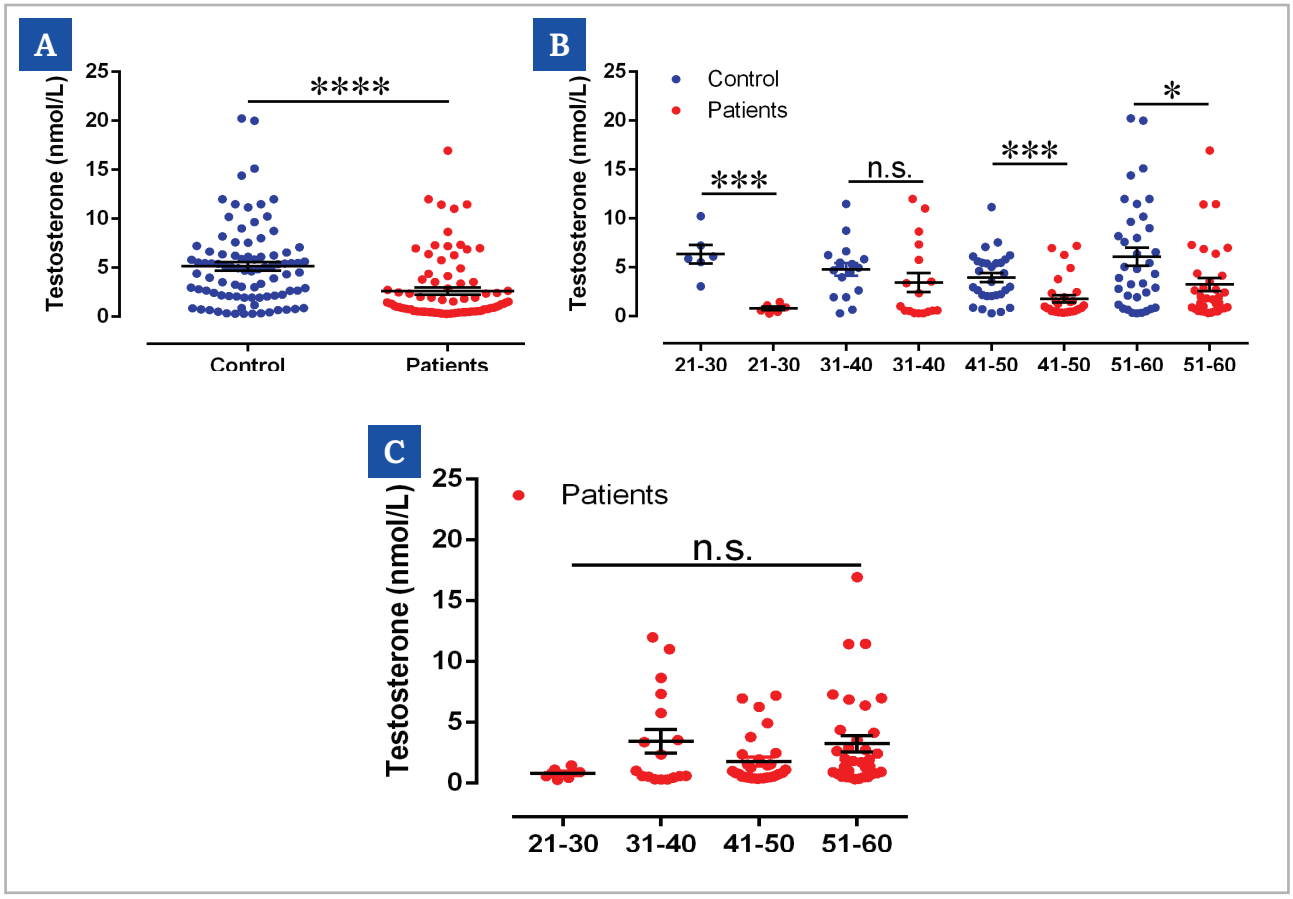

Figure 3: Testosterone decreased in patients with diabetes. A – Left, representative graph showing healthy control *vs*. patient's testosterone levels. B – Graph showing testosterone readings across different age groups. Red dots representing the patients, while the blue ones referring the control. P-values were calculated by Student t-test or ANOVA multiple comparison test with recommended option tukey. C – Graphs represent the group-wise testosterone analysis. Error bars indicate S.E.M., ***p b 0.05 for the indicated comparison.

In contrast, acute stress induces a rapid increase in serum concentrations of cortisol followed by a decrease in testosterone serum concentrations. Earlier records have shown that the administration of dexamethasone, a synthetic glucocorticoid, causes a decrease in the serum concentrations of testosterone. As far as the mechanism of glucocorticoids' action in suppressing testosterone concentrations is concerned, it has been reported that cortisol inhibits testosterone secretion at the level of testes since glucocorticoids decrease the secretion of testosterone from testes. In addition, glucocorticoids have been reported to decrease GnRH, FSH, LH, and gonadal steroids. In contrast, corticotropin-releasing hormone (CRH) has been shown to inhibit the release of GnRH from the hypothalamus [21]. Elevated cortisol levels in patients with T2DM are known to be related to low testosterone levels [22, 23].

An investigation into the suppression of testosterone concentrations by increasing cortisol concentrations revealed that chronic stress is associated with a decline in testosterone serum concentrations. In contrast, acute stress induces a rapid increase in serum concentrations of cortisol followed by a decrease in testosterone serum concentrations. In the present study, insulin resistance in type 2 diabetes mellitus may cause alteration in the hypothalamic-pituitary-gonadal axis. This can lead to a decrease in the formation of luteinizing hormone and hormone stimulating follicles which can cause a decrease in testosterone formation of the Leydig cell of the testis. In addition, it has been reported that the administration of dexamethasone, a synthetic glucocorticoid, causes a decrease in the serum concentrations of testosterone. Recent findings demonstrated that weight loss had a greater effect on testosterone levels in obese compared to non-obese men [24].

Thus, it seems reasonable that obese candidates who lost weight had lower testosterone levels. The positive correlation between testosterone and cortisol in our study is in line with Smith *et al*. drawing a different conclusion that the high level of cortisol and testosterone positively correlates with metabolic factors such

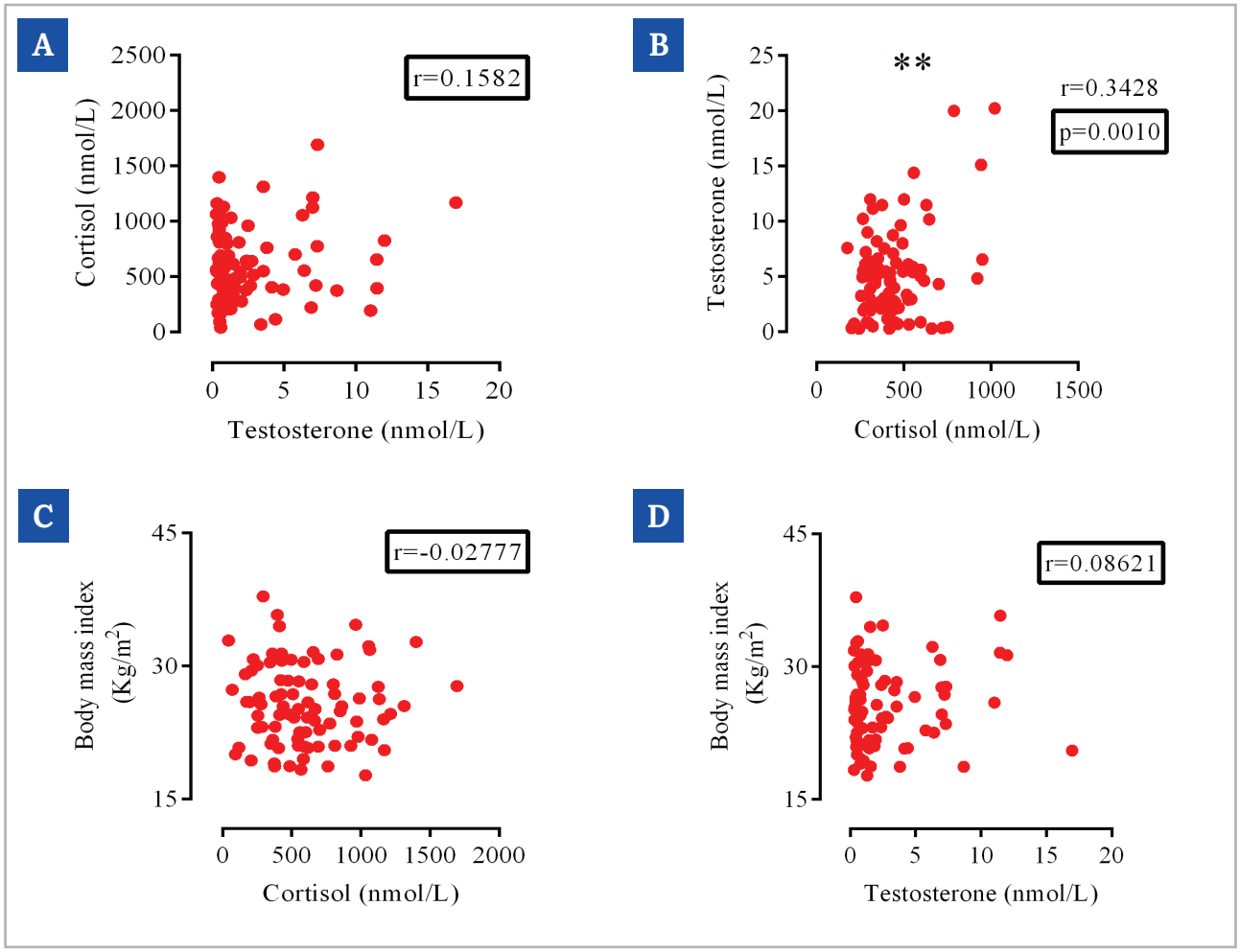

Figure 4: Correlation between Mean Plasma Concentrations of Cortisol and testosterone and BMI in patients *vs.* normal subjects. A – Morning cortisol *vs.* testosterone. B – Morning cortisol *vs.* testosterone in normal subjects. C – Patients BMI *vs.* cortisol in T2D patients. D Correlation analysis of BMI *vs.* testosterone in T2D patients.

as insulin resistance syndrome [25]. The association between testosterone and cortisol levels and BMI was investigated. It was noted that cortisol levels and BMI positively correlate with serum testosterone levels. Additionally, cortisol levels demonstrated a significant positive correlation with serum testosterone levels in healthy subjects. Previous studies did not show similar results, but clues could be found in studies on BMI and testosterone levels [26].

The levels of cortisol and BMI were negatively correlated in the current study. In obesity, the serum cortisol readings are generally normal, however, some literature suggests that obese subjects may have lower than expected cortisol levels [27], while several studies over the last several years have tried to demonstrate the opposite [28, 29]. The majority of study participants had average socio-economic status and low educational attainment. The most recent National Diabetes Statistics Report showed that prevalence varied considerably by educational level, which is an indicator of socio-economic status. Specifically, 12.6% of adults with less than a high school education were diagnosed with diabetes *versus* 9.5% of those with high school education and 7.2% of those with more than high school education [30]. Earlier investigations were subject to numerous limitations, such as the inclusion of participants from single or random age groups with no control groups and an average disease period that was inadequate to study the relationship between serum testosterone and cortisol levels and BMI in T2DM patients.

The strength of our study was that we included age-matched healthy controls and studied the relationship between testosterone and cortisol levels and BMI in patients with T2DM. The current study's limitations included its small sample size and single-location setting. Other proteins, such as sex hormone-linked globulin, may cause changes in serum testosterone levels, but these were not included in our study. It may be very helpful to recruit a larger population with more diabetic individuals or to increase the observation time.

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Although our study cannot provide strong evidence for causal inference, the interesting associations observed in the current study can still provide useful clues for future investigations.

Conclusions

Testosterone is associated with maintaining normal cortisol levels, especially for patients with T2DM. Given the many adverse health effects, T2DM may lead to other worse conditions. In conclusion, most of the T2DM patients had low testosterone levels, whether they had high or low cortisol levels, but they were significantly lower in T2DM patients compared to non-diabetic healthy subjects and positively correlated with cortisol and BMI. Future studies should focus on the potential causal relationship between BMI change and testosterone and cortisol reference with a larger sample to offer more evidence-based recommendations about their association.

Conflict of interest

The authors declare no conflicts of interest.

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