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Research Article

The Correlation Study Between Testosterone Levels and Hba1c In Type 2 Diabetic Patients Compare with Healthy Persons

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Abstract:

The purpose of this study was to evaluate the the connection between hemoglobin glycated (Hba1c) and testosterone levels in the patients of type 2 diabetes mellitus compare with healthy person, also determine the significant differences between this parameter among healthy and diabetic patients, after assumed whole blood and serum specimens from laboratory of Diabetes and Endocrinology Center in Marjan hospital and privately owned in Babylon City starting in October 2022 to February 2023.

The outcomes showed non-significant (P>0.05) variations between those with diabetes and control groups in age , which refer to all persons in the same age, also it revealed significant increase (P < 0.05) in Hba1c level in diabetic group compare with healthy men, which may refer to disease effect on these patients and lead to elevation in Hba1c in diabetic group, also it was revealed significant decrease (P < 0.05) in diabetic group compare with control in Testosterone level, in addition to significant increase (P<0.05) in body mass index in diabetic men compare to control group, and The results showed negative correlation between glycated hemoglobin and testosterone levels in both healthy and diabetic men.

The research was concluded that HbA1c value is associated with testosterone levels in diabetic patients.

Key words: Testosterone, Type 2 Diabetic Patients and fertility.

Introduction

One group of metabolic diseases known as diabetes mellitus (DM) is typified by problems controlling blood sugar levels [1]. Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose levels resulting from either a total or relative lack of insulin in conjunction with insulin resistance, -cell dysfunction, or both.

One of the diseases with the fastest rate of growth in the world is diabetes. Diabetic patients face severe macrovascular consequences such as heart disease as well as microvascular issues such as diabetic kidney disease, diabetic retinopathy, and neuropathy, which can result in increased mortality, kidney failure, blindness, and a lower overall quality of life [2].

Several genetic studies have established a clear hereditary component to diabetes and its aftereffects. According to Cole and Florez (2020) [3], the onset of vascular issues cannot be predicted solely by clinical risk factors and glucose control.

Diabetes mellitus (DM) is a long-term medical condition marked by inadequate synthesis of insulin or inadequate utilization of insulin. Increased blood sugar levels in long-term untreated diabetes mellitus harm the heart, blood vessels, eyes, kidneys, and nerves [4].

Though not perfect, glycated hemoglobin (HbA1c) is a biomarker that plays a crucial role in the diagnosis and follow-up of patients with diabetes. In order to diagnose and track diabetes, glycosylated haemoglobin, or HbA1c, is essential [5].

For evaluating diabetic complications, glycosylated hemoglobin (HbA1c) has been the only substitute marker available. However, HbA1c is frequently reported to have limitations, including the inability to provide comprehensive information on short-term glycemic control and its susceptibility to interference from a variety of clinical conditions, including anemia, pregnancy, and liver disease. As a result, HbA1c by itself might not accurately reflect a patient's true glycemic status [6]

Testosterone is one of the most important male hormones in men. It is produced by Leydig cells in the testes. The pituitary gland and hypothalamus control the production of male hormones and sperm. There are many places where testosterone is produced, as follows:

About 90-95% of testosterone is produced in the more than 500 million Leydig cells found in the testes in men. 5% of testosterone is produced in the reticular region of the adrenal cortex. It is also made in much smaller quantities in women by the thecal cells of the ovaries, than the placenta. It can also be produced in very small amounts in the skin of both sexes [7].

Luteinizing hormone and follicle-stimulating hormone produce testosterone, which are important hormones sent from the pituitary gland and act on the testicles. Stages of testosterone development with age [8].

This research was aimed to survey about the relationship

between testosterone level and Hba1c level in diabetes and healthy men also comparison between these relationships in groups.

Materials and Methods

Patients or subjects

Serum and whole blood specimens were collected from laboratory of Diabetes and Endocrinology Center in Marjan hospital and private in the Babylon City during the period from October 2022 to February 2023. The study was included 50 samples, that composed from 30 samples from diabetic patients type 2and 20 samples from healthy person.
The tools were employed in the present research explained in the table (1).

Table (1) materials and method equipment.

Equipments	Company/ origin
1- Plane Centrifuge	Hettic/ Germany
2-Micropipette, 100-1000 µl	Dragon lab / china
3-refrigerator	Samsung / china
4-Micropipette, 10-100 µl	Dragon lab/ china
5- fluorecare devise/ testosterone test	Microprfit-bio / Germany
6- ichroma devise HbA1c test	Medlap/ Korean

Chemical materials

1- **HbA1c kit:** uses to determine HbA1c levels in different samples, specific kit which manufactured by Ichromax: Biotech-medical / Korean company.

A- Principle of the method

The test makes use of a sandwich immune-detection technique, whereby the immobilized antibody on the test strip captures the detector antibody in the buffer after it binds to the antigen in the sample to form antigen-antibody complexes and migrates onto nitrocellulose matrix. The amount of antigen in the sample increases the amount of antigen-antibody complexes, which intensifies the fluorescence signal on the detector antibody. The hemoglobin glycated content with regard to percentage of blood's total hemoglobin is displayed by the IchromaTM test instrument.

B- Procedure

1) The detection buffer tube was filled with 100 µL of hemolysis buffer.
2) The blood sample (5 ml) was added into the detection buffer tube.
3) After closing the detection buffer tube's lid and giving the sample a good shake for around fifteen times, it was thoroughly mixed.
4) The cartridge half was removed from the i-Chamber slot.
5) The sample mixture was pipetted out (75 Ml) and load it into a sample well in the test cartridge.
6) The windows display the flow of the sample mixture. (Roughly ten seconds)
7) The cartridge was placed inside the i-Chamber.
8) The cartridge was removed from the i-Chamber after being there for 12 minutes.
9) The sample-loaded cartridge was placed into the ichromaTM test device's cartridge holder for scanning. Before inserting the cartridge all the way into the cartridge holder, make sure it is oriented correctly. The cartridge has an arrow marked on it specifically for this use.
10) the process was started after chose start in ichroma
11) The test result was readed on the display screen of the instrument for ichroma™ tests.

Reference value

The ichromaTM test instrument automatically computes and displays the test result HbA1c concentration of the test sample in terms of %
Normal range = 4.5-6.5 %

Testosterone evaluation

Principles of test:

The fluorecare Testosterone uses a competitive immunodetection method to measure the amount of Testosterone in human serum. It is based on the Immunochromatographic assay principle. After adding human serum or plasma to the specimen adding hole, the fluorescence-labeled testosterone antibody on the bonding pad combines with the testosterone in the specimens to be tested to form the testosterone antibody complex. The testosterone antibody complex will diffuse across the nitrocellulose membrane as a result of the Chromatogaphy's effect. The coated antigen in the detection line area and the testosterone antibody complex in the test line (T) region must compete with one another; the higher the concentration of testosterone, the less the complex aggregation on the fluorescent band's color at a given wavelength, the weaker the detection line. The concentration of the substance to be measured in the specimens has a negative correlation with the fluorescence antibody signal intensity. When the second antibody catches the fluorescently-labeled antibody, it diffuses to the quality control line and forms a fluorescent band at that particular wavelength. The instrument can be used to interpret the results after the Reaction is finished. The instrument will analyze the optical densities of the test and control lines. The device then uses the previously established calibration curve to determine the testosterone concentration and shows the results in ng/mL.

Procedure:

1. the test card was taken remove from the fridge and allow to come to room temperature (20 -25 C˚).
2. the tool was turned on based on the instruction, the incubator was opened (the temperature to 25 C˚, and time to 15 minutes).
3. The lot number of the diagnostic kits and the consistency of the lD chip were examined. When inserting the lD chip, avoid touching its insertion end.
4. After tearing open the foil pouch along the splice, the test card was placed on the flat operation table. The test card needs to be completed in an hour.
5. the pipette was holding vertically, and 70 µl from sample was added or the benchmark item devoid of air bubbles to the diluent and mix well with diluent. Then 70 µl mixture was Applied to the sample well on the test card.
6. test card was placed into the incubator for 15 minutes before

removing.

7. The test card was placed into the analyzer's test card holder, and the test was initiated in accordance with the analyzer's instruction manual. The outcome will automatically appear on the screen a few seconds later. It is possible to print off and preserve the outcome.

8. The test card ought to be handled with consideration for any possible biological hazard.

Reference range:

Sex	Range (ng/mL)
Male	1.40-9.23
Female	0.1-0.6

Body mass index calculation

Body mass index was calculated by equilibrium:

$BMI = \frac{weight}{length^2\ (by\ meter)}$ [9].

Statistical analysis

The Independent Sample T test experiment design, which expresses these relations by linear equations and correlation coefficients for each relationship, was used to compare the quantity of testosterone in different groups and infer the significance, mean, and standard deviation (SD) using the well-known statistical system, statistical package for social science (SPSS) (version 22.0). These variables represent the extent of the two axes and the characteristics of the linear relations [10].

Results and Discussion

Table 2 significant differences (P<0.05) between control and diabetic group in the parameters of study

Parameters	Diabetic patients Mean ± SE	Control Mean ± SE	SIG	P value
Age	38.65 ± 2.63	35.22 ± 5.43	0.38	0.05
HbA1c mg/dl	7.83 ± 0.36	4.662 ± 0.123	0.02	
Testosterone ng/ml	1.0643 ± 0.042	3.944 ± 1.621	0.0133	
BMI Kg/cm^2	30.532 ± 4.63	20.1333 ± 2.774	0.0501	

The outcomes showed non- notable variations (P>0.05) between diabetic and control groups in age , which refer to all persons in the same age, also it revealed significant increase (P < 0.05) in Hba1c level among those with diabetes compare with healthy men, which may refer to disease effect on these patients and lead to elevation in Hba1c in diabetic group, the HbA1c is a useful diabetes biomarker because it provides information on average blood glucose levels over the last few months [11], Multiple variables can influence HbA1c levels, including sugar intake, exercise, and medication adherence. HbA1c might likely predict dyslipidemia and CVD in some studies, HbA1c is well now as predictor for hyperglycemia due to accumulation of glucose on the Hb [12].

Also it was showed a significant drop (P < 0.05) in the group of diabetics compare with control in Testosterone level, these findings may allude to how diabetes in diabetics led to oxidative stress patients, Oxidative damage to the hypothalamus, which secretes gonadotrophin-releasing hormone (GnRH), which stimulates the pituitary gland to release LH and FSH, could be the cause of the decrease in LH, FSH, and testosterone [13]; Another study found that both sexes with type 2 diabetes have lower levels of LH and FSH, clinical research revealed that 25% of males with type 2 diabetes also have low levels of FSH and LH. Earlier studies revealed that 33% of patients with hypogonadism also had significantly lower levels of FSH and LH [14].

Patients of T2DM tended to have significantly lower testosterone level when compared with non-diabetic individuals [15]. Other study indicates that men's risk of type 2 diabetes can be significantly reduced by having higher testosterone levels, and that there may be a reverse causal relationship between men's risk of type 2 diabetes and low testosterone levels [16].

Pituitary hormones are mainly responsible for controlling testicular function. Whereas the luteinizing hormone governs Leydig cell activity, the follicle-stimulating hormone controls spermatogenesis. Diabetes has been linked to decreases in prolactin, growth hormone, LH, and FSH serum levels [17].

The results showed significant increase (P<0.05) in body mass index in diabetic men compare to control group, that may refer to increase storage of glucose after convert it to fat which lead to increase BMI, obesity (body mass index [BMI] ≥ 30 kg/m2) is a worldwide epidemic affecting people of all ages, and is a major risk factor for type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD), Paul et al., (2017) resulted as patients with T2DM had significantly higher mean BMI levels by about 5 kg/m2 at diagnosis (32.2 kg/m2) compared to the matched controls (27.4 kg/m2),

The precise mechanism underlying the relationship between DM and BMI is still unknown. In certain patients, it might be secondary to inadequate glycemic control and weight loss, placing them in the normal-weight category, those who are diagnosed with the disease at a lower body mass may have an alternative, possibly more aggressive, pathophysiology than those who develop it when obese. These differences could include an early pancreatic islet failure, a stronger genetic predisposition to insulin resistance, or an increased sensitivity

to visceral fat accumulation. There have been reports of this alleged "obesity paradox" in relation to long-term conditions like coronary artery disease and chronic heart failure [18].

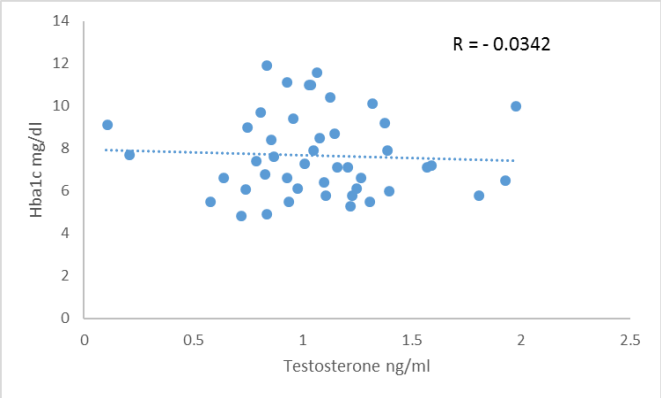

Figure 1 negative correlation between Hba1c and Testosterone in diabetic group

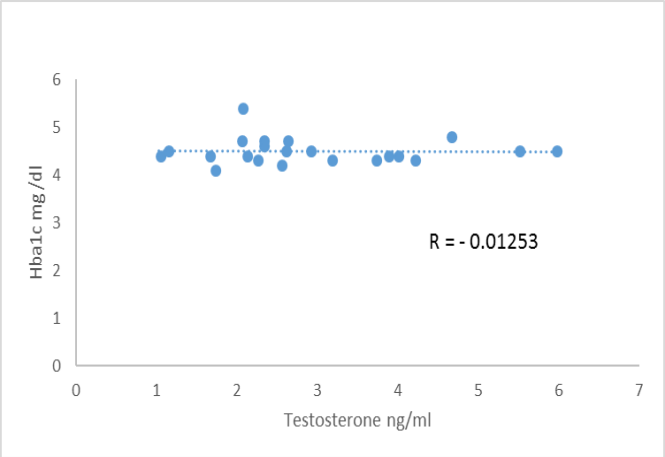

Figure 2 negative correlation between Hba1c and Testosterone in control group

The results showed negative correlation between glycated hemoglobin and testosterone levels in both healthy and diabetic men figures 3-1 and 3-2 consequently, which may refer to normal negative relationship in control group but in diabetic group may it refer to respond of diabetic persons to drug which lead to regulate blood glucose level and make decrease level of Hba1c, According to a number of studies, men who have low testosterone may be more susceptible to type 2 diabetes (T2D) and may even be able to predict when diabetes will manifest [19].

Other study reported low plasma testosterone levels are commonly observed in men with T2D and insulin resistance, also low levels of sex hormone binding globuline (SHBG) were believed to have a part in the emergence of insulin resistance and, eventually, type 2 diabetes. When compared to healthy men, a significant number of T2D patients had lower testosterone levels [20].

And clinical research also proposed that testosterone supplementation could reduce insulin resistance and atherosclerosis in hypogonadal men. These findings imply that a lack of testosterone is a major factor in a number of diseases, including metabolic disorders, type 2 diabetes, and obesity [21]. According to another study revealed men with diabetes always had lower total testosterone levels than non-diabetic controls, and men with higher testosterone levels had a lower risk of developing diabetes incidentally [22].

Type 2 Diabetes mellitus was found to be independently linked to low total testosterone levels in men in a more recent meta-analysis of prospective and cross-sectional studies. Additionally, testosterone replacement therapy was linked to a significant reduction in men's fasting plasma glucose [23], HBA1c, fat mass, and triglycerides in the same meta-analysis of the metabolic effect of testosterone replacement therapy. Even after adjusting for BMI, diabetic men still have lower testosterone levels, despite the obvious link between low testosterone and obesity [24].

Conclusions and Recommendations

Conclusions

1- The research was concluded that HbA1c value is associated with sexual deficiency in diabetic patients
2- Negative correlation was conducted between testosterone and Hba1c in diabetic group.

Recommendations (future studies)

1- Repeat the study in large number of samples in cohort study instead of case control study.
2- Study the relationship between Hba1c and other parameters such as lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in diabetic patients.
3- Comparison study the Hba1c and luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in different type of diabetes mellitus.
4- Comparison study between different types of diabetic drugs and how that effect on sexual hormones activity.

Ethical approval:

Ethics The human study was approved by the Al-Qasim Green University, Babylon Province, Iraq Review Board.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' contributions

All authors had 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND 2. Drafting the work or revising it critically for important intellectual content; AND 3. Final approval of the version to be published; AND 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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