EVALUATION OF FERTILITY HORMONES IN TYPE 2 DIABETIC MALE SUBJECTS IN NAUTH, NNEWI.

ABSTRACT

Background and Aim of study: To Assess the levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone among type 2 diabetic male subjects in NAUTH, NNEWI.

Methodology: This was a cross sectional study carried out at Endocrinology unit, NAUTH Nnewi. A total of 134 participants were recruited for this study which comprised of 67 male type 2 diabetics mellitus subjects and 67 apparently healthy controls .The levels of testosterone, LH, FSH and glycated hemoglobin were analysed using ELISA and colorimetric assay methods respectively.

Results: Results from this findings showed that the mean levels of glycated hemoglobin (HbA1c), LH and FSH were significantly higher in test subjects compared with that of the control subjects ($p<0.05$). Conversely, the mean level of testosterone was significantly lower in the type 2 diabetic male subjects compared to that of the control subjects ($p<0.05$) In male type 2 diabetic participants, there was a significant positive correlation between the mean levels of HbA1c and the mean levels of LH ($r=0.385$, $p=0.001$) and FSH ($r=0.535$, $p<0.001$). Nevertheless, among the male type 2 diabetic participants, there was no significant correlation ($p>0.05$) between the mean HbA1c levels and testosterone. In the control group, there was a significant positive correlation ($r=0.461$, $p<0.001$) between the mean values of HbA1c and FSH.

Conclusion: This suggests there is hypogonadism which is indicative of an alteration in the hypothalamic-pitutary -gonadal axis and this could lead to infertility in type 2 diabetic male subjects.

Key words: Diabetes mellitus, glycated hemoglobin/HbA1c, FSH, LH, Testosterone.

INTRODUCTION

Diabetes mellitus (DM) is one of the metabolic disorders which is characterized by hyperglycemiaresulting from lack of insulin synthesis and secretion or reduced sensitivity of tissues to insulin[1] . According to WHO (2016), the 7th leading cause of death is diabetes and as of 2021 its been estimated that about 537 million people had diabetes mellitus with about type 2 diabetes prevailing more[2]. Futhermore, its been estimated that by 2045, approximately 783 million adults, or 1 in 8 people, will be living with diabetes, representing a 46% increase from the current figures[2]. Reports have shown percentage prevalence of diabetes mellitus in Nigeria to be within 0.8-4.4% with its rural and urban areas between 4.6-7% [3,4]. The pooled prevalence of Diabetes mellitus in the six geopolitical zones of Nigeria were 3.0% in the north-west, 5.9% in the North east, 3.8% in the North central zone, 5.5% in the south west, 4.6% in the south-east and 9.8% in the south south zone[5] .

According to Berta , Reproductive hormones are secreted from the anterior pituitary gland and they are :The follicle-stimulating hormone (FSH) , luteinizing hormone (LH), prolactin, growth hormone (GH), adrenocorticotropic hormone (ACTH) and thyroid-stimulating hormone (TSH). LH, FSH and testosterone are major hormones of reproduction which directly control many aspects of gondal development. Male fertility can be assessed by measuring the serum levels of male fertility hormones such as: luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone[12]. FSH receptors are located on the membrane of Sertoli cells, while those of LH are on the Leydig cells. They coordinate to synthesize testosterone, maintain normal spermatogenesis, sperm health and density[11]. The gonadotropin releasing hormone (GnRH) secreted by the hypothalamus regulates the release and secretion of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from anterior pituitary that in turn regulate testicular functions [12]. LH controls the production of testosterone by the leydig cells which is located on the interstitium of the testis[28].Testosterone is essential for male virilization as it combines with FSH to trigger and maintain spermatogenesis[28]. There will be a joint action of testosterone and FSH on the sertoli cells this will limit the wall of seminiferous tubules that support germ cells undergoing development to become mature sperm.For the process of spermatogenesis to be initiated and continously, leydig cells must secrete more LH during purberty in order to maintain an increased amount of intratesticular testosterone, which is necessary for male virilization and the maintenance of spermatogenesis in the male external genitalia[28]. In males, intratesticular hormone is actually 100 times more circular than peripheral blood[28]. Testosterone also stimulates erythropoietin which is responsible for higher levels of heamatocrit in males than in females. Testosterone tends to drop as age increases and because of this there is reduction in testicular size, drop in libido, lower bone density, decline in muscle mass, increased fat production and decreases in erythropoietin and thereafter leading to anemia[40]. These gonadal steroids as well as the pituitary gonadotropins, via feedback regulatory mechanisms, further establish physiological homeostasis and maintains normal reproductive functions[11].

Prior reports have revealed an association between low serum testosterone levels and diabetes mellitus[13,14]. There has been previous research done in ascertaining the prevalence of hypogonadism in male type 2 subjects and these showed low levels of testosterone and elevated levels of LH in male subjects respectively[15]. Hypogonadism has been characterized by Low testosterone levels followed by other features seen in hypogonadism which are : erectile dysfunction, poor morning erection, low libido etc[17-19]. About 6.0 -26% of male subjects with diabetes mellitus has been noted to have low testosterone levels[16]. Studies by Al fartosy showed an increased HbA1C levels is associated with LH, FSH and low testosterone levels in research on correlation of insulin resistance with HbA1C .This could be due to an imbalance between reactive oxygen species and antioxidant capacity which could result in oxidative stress[26]

Glycosylated hemoglobin/glycated hemoglobin (HbA1c) can be directly correlated to glucose levels and complications, and is recommended by international guidelines as the preferred measure when evaluating the overall control of diabetes and the patient's risk for complications [21]. Glycated hemglobin is the most preferred assay for blood glucose determination because it provides a long term measure of the patients blood glucose levels. It also provides the physician with a reliable means of monitoring patients hyperglycemia without the need to request for fasting prior to testing like in fasting plasma glucose [27]. Hence, close monitoring of HbA1c levels is recommended for all diabetic patients and those with the potential for developing diabetes. It is also suggested that diabetic and non- diabetic patients with raised HbA1c levels should be clinically checked and monitored as a preventive intervention to avoid developing T2DM[22] .There is evidence according to Shewarni et al which revealed elevated levels of HBA1c in diabetic male patients. The most significant modifiable risk factor for diabetes mellitus is obesity, and the diagnosis and definition of obesity are based on anthropometric measures[23].

Waist circumference, waist to hip ratio, waist-to-height ratio, weight, height, and BMI[23] are measurements obtained from anthropometry; however, due to the historical use of BMI[23], BMI is still primarily utilised among these anthropometric measurements in the classification of obesity[24].Shi et al reported high levels of BMI in male diabetic patients with erectile dysfunction .Elevations in anthropometric measurements have also been linked to hyperglycemia[24]. Studies by Cheung et al. has revealed that one of the features of diabetes mellitus in men which causes low levels of testosterone is obesity[20] .The mechanism behind this is that ,in obese men during peripheral conversion of testosterone to oestrogen there will be an attenuation in amplitude of leutinizing hormone pulses this could subsequently inhibit testosterone production, causing low testosterone levels[20]. Al hayek et al. conducted studies to assess the prevalence of low testosterone levels in male diabetic subjects, which revealed that approximately 48.3% of diabetic male subjects were obese and 39.3% were overweight, with a large percentage having been diabetic for more than ten years[15]. In addition, they were taking oral antidiabetic medications and insulin. It was also

discovered that men aged 30-70 years were recruited for the analysis, with a focus on people aged 60-69 years[15].

Long term complications of type 2 diabetes develop gradually, with infertility being one of them[6]. Infertility is a disease characterised by failure to establish a clinical pregnancy after 12 months of regular and unregular unprotected sex[7].Males account for roughly 20–30% of infertility cases, while females account for another 20–30%, or about 50% of cases overall.[7]Studies have shown there are higher risk of infertility from men who are diabetic when compared with control subjects[8] and evidence from studies by Ding et al. revealed the impact of diabetes mellitus on reproductive system with the following conditions being generated : dysfunction of hypothalamic pituitary gonadal axis, decreased testosterone and synthesis secretion, testicular failure, spermatogenesis disorder, erectile dysfunction, ejaculatory disorder etc .It is therefore imperative to evaluate the LH,FSH and testosterone and glycated hemoglobin levels in type 2 diabetic male subjects.

MATERIALS AND METHOD

Study design

This was cross-sectional study designed to assess some fertility hormones among type 2 diabetic male subjects in NAUTH Nnewi, Nigeria. A total number of One hundred and thirty-four (134) Participants were recruited randomly for this study. This comprised of Sixty-seven (6)7 type 2 diabetic male subjects and who were aged 40-65 years selected from endocrinology unit NAUTH, Nnewi and Sixty-seven (67) apparently healthy controls who were aged 40-65 years selected from staff of Nnamdi azikiwe university teaching hospital (NAUTH) Nnewi,Anambra state. Information on socio-demographic, medical history and lifestyle was obtained using a questionnaire. Body mass index (BMI) was derived using the height and weight measurement of subjects.

Ethical Clearance

The ethical approval for this research was obtained from the ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi (NAUTH) with reference no: NAUTH/CS/66/vol.15/VER.3/338/2021/112.

Informed Consent

Consent was sought and obtained from subjects prior to study. A written consent form was used.

Exclusion Criteria

These were Individuals outside the age range of 40-65 years and Subjects with history of chronic diseases such as: chronic obstructive pulmonary disease, hypertension and thyroid disorders, Smokers and chronic alcohol drinkers and Known infertile subjects.

Specimen Collection

Five millimeters (5mls) of whole blood was collected from the subjects using the venipuncture technique, 3mls of the blood sample was dispensed into plain sample container and 2mls dispensed in EDTA container. The sample dispensed into the plain sample container was allowed to stand for 10mins to clot and centrifugation performed at 4000rpm for 5minutes. The serum was separated and stored at -20◦C until analysis of testosterone, LH, FSH. Sample in the EDTA container was used for the assay of Glycated Hemoglobin to ascertain glycemic control.

Laboratory procedures

Determination of Glycated Haemoglobin (HbA1c)

Glycated Hamoglobin was determined by Immunoturbidimetric assay method as described by Metus *et al.*[30].

Determination of Luteinizing Hormone Levels

LH was determined using sandwich enzyme linked immunosorbent assay (ELISA) as described by Benard and Ongaro[31].

Determination of Follicle Stimulating Hormone Levels

FSH was determined using competitive enzyme linked immunosorbent assay(ELISA) was used as described by Ongaro *et al.*[32]

Determination of Testosterone Hormone

Testoterone was determined using Competitive enzyme immunoassay method (ELISA) as described by Tietz [33].

Anthropometric measurement

The weight and height of each participant were measured using a standard beam balance scale and a stadiometer respectively. Body mass index (BMI) was calculated as weight (kg) divided by height squared in meters.

BMI ($Kg/m^{2)}$ = $Weight(Kg)/Height^2$ ($m^{2)}$.

Statistical Analysis

Statistical package for social sciences version 23.0 was used for data analysis.The data obtained were analyzed using ANOVA, Independent t-test and Pearson Correlation. Results were deemed significant at $p < 0.05$.

RESULTS

The result of analysis of variance showed the mean (± SD) value of BMI and age in male type 2 diabetic subjects and control group. The mean body mass index (BMI) of type 2 male diabetic subjects was observed to be significantly higher compared with that of the control subjects (30.57±4.83 VS 25.71±2.67; P=0.001). Whereas, the mean value of age did not show any significant difference in type 2 diabetic male subjects compared to control group (P=0.171) See table 1.

The mean serum level of HbA1c was observed to be significantly higher in type 2 male diabetic subjects compared with that of the control subjects (8.56±1.22 Vs 5.25±0.31; P =0.001). Also, the mean serum levels of LH and FSH was noted to be significantly higher in type 2 diabetic male subjects when compared with control group (10.74±4.50 Vs 4.88±1.36; P =0.001) and (15.13±7.26 Vs 6.89±2.92; P=0.001) respectively. However, the mean serum levels of

testosterone was observed to be significantly lower in type 2 diabetic male subjects in comparison to the control group (2.89±1.05 Vs 2.89±1.05; P=0.001) . See Table 2

There was a significant positive correlation between the mean levels of HbA1c with mean serum levels of LH (r= 0.385, p=0.001) and FSH (r= 0.535 ; p=0.001) in type 2 diabetic male subjects . However, there was no significant correlation was noted in mean serum levels of HbA1c with mean serum levels of testosterone in the type 2 diabetic male subjects and control group (p>0.05). See table 3.

TABLE 1: Age and BMI in type 2 diabetic male subjects and control group (Mean± SD)

VARIABLE	TEST GROUP **(n=67)**	CONTROL GROUP **(n=67)**	P-VALUE	SIGNIFICANCE
BMI (KG/M^2)	30.57±4.83	25.71±2.67	0.001	Significant
AGE (YEARS)	51.75±4.91	50.120±5.99	0.171	Not Significant

Table 1: Showed the mean body mass index (BMI) of type 2 male diabetic subjects (30.57±4.83 KG/M^2) was observed to be significantly higher compared with that of the control subjects (30.57±4.83 VS 25.71±2.67; P=0.001). Whereas, the mean value of age did not show any significant difference in type 2 diabetic male subjects compared to control group (P=0.171)

*Statistically significant at p≤0.05 * BMI= Body mass index * SD= Standard deviation

***** n= number of subjects in group *

TABLE 2: Levels of glycated hemoglobin (HbA1c), luteinizing hormone, follicle stimulating hormone and testosterone in male diabetic and male control subjects (mean ±SD).

Parameter	DIABETIC n=67	CONTROL n=67	t-value	p value
HbA1c (%)	8.56±1.22	5.25±0.31	21.452	0.001*
LH (mIU/ml)	10.74±4.50	4.88±1.36	10.214	0.001*
FSH (mIU/ml)	15.13±7.26	6.89±2.92	8.620	0.001*
TESTOSTERONE (ng/ml)	2.89±1.05	5.08±1.30	-10.702	0.001*

Table 2 : Showed the mean serum level of HbA1c, LH and FSH was observed to be significantly higher in type 2 diabetic male subjects when compared with control group (8.56±1.22 Vs 5.25±0.31; P =0.001) , (10.74±4.50 Vs 4.88±1.36; P =0.001) and (15.13±7.26 Vs 6.89±2.92 ;P= 0.001) respectively. However, the mean serum levels of testosterone was observed to be significantly lower in type 2 diabetic male subjects in comparison to the control group (2.89±1.05 Vs 2.89±1.05; P=0.001) .

* - Mean difference significant at p≤0.05

Key:

n – Number of subjects in the group

SD – Standard Deviation

HbA1c – Glycated Hemoglobin

LH – Luteinizing Hormone

FSH – Follicle Stimulating Hormone

TABLE 3: Correlation of mean serum levels of glycated hemoglobin with mean serum levels of luteinizing hormone, follicle stimulating hormone and testosterone in type 2 diabetic male and control subjects

	DIABETIC (n=67)		**CONTROL (n=67)**	
Parameter	**R**	**P value**	**R**	**P value**
HbA1c & LH	0.385	0.001**	0.000	0.997
HbA1c & FSH	0.535	0.001**	0.461	0.001**
HbA1c & TESTOSTERONE	-0.043	0.731	0.168	0.173

Table 3 : Showed there was a significant positive correlation between the mean levels of HbA1c with mean serum levels of LH (r= 0.385, p=0.001) and FSH (r= 0.535 ; p=0.001) in type 2 diabetic male subjects . However, there was no significant correlation was noted in mean serum levels of HbA1c with mean serum levels of testosterone in the type 2 diabetic male subjects and control group (p>0.05).

*Correlation significant at p<0.05, **Correlation significant at p<0.01

Key:

r - Pearson Correlation Co-efficient

n – Number of subjects in the group

HbA1c – Glycated Hemoglobin

LH – Luteinizing Hormone

FSH – Follicle Stimulating Hormone

DISCUSSION

The mean serum values of BMI and age in type 2 diabetic male subjects were observed to be significantly higher compared with that of the control subjects. This is in line with a study carried out by Gary et al. in determination of the effects of elevated body mass index (BMI) on type 2 diabetes mellitus (DM) onset and its complications among elderly. There were Elevated BMI values observed to be associated with progressively higher risk for all diabetes mellitus complications[34].BMI is used to compare percentages of body fat, and elevated BMI values are associated with obesity[43]. A BMI of 27.3 kg/m/^{2} for women and greater than 27.8 kg/m/^{2} for males is considered obesity.[42].A BMI greater than 27.5 kg/m2 is classified as obesity and identified as a risk factor for diabetes mellitus , according to WHO categorization that was adopted by NIH panel experts[43] Previous research, as reported by Logue et al. indicated that men with diabetes mellitus had increased BMIs, this is consistent with these findings. There has also been studies done by Al hayek et al. in prevalence of low testosterone levels in male diabetic subjects showed that about 48.3% of diabetic male subjects were obesed and 39.3% were overweight.Several evidences on the link between hypogonadism and type 2 diabetes has found that obesity is associated with low levels of testosterone[50] The results of this study showed that the type 2 diabetic male subjects' mean HbA1c levels were considerably higher than those of the control subjects. This aligns with cohort studies examining HBA1C variability in type 2 diabetic participants, which revealed higher HBA1C levels in male participants with diabetes[44]. Glycated hemoglobin or hemoglobin A1c is stated by the international federation of clinical chemistry working group (IFCC) 1 as Standard of Care (SOC) which is used for assessing and monitoring of history of blood glucose level[38]. It is used as an index of glycemic control. Glycated hemoglobin is an irreversible non enzymatic addition of a sugar residue to the hemoglobin, the rate of production is directly proportional to the glucose concentration. Monitoring of HbA1c is suggested by the American Diabetic association, American diabetic federation, and European association for the management of diabetes[39]. So HbA1c is now routinely obtained as the most prominent, single and independent parameter of metabolic control. It is a risk factor for the growth and development of diabetic complications and significantly used in treatment and

management[39]. Analysis of HbA1c in blood gives evidence about individual's average blood glucose levels in the period of 120days [39].

The serum levels of LH and FSH were significantly higher in the test subjects as compared to the control group. Conversely, serum level of testosterone was observed to be significantly lower in type 2 diabetic subjects when compared with that of the control subject. This could be due to the effects of insulin resistance on the hypothalamus, pituitary glands and gonads, this causes less secretion of the gondalsteroids[4,5]. Futhermore, the aromatase enzyme, which converts testosterone to estradiol, may be the cause of the noticeably reduced testosterone levels. The ratio of testosterone to oestrogen is also an indicator of type 2 diabetes mellitus[46]. Additionally, it has been observed that insulin resistance lowers the quantity of leydig cells, which in turn alters the hormone testosterone concentration[47]. In diabetic patients, oxidative stress can also impair pituitary and hypothalamic function[48], giving rise to a condition called hypogonadism[49]. The findings of this study are consistent with those of investigations conducted by Ezekiel et al. to ascertain the pattern and prevalence of hypogonadism in males with type 2 diabetes mellitus who are Nigerian. It was discovered that the mean testosterone of type 2 diabetic men was significantly lower compared to the controls, the mean LH and FSH levels were significantly higher in type 2 diabetic men than the controls .Prior research were consistent these reports [36 37].

The mean serum levels of HbA1c was significantly correlated positively with LH and FSH. However, the mean serum levels of HbA1c was not significantly correlated with testosterone in the test subjects and control subjects. This is in agreement with findings by Shewani et al which stated that as glycated hemoglobin (HbA1c) increases, LH and FSH increases and vice versa in type 2 diabetic male subjects. Several studies have indicated association of HbA1C with LH and FSH[13,14,26.] This is attributed to the interaction of gonadotrophin hormones and gonadal steroid hormone and their feed back mechanism at the hypothalamic pituitary axis in order to maintain homeostasis and spermatogenesis, produce healthy sperms with great density and regular testicular function especially diabetic conditions which is characterized by hyperglycemia[11,12].

Hyperglycaemia causes tissue damage through multiple mechanisms including increased flux of glucose and other sugars through the polyol pathway, increased intracellular formation of advanced glycation end products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine

pathway [11]. These pathways eventually lead to oxidative damage to testis, sperm. Testicular testosterone production is acutely reduced as a result of testicular dysfunction[11]. A decrease in testosterone levels increases the secretion of LH and FSH via negative feedback to hypothalamus and pituitary gland [11]. Further studies with adequate sample size are needed to validate the findings of this study. Also, Fertility hormonal assay should be included in the panel of tests conducted for diabetic individuals males.

Conclusion

There were elevated levels of leutinizing hormone (LH), Follicle stimulating hormone (FSH) and significantly low levels of testosterone, as well as high levels of HBA1c (hyperglycemia) were revealed in this study. This is an indication of alteration in the hypothalamic-pitutary-gonadal axis which could lead to male infertility.

Ethical approval: All authors hereby declare that the experiments have been examinedand approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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