

MALE HYPERGLYCEMIC-INDUCED INFERTILITY: AN INTEGRATION OF SOME BIOCHEMICAL FACTORS

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ABSTRACT: *Concern for hyperglycemia due to diabetes mellitus and its complications in male diabetics prompted the need for this study. Making use of body fluids such as urine, seminal fluid, serum sample and excised tissues, we examined factors that can potentiate complications. Batteries of tests conducted includes insulin, glucose, glycated haemoglobin, urea, creatinine and some male sex hormones, FSH, LH and testosterone. Spectrophotometric, radioimmunoassay and histopathological approach were adopted for the analytical methods. A total of 100 subjects were studied out of which 50 were non diabetics as defined by their glucose concentration (3.5-5.6 mmol) and 50 diabetics with glucose concentration ranging from (15.5-35.5 mmol/l). Our findings portray a correlation among the factors and elicit a nexus that interrelate to cause complication in diabetes. Values of the analyte measured after a 24 hour fast showed a significant difference ($p < 0.05$) between the diabetics and non diabetics while no statistical significant difference was observed among the normal subjects. We submit that variation in the values of biochemical parameters measured and their interactive interplay act in uncommon way to exacerbate diabetic complications and reduce fertility in males. Inhibiting these factors highlighted from rising will improve fertility among male diabetics.*

KEYWORDS: Hyperglycemia, Diabetes, Infertility, Biochemical Factors.

INTRODUCTION

Global consideration now put diabetes mellitus ranking among the most common non infectious diseases. With a rising prevalence and sustained rising mortality and morbidity, there is now a heightened interest for further study to elucidate the causes of complication. There is justifiable fear that the number of diabetes may outstrip previous estimates significantly (Wild et al, 2004). Type 1 diabetes occurs due to lack of insulin and may be potentiated by hereditary, immunity and environmental factors. It is characteristically common in younger groups although it may occur at all ages. Type 2 occurs as a result of relative lack of insulin and is

enhanced by hereditary obesity, insulin secretion and insulin receptors. It also occur in middle age and the elderly.

Traditionally, diabetes mellitus is considered as hyperglycemia due to absolute or relative deficiency of insulin secretion. Studies of previous literature have shown that a myriad of environmental factors contribute immensely to enhance the development and sustenance of diabetes. One of the factor is industrialization which is attributed to cause increased calorific consumption resulting in overweight, body mass index (BMI) ≥ 25 kg/m², obesity ≥ 30 kg/m² and physical inactivity (Rosen et al, 2009). The mortality rate of diabetics is enhanced as a resulting macrovascular and microvascular disease that develop as a result of complication that results in nephropathy, neuropathy and retinopathy (Vinik et al, 2003). To these groups are added further complex medical issues that have to do with coronary heart disease, loss of sight, libido reduction and foot ulcers that has resulted in several foot amputations (Wessels et al, 2011). As shown by (Jones et al, 2011 and Dong et al, 2011), there is evidence associating several dysfunction in both sexes as a result of hyperglycemia due to diabetes mellitus. Determination of biomedical parameters are useful for detecting and assessing the effects of environmental or toxicological stress on animals. Data on biomedical values are available to a limited extent in literature. It is however known that extensive experimental evidence is available that sex steroid and insulin interact in their actions on tissues (Agbaje et al, 2007). Steroids are also known to promote insulin resistance that involves reduction in insulin receptor, reduced expression and translocation of insulin – responsive glucose transporters and defects of the insulin signaling pathway distal to the insulin receptor all of which could be explained from a molecular viewpoint (Rolo and Palmeria, 2006). Elevated serum levels of sex steroids associated with normal puberty are linked to a decrease in insulin sensitivity which is related to peripheral glucose metabolism (Campos, 2012).

In this study we examined if hyperglycemia responsible for complications in diabetes interplay with some biomedical markers and tissue cells in sexual dysfunction to exacerbate infertility.

MATERIALS AND METHODS

At the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria, blood sample (serum and plasma), urine and seminal fluid were collected from diabetic patients (n=50) whose blood glucose level were above Threshold (>10.0 mmol/l) with presence of glucosuria. Control subjects (n=50) for the study were those with glucose level ranging between 3.5-6.7 mmol/l with no glucosuria. All samples were overnight fasting samples. Consent were obtained from all subjects.

ANALYTICAL METHODS

The concentration of blood glucose was determined by the glucose oxidase method with the use of Randox Kits (London) and values measured with spectrophotometer SPEC 22D+ set at 540nm. Urine samples were analysed with N-multistix, Macherry-Nagel (Germany) for glucose. Urea and creatinine were determined colorimetrically with corning colorimeter using urease and Jaffe methods respectively. Glycated heamoglobin was determined by the enzyme endoproteinase Glu-C. In a second step the glycated and non glycated N-terminal hexapeptide of the β -chain were separated and quantitated using ion-exchange high performance liquid

chromatography (HPLC-Esi/ms) approach with Uv-detection. The percentage of HbA1c was determined as a ratio of the glycated to the non-glycated B-N terminal hexapeptide of the haemoglobins. Cx9 automated machine (Beckman) was used with Beckman Assay reagent for the automated analysis of insulin antibodies. Separation of bound from free ligand was accomplished by double antibody precipitation. Luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin and testosterone were all determined by radiomunoassay techniques. For the histological examination of diabetic foot ulcer, tissue samples were excised from the spot and immediately transferred to 10% formalin. The samples were sliced to facilitate complete fixative penetration. Samples from both the normal and diabetic subjects were then processed with paraffin infiltration and embedded in paraffin. The formalin-fixed-paraffin embedded tissue blocks were cut with a rotary microtome into 4-6 μ m thick, and were placed in positively charged glass slides. They were then dried and stained with haematoxylin/eosin for histopathological examination.

RESULTS

Analysis of biochemical parameters are shown in Tables 1,2 and 3 respectively for hormones, other metabolites and semen analysis. Values obtained are mean SD \pm SEM of triplicate determination. In plates 1 and 2 histological pictures of foot ulcer collected from some of the patients are as shown.

| Hormones | Non-diabetics (n=50) | Diabetics (n=50) |
|------------------------|----------------------|------------------|
| LH (iU/L) | 6.8 \pm 3 | 3 \pm 0.5 |
| FSH (iU/L) | 10.2 \pm 2 | 5 \pm 2 |
| Prolactin (μ g/l) | 8.5 \pm 3 | 6 \pm 0.5 |
| Testos (mmol/l) | 205 \pm 5 | 135 \pm 5 |
| Insulin (pmol/l) | 35 \pm 5 | 5 \pm 3 |

Table 1: Biochemical profiles of hormones in diabetic and non-diabetic subjects. Values are mean \pm SEM of triplicate determination.

| Metabolites | Non-diabetics (n=50) | Diabetics (n=50) |
|---------------------------|----------------------|------------------|
| cholesterol (mmol/l) | 4.0 \pm 0.6 | 10 \pm 0.6 |
| Glucose (mmol/l) | 4.8 \pm 0.3 | 22 \pm 3 |
| HbA1c (mmol/l) | 42 \pm 3 | 98 \pm 3 |
| Urea (mmol/l) | 3.5 \pm 2 | 8 \pm 2 |
| Creatinine (μ mol/l) | 85 \pm 5 | 110 \pm 5 |

Table 2: Biochemical profiles of some metabolites. Values are mean \pm SEM of triplicate determination.

| Semen analysis | Non-diabetics (n=50) | Diabetics (n=50) |
|------------------------|----------------------|----------------------|
| Total count (cells/ml) | 35x610 ⁶ | 10.5x10 ⁶ |
| Active motility (%) | 80 | 40 |
| Sluggish cells (%) | 15 | 35 |
| Dead cells (%) | 5 | 25 |

Table 3: Cell count and motility profile of semen of diabetics and non-diabetics. Values estimated based on sample collected from subjects after five (5) days abstinence and counted with improved nauber counting chamber.



Plate 1

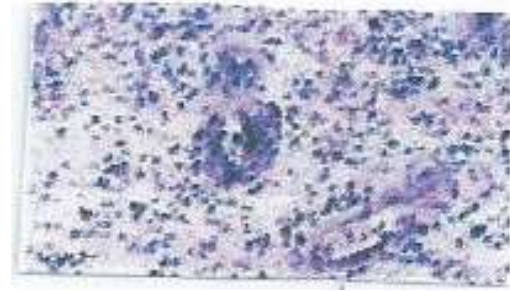


Plate 2

From the histological results we noticed from plate 1 that there is evidence of marked inflammation of the tissues. We observed the presence of granulating tissues from the bottom and margin of the ulcer in plate 2. These patterns are suggestive of acute lesion. Foot ulcers are typical complications of diabetes mellitus.

DISCUSSION

The rising incidence of diabetes and the associated complication especially in impairment of reproductive function in male informed this study. Our findings from the profiles of hormones, other metabolites and semen analysis from subjects investigated have shown a correlation to suggest that that these metabolites interact to potentiate the complications of diabetes. We observed a marked variation in the values of hormones, the metabolites and semen analysed from the diabetic and non-diabetics. Testosterone level for diabetics were markedly reduced, while cholesterol, glucose and glycated hemoglobin were elevated. A similar observation was made for the sperm viability assessment which show \ reduction among the diabetics. The genetic basis for this is not well known, nevertheless the coexistence of genetic and environmental factors have earlier been suggested (Guigliano et al, 2010).

The parameters measured in this study could be used alone or in combination with others from previous study or even future study to better diagnose infertility or erectile dysfunction. There has been continuous research effort to confirm whether hyperglycemia is a potential risk factor among men. As shown by (Seftel et al, 2004 and Lu et al, 2009) an association exist between poor glycemic control which is exhibited by raised level of glycated haemoglobin. The triad of hyperglycemia, hyperlipidaemia and hypertension operates in tandem with diabetes. Insulin resistance, hypogonadism and vasculopathy all contribute to infertility in men. It has been shown by (La Vignera et al, 2009) that in diabetes, the atherosclerotic damage in blood vessels reduces blood flow to the penis. Hyperglycemia is known to effect the endothelium causing endothelial dysfunction. This is expressed in form of a reduction of the bioavailability of nitric oxide (NO). Nitric oxide functions to relax the vascular smooth muscle of corpora cavernosa. The chemistry of this action involves increase in glycated end products and elevation of oxygen free radicals that cause a reduction in the bioavailability of nitric oxide (NO). Escrig et al, (2002) had earlier observed an elevation of microparticles which is a marker of endothelial dysfunction in diabetics. Again it has been shown that both somatic and autonomic neuropathies have played a role in male infertility due to the defect of sensory impulse from penis (Malavige and Levy, 2009). Further extensive evaluation of previous studies have

elucidated in previous studies a subnormal concentration of testosterone in type 2 diabetic men and reduced luteinizing hormone (Esposito and Guigliano, 2011, Dandona and Dhindsa, 2011). Our findings in this work collaborate these as could be seen from the results. Testosterone is shown to modulate most component of the male hormone. In diabetes the mechanism involved in testosterone deficiency includes reduced sex hormone-binding globulin which is caused by resistance to insulin, increased aromatase activity in visceral adipose tissue leading to an augmented conversion of testosterone to estradiol, leptin resistance causing reduced secretion of LH and testosterone. In addition to these, elevated levels of inflammatory mediators which may surpass the secretion of gonadotropin releasing hormone and LH are added as factors. (Ahmed, 2005 and Isidori et al, 2014) .Whereas we could not measure the reactive oxygen specie, it has been shown (Kim and Moley, 2008) that spermatozoa can be readily damaged by oxygen specie. This is attributed to the fact that the plasma membrane of spermatozoa may account for defective sperm function. In addition to this, sperm cells are known to contain high concentration of polyunsaturated fatty acids and their cytoplasm contain low concentration of scavenging enzymes. As could be seen from the result the sperm count of diabetics were reduced with poor motility an indication that the sperm cells are not normal. Moreover several of the cells are not active.

We deduced that these findings are products of the hyperglycemia which have subtly brought about molecular changes that is important for sperm quality and function. As could be seen from the result, conventional sperm parameters have been altered. We can use mechanism of sperm damage, endocrine disorders, neuropathy and oxidative stress to explain results obtained. The increased oxidative stress in diabetes has the potential to damage sperm nuclear and mitochondria DNA. Moreover it is known that while disorders in spermatogenesis and germ cells apoptosis in type 1 diabetes may be caused by local autoimmune damage, obesity and insulin resistance may affect sperm parameters and reduce testosterone levels in patients with type 2 diabetes (La Vignera et al, 2011). We observed oligospermia among the diabetics in which some cases were marked. We deduced that the low testosterone, LH and FSH levels are indicative of gonadal dysfunction. Histological examination of lesion collected from some of the diabetics show a marked morphological different from the non-diabetics.

CONCLUSION

In conclusion, developing ingenious ways to counteract the effect of parameters investigated in this study will enhance management of diabetics. Hormones and the named metabolites has been shown in this study to be acting in an uncommon way to increase the complication of diabetes.

REFERENCES

- Agbaje, I.M., Rgers, D.A., McVicar, C.M., McClure, N., Atkinson, A.B., Mallids, C., Lewis, S.E. (2007):
Insulin dependent diabetes mellitus: implication for male reproductive function. *Hum Repro.* 22, 1871-1877.
- Ahmed, R.G. (2005): The physiology and Biochemical effect of diabetes on the balance between oxidative stress and antioxidant defence system. *Med. J. Islamic World Acad. Science* (15):31-42.

- Armstrong, D.G., Ngyugen, H.C., Larvary, L.A., Van Schie, C.H.M., Brulton, A.J.M., and Harkless, L.B.
(2001): Off-loading the diabetic foot wound: a randomized clinical trial. *Diabetes care.* 24:1019-1022.
- Campos, C. (2012): Chronic hyperglycemia and glucose toxicity: Pathology and clinical sequale. *Posraduate Med.* 124(6):90-97.
- Dandora, P., and Dhindsa, S. (2011): Update: Hypogonadotropic hypogonadism in type 2 diabetes and obesity. *J. Clin Endocrinol Metab.* 96(9):2643-2651
- Dong, J.Y., Zhang, Y.H., Qin, L.Q., (2011): Erectile dysfunction and risk of cardiovascular disease: metaanalysis of prospective cohort studies. *J. AM Coll Card.* 58(13) 1378-1385.
- Escrig, A., Mann, R., Abren, P., Gonzalez-Mora., and Mas, M.(2002): Changes in mating behavior, erectile function, and nitric oxide levels in penile corpora cavernosa in streptozotocin-diabetic rats. *Biol Repro.* (66):185-189.
- Esposito, K., and Guigliano, D. (2011): Obsity, the metabolic syndrome, and sexual dysfunction in men. *Clin Pharmacol Ther.* 90(1):169-173.
- Frykberg, R.G., Lanery, L.A., Pharm, H., Harvey, C., Harkles, I., and Verves, A. (1998): Role of neuropathy and high foot pressure in diabetic ulcers. *Diabetes Care* 21:1714-1719.
- Guigliano, F., Maiorino, M, Bellastela, G., Gicchvio, M., Guigliano, D., Espisto, K (2010). Determinants of exrectile dysfunction in type 2 diabetes *Int J Impot Res.* 22 (3):204-209
- Isidori, A.M., Buvat, J., and Corona, G (2014); A critical analysis of the role of testosterone in erectile function from pathophysiology to treatment – a systemic review. *Eur Urol.* 65(1):99-112
- Jones, T.H., Aner, S., Behre, H.M, (2011): TIME 2 Investigators Testosterone placement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIME 2 study). *Diabetes Care.* 34(4):828-837
- Kim, S.T., and Moley, K.H. (2008): Paternal effect on embryo quality in diabetic micl is related to poor sperm quality and associated with deacresed glucose transporter expression. *Reproduction* (136):313-322.
- La Vignera, S., Di Mauro, M., Condorelli, R., La Rosa, S., Vicari, E. (2009): Diabetes worsens spermatic oxidative stress associated with inflammation of male accessory sex glands. *Clin ter* (160):363-366.
- La Vignera, S., Condorelli, R.A., Di Mauro, M., D'Agata, R., Vicari, E., and Calogero, A.E., (2011): Seminal vesicles and diabetic neuropathy ultrasound evaluation. *J. Androl* (32):478-483.
- Lu, C.C., Jiann, B.P., Sun, C.C., Lam, H.C., Chu, C.H., and Lee, J.K. (2009): Association of glycemic control with risk of erectile dysfunction in men with type 2 diabetes. *J Sex Med.* 6(6):1719-1728

- Malavige, L.S., and Levy, J.C. (2009): Erectile dysfunction in diabetes mellitus. *J. Sex Med.* 6(5): 1232-1247.
- Rolo, A.P. and Palmeria, C.M. (2006): Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol.* 212(2): 167-78
- Rosen, R.C., Wing, R.R., Schneider, S., (2009): Erectile dysfunction in Type 2 diabetic men: relationship to exercise fitness and cardiovascular risk factors in look AHEAD trials *J. Sex Med.* 6(5): 1414-1422
- Seftel, A.D., Sun, P., Swindle, R. (2004): The prevalence of hypertension, hyperlipidaemia, diabetes mellitus and depression in men with erectile dysfunction. *J. Urol* 171 (6 pt 1):2341-2345.
- Vinik, A.I., Maser, R.E., Mitchell, B.D., Freeman, R. (2003): Diabetic autonomic neuropathy. *Diabetes Care* 26(5):1553-1579
- Wessels, H., Penson, D.F., Cleary, P. (2011): Diabetes Control and complication trial/epidemiology of diabetes interventions and complications research group. Effect of intensive glycemic therapy on erectile function in men with type 1 diabetes. *J. urol.* 185(5):1828-1834
- Wild S., Roglic, G., Green, A., Sicree, R., King, H, (2004): Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27(5):1047-1053

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