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Estimation of Serum Cortisol in Type-2 Diabetic Patients under Control

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Abstract: Background: Cortisol is a steroid hormone, in the glucocorticoid class of hormones that functions to increase blood sugar through gluconeogenesis. Diabetogenic hormones such as cortisol play a role in the complications of diabetes mellitus and abnormally high cortisol exerts anti-insulin effect that leads to reduction in insulin sensitivity which is a major factor contributing to development of type2 diabetes mellitus. **Aim**: This study aimed at evaluating the serum level of Cortisol in type-2 diabetes mellitus patients. **Materials and Methods:** Samples from thirty (30) confirmed drug treated type2 diabetic subjects attending clinic at federal teaching hospital Abakaliki and fifteen (15) non-diabetic volunteers were analyzed of serum cortisol level using enzyme linked immunosorbent assay (ELISA) method. The data generated was analyzed using statistical software IBM SPSS version 20.0. The results were expressed as mean$_{\pm}$ SEM. Data from this study was analyzed using T-paired test analysis. **Results and Discussion:** This study showed that the serum cortisol level was normal and had no significant difference (p=0.13) in treated type2 diabetic subjects (2.94$\pm$0.73nmol/l) compared to that of the control subjects (1.83$\pm$0.06). Also, the mean levels of serum cortisol in comparison with the duration of treatment showed that type2 diabetic subjects with treatment duration of 1-2years showed higher significant difference (P=0.03) in their mean levels of cortisol (2.51$\pm$0.54) compared to subjects with treatment duration of 6-10years (0.82$\pm$0.05). **Conclusion: S**erum cortisol level in a well drug treated type2 diabetic subjects was observed to be normal with an inverse correlation with duration of treatment.

Keywords: Cortisol, Diabetic Mellitus, Insulin, Treatment, Type-2

Introduction

Diabetes is a major threat to global public health, characterized by chronic hyperglycemia resulting from impaired insulin action/secretion or both and it is classified into two major categories; type-1 and type-2 [1]. The number of diabetic patients is rapidly increasing all over the world, according to a projection of the International Diabetes Federation

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(IDF), 366 million people had diabetes in 2011, which will increase to 552 million by 2030 [2].

There is however, compelling data to show an increasing incidence and prevalence of diabetes mellitus in the continent [3]. The estimated prevalence of diabetes in Africa is 1% in rural areas, and ranges from 5% to 7% in urban sub-Saharan Africa [3]. The prevalence of diabetes mellitus in Nigeria has increased from 2.2% as reported by Akinkugbe in 1997 from a national survey to 5.0% by 2013 estimates of the International Diabetes Federation [4].
The United Nations (UN) recognizes diabetes as a chronic debilitating and costly disease associated with severe complications, which poses severe risks to families, member states and the entire world; and serious challenges to the achievement of internationally agreed developmental goals, including the Millennium Development Goals (MDGs) [5].

According to the World Health Organization (WHO), there are approximately 347 million diabetics worldwide, the number of diabetics had been doubled in the last few years and WHO projects reported that, diabetes death will increase by two thirds between 2008 and 2030 [6]

Type 2 diabetes mellitus also referred to as adult-onset diabetes, is the result of interaction between genetic and environmental factors, leading to heterogeneous and progressive pancreatic β-cell dysfunction [7]. Overweight and obesity are major contributors to the development of insulin resistance and impaired glucose tolerance. The inability of β cells to secrete enough insulin produces type 2 diabetes. Glucose concentration in the blood is maintained within a fairly narrow interval under diverse conditions (feeding, fasting, or severe exercise) by insulin, glucagon, cortisol and other hormonal actions. The effects, mechanism of action, counter regulatory action of these hormones will be studied.

Cortisol is a steroid hormone; in the glucocorticoid class of hormones, it is sometimes called the stress hormone because its level in the body spike during times of high stress. It is produced by the zona fasciculate of the adrenal cortex within the adrenal gland [8]. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system and to aid in the metabolism of fat, protein and carbohydrates [9].

Cortisol may play an important role in the development of type 2 diabetes, it is possible that even small increase in cortisol within the range of normal, may have a detrimental influence by worsening diabetes and increasing complications [10]. Cross-sectional studies of the association of diurnal cortisol and diabetes suggest a flattening of the diurnal cortisol curve in individuals with diabetes compared to those without diabetes. In a small study of 30 subjects, diabetic individuals showed a blunted cortisol awakening response (CAR) compared to non-diabetic individuals [11]. This finding was confirmed by the Cooperative Research in the Region of Augsburg (KORA)-F3 study, which also found higher bedtime cortisol levels in participants with diabetes compared to those without diabetes, as well as a trend toward lower wake-up cortisol levels [12]. High levels of cortisol decrease metabolism of glucose and increases mobilization and metabolism of fats. Decreased metabolism of glucose contributes to increased blood glucose levels, and increase blood fat level contributes to insulin resistance. Increased levels of blood glucose and blood fats are classic symptoms of diabetes. When blood cortisol levels are too high, insulin will not lower blood sugar [13]. Based on this model, any long-term condition of excess stress can potentially increase the risk of diabetes and decrease the effectiveness of insulin treatment of diabetes. To effectively treat diabetes, sources of stress or inflammation, including those related to the diet or digestion should be addressed [14]. There are conflicting reports about the level of cortisol and the chances of developing diabetes mellitus [10, 11]. This study therefore aimed at evaluating the serum level of Cortisol in type-2 diabetes mellitus patients.

Materials nd Method

Study Area

Participants for this study were drawn from the General out Patient Department of Teaching Hospital, Abakaliki.

Study Population

A total of thirty (30) type2 diabetic subjects and fifteen (15) non-diabetic participants within the ages of 25 to 80years, were recruited for this study. The subjects included for this study were confirmed type-2 diabetic subjects attending Federal Teaching Hospital, Abakaliki Endocrinology unit. Apparently healthy individuals who are non-diabetic were used as control.

Ethical Approval. The Research and Ethical Committee of Federal teaching hospital Abakaliki/Ebonyi State University, gave ethical clearance for the study. Informed written consent was obtained from form the subjects.

Exclusion criteria

Participants who are below 25years and above 80years were excluded. Subjects who took medication before coming to the hospital on the day of sample collection. Participants with type I diabetic

mellitus, proliferative retinopathy, severe neuropathy and highly stressed subjects were excluded in this study.

Statistical method
The data were analyzed using Statistical Package for Social Science IBM SPSS-package version 20.0. The mean serum cortisol level was compared using student paired T-test and the results were expressed in Mean±SEM.

Sample collection
The Samples were collected in Ebonyi in eastern Nigeria. Analysis of the samples was performed in the Genbuk biomedical Chemical Pathology Laboratory of mgbukobe, Ebonyi state, Nigeria. On arrival of the participants to the hospital, they were randomly selected and their consents were sought via the informed consent form. Blood samples were collected in the morning from the subjects. A 5ml syringe and needle was used to collect blood sample (5ml) from the participants once by venipuncture and then placed into plain (red capped) container for the evaluation of the serum cortisol level.

Sample preparation
Blood samples collected from the patients that gave consent were taken to the laboratory within two (2) hours of collection via road transport where they were centrifuged at 3000r.p.m. (revolution per minute) then the serum was separated using a pasture pipette. After sample separation, the samples were stored at a freezing temperature of 4C.

Sample analysis
Cortisol can be estimated from blood, serum, faces, sweat, urine, hair and saliva. There is usually a good correlation between cortisol concentrations in saliva and serum. For the purpose of this study, serum was used; we employ Cortisol Enzyme-Linked Immunosorbent Assay (ELISA). This method is principled on the competition between cortisol and cortisol acetylcholinesterase (AChE) conjugate (cortisol tracer) for a rationed number of cortisol-specific mouse monoclonal antibody binding sites [17, 18]. Measurement of Cortisol (human Cortisol ELISA Kit)

Procedure
50ul of standard was added to the standard wells. To the sample well, sample dilution of 40ul was added followed by 10ul of sample (serum).100ul of Horse radish peroxidase (HRP)-conjugate reagent was added to each well except blank well. The plate was closed with closure plate membrane and incubated for 60minutes at 37C. A twenty (20) fold wash solution (washing buffer) was configurated and used in washing the wells after the closure plate membrane have been uncovered upon the completion of the incubation time. The washing was repeated for five times and then dried by pat. 50ul of chromogen solution A and chromogen solution B was added to each well with the light preservation evaded for 15 minutes at 37C. 50ul of stop solution was added to each well and this produced a color change (from blue to yellow color). The blank was taken as zero, while the absorbance of the preparation was read at 450nm after adding stop solution and within 15minutes.

Result
Comparison of means of demographic variables in type-2 diabetic and control subjects;
The total number of study subject were forty-five (45) as presented in table-1. Thirty were drug treated type2 diabetic subjects of which 18(60%) were males while 12(40%) were females. The remaining thirty were control subjects of which 8(53%) were male while 7(47%) were females. Also, there was significant difference in the mean of age of drug treated type2 diabetic subjects and the control subjects ($p<0.05$).

Table-1
Comparison of means of demographic variables in type-2 diabetic and control subjects.

Characteristics	type2 diabetic subjects n=30	control subjects n=15	p-value	Remarks
NO of males (%)	18(60%)	8(53%)	_	_
NO of females (%)	12(40%)	7(47%)	_	_
Age (Years)	55.2±2.60	39.6±2.30	p<0.05	S*

Comparison (Mean±SEM) with control.
Statistical method; Paired T-test analysis.
S*=significant.

Comparison of serum cortisol levels of type2 diabetic and control subjects
Table-2 shows a comparison of means of serum cortisol level in drug treated type2 diabetic and control subjects. The mean serum cortisol level was normal and there was no significant difference (p=0.13) compared to control subjects.

Table-2
Comparison of serum cortisol levels of type2 diabetic and control subjects

	Type2 diabetic subjects n =30	control subjects n=15	p-value	Remarks
Cortisol(nmol/l)	2.94±0.73	1.83±0.06	P=0.13	NS*

Comparison (Mean±SEM) with control.
Statistical method; Paired T-test analysis.
NS*= No-significance.

Mean levels of serum cortisol in comparison with duration of treatment in type2 diabetic subjects.
Table 3 shows the comparison of the mean levels of serum cortisol based on different durations of medication. Subjects that have received the medication for the duration of 1-2years showed no significant difference (P=0.5) when compared to subjects that have received treatment for the duration of 2-4years. However, subjects that have received treatment for duration of 2-4years when compared those that have received treatment for the period of 6-10years showed significant difference (P=0.03). Also, the comparison of subjects with the treatment duration of 1-2years against those of 6-10years showed a significant difference (P=0.03).

Table-3
Mean levels of serum cortisol in comparison with duration of treatment in type2 diabetic subjects.

	Duration of (1-2) years n =15	Duration of (2-4) years n =6	P-value	Remark
cortisol (nmol/L)	2.51± 0.54	2.03± 0.10	P=0.5	NS*
	Duration of (2-4) years n=6	Duration of (6-10) years n=9		
Cortisol	2.03±0.10	0.82± 0.05	P=0.03	S* (nmol/l)
	Duration of (1-2) years	Duration of (6-10) years		
Cortisol	2.51± 0.54	0.82± 0.05	P=0.03	S* (nmol/l)

Comparism (mean±SEM)
Statistical method;Paired T-test analysis
S*=Significant.
NS*=non-significant.

Discussion
This study demonstrated the effect of duration of medication on the serum cortisol level of type2 diabetes mellitus subjects.

Serum cortisol level in type2 diabetic subjects was found to be normal with no significant difference (p=0.13) compared to control subjects. The normal cortisol level observed in type2 diabetic subjects receiving medication is perceived to be due to the antidiabetic medications administered to the them in the hospital which includes; pioglitazone (Actos), metformin(glucophage), Glibenclamide (daonil), insulin, canagliflozin.

However, comparison of the mean of serum cortisol level based of the different duration of treatment indicated that although all the samples were all normal but the subjects with Treatment duration of 1-2years had a significantly higher (P=0.03) mean serum cortisol level (2.51±0.54) compared to subjects with treatment duration of 6-10years(0.82±0.05) although there was no significant difference (P=0.5) in the mean serum cortisol level of subjects with treatment duration of 1-2years (2.51±0,54) when compared to subjects with treatment duration 2-4years (2.03±0.10). This shows an inverse correlation between serum cortisol level and treatment duration in type2 diabetic subjects.

The findings from this study were consistent with the invivo studies of [15], which showed that pioglitazone (Actos) a member of the thiazolidinedione family and metformin a member of the biguanide family contributes to maintenance of normal cortisol level in type2 diabetes mellitus. This finding was also consistent with invivo study of [16] (Kota Nishihama, *et al*, 2018) which demonstrated that canagliflozin a member of the sodium glucose transporter2 (SGLT2) inhibitors contributes to reduced serum cortisol levels in hypercortisolemic type2 diabetic subject over long duration of administration.

There are limitations to this study being a baseline study in this part of the country for further research, only small groups were studied. However, the main findings of the study were consistent, that is, serum cortisol level in type2 diabetic subjects receiving drug treatment appropriately was found to be normal

with no significant difference compared to control subjects and also longer duration of medication produces a lowering effect on serum cortisol level of type2 diabetic subjects.

Conclusion

The serum cortisol level in a well drug treated type2 diabetic subjects was normal with an inverse correlation with duration of treatment.

Authors' Contributions

This work was carried out in collaboration between all Authors read and approved the final manuscript.

Conflict of Interest

Authors declare no conflicts of interest.

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