British Journal of Medicine & Medical Research
9(7): 1-7, 2015, Article no.BJMMR.19286
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Serum and Salivary Cortisol Levels in Diabetics Attending University of Calabar Teaching Hospital, Calabar, Nigeria

Bassey, Iya Eze[1*], Gali, Rebecca Mtaku[2], Ogbolu, Ifechukwude Augustina[1], Essien, Okon Ekwere[3], Usoro, Chinyere Adanna Opara[2] and Emeribe, Anthony Uchenna[1]

[1]Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.
[2]Department of Medical Laboratory Science, College of Medical Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria.
[3]Department of Internal Medicine, Faculty of Medicine and Dentistry, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors UCAO and BIE initiated and designed the research, Authors OIA and BIE did the analysis and interpretation of data. Authors BIE, OIA and EAU wrote initial draft manuscript while authors GRM and EOE critically reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/19286
Editor(s):
(1) Rashmi K. Ambasta, Delhi Technological University, Delhi, India.
Reviewers:
(1) Jaspinder Kaur, Department of Obstetrics and Gynaecology, Punjab Institute of Medical Sciences, Jalandhar, India.
(2) Anonymous, Benghazi University, Libya.
Complete Peer review History: http://sciencedomain.org/review-history/10092

Original Research Article

Received 1st June 2015
Accepted 25th June 2015
Published 8th July 2015

ABSTRACT

Aims: To investigate if salivary cortisol can be used as an alternative to serum cortisol in the management of diabetes mellitus.
Study Design: The design of the study was cross sectional.
Place and Duration of Study: Diabetic Clinic of the Department of Internal Medicine and the Department of Chemical Pathology, University of Calabar Teaching Hospital between June 2009 and July 2010.

**Corresponding author: Email: iyantui@yahoo.com;*

Methodology: Fifty five (55) type II diabetic patients and thirty three (33) non-diabetic controls (45 men, 43 women; age range 30 - 69 years) were recruited for this study. The levels of salivary cortisol, serum cortisol, fasting plasma glucose and glycated haemoglobin were determined. Serum and salivary cortisol was determined using enzyme immunoassay; fasting plasma glucose using colorimetric method and glycated haemoglobin using cation-exchange resin separation method.
Results: The mean serum and salivary cortisol levels were significantly higher (p=0.000) in diabetics as compared to the controls. The salivary cortisol was about 70% lower than the serum cortisol in each group. There was no significant difference between the percentage difference between the serum and salivary cortisol levels in both the diabetics and controls. There was a significant positive correlation between serum cortisol and salivary cortisol in both the diabetic subjects (r = 0.362, p=0.007) and controls (r =0.406, p= 0.019).
Conclusion: Cortisol levels in saliva reflected those in serum in both diabetics and controls therefore salivary cortisol may be used in place of serum cortisol in the management of diabetes.

Keywords: Cortisol; type II diabetes; saliva; serum.

1. INTRODUCTION

A high prevalence of subclinical hypercortisolism has been suggested in patients with type II diabetes mellitus with poor metabolic control and several observations have suggested that in type II diabetes patients, subclinical hypercortisolism may be more frequent than previously expected [1]. Elevated levels of cortisol have been reported in type 2 diabetics [2].

The important role cortisol may play in the development of type 2 diabetes has renewed interest in adrenal function in diabetic conditions. It is probable that small increases in cortisol levels, even within the range of normal, may cause a deteriorating effect on diabetes itself, thereby increasing the risk of diabetes-related complications [3]. Diabetes (mainly type 2 diabetes) has been shown to be associated with cortisol levels because hypothalamic-pituitary-adrenal activity is enhanced in patients with diabetes complications and the degree of cortisol secretion has been shown to be related to the presence and number of diabetes complications [4]. High levels of cortisol in the body increase blood glucose levels through glycogenolysis and also increase mobilization and breakdown of blood lipids. This leads to hyperglycaemia and hyperlipidaemia. Hyper-lipidaemia contributes to insulin resistance. Hyperglycemia and hyperlipidaemia are classic symptoms of diabetes mellitus. An elevated level of cortisol also antagonizes the effect of insulin on blood glucose [5].

Studies have shown that the compliance to tests and therefore good management of diabetes is reduced due to anxiety, fear and weariness of diabetic patients over repeated blood sampling for tests [6]. Anxiety has been linked to worse glycaemic control in diabetics. Therefore, less invasive sampling methods may reduce anxiety and indirectly help in achieving improved diabetes control.

It has been shown that salivary cortisol measurement can be an alternative to serum cortisol as a marker for adrenocortical function following insulin tolerance test, corticotrophin-releasing-hormone stimulation and adreno-corticotrophin hormone stimulation [7]. Salivary cortisol testing is non-invasive, easy to perform, accurate, convenient and cost effective. Salivary steroid levels can reflect the circulating level of free steroid rather than total circulating levels, which are confounded by the presence of circulating high affinity binding proteins [8,9]. It offers the attractive option of a stress-free and real-time repeated sampling where blood collection is either difficult or undesirable [6]. The aim of this study therefore was to determine if salivary cortisol can be used as an alternative to serum cortisol in the management of diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Subject Selection

Eighty-eight subjects of Nigerian origin were selected in this study. Fifty-five (55) of these were confirmed type II diabetic patients (males and females) attending the University of Calabar Teaching Hospital (UCTH) Diabetic clinic. They were recruited as test subjects. Thirty-three apparently healthy non-diabetic subjects from Calabar metropolis were recruited as controls.

This was a cross sectional study and consecutive sampling method was used. Ethical approval was obtained from the ethical committee of the University of Calabar Teaching Hospital. The purpose and nature of the research was explained to the participants and informed consent was obtained from them.

2.1.1 Inclusion criteria

All the subjects recruited for the study were 30years of age and above.

2.1.2 Exclusion criteria

Type 1 diabetics, terminally ill type 2 diabetics, as well as anyone who did not consent to participating in the study were excluded from the study.

2.1.3 Sample size calculation

The number of samples used in this research was determined using the formular below

$$N = \frac{Z\alpha^2 pq}{d^2}$$

where

N = desired sample size
Zα = the α level of the coefficient interval at 95% (1.96)
p = proportion of non-occurrence
q = (1-p) proportion of non occurrence
d= precision

Substituting the expected occurrence of p= 2.2% i.e. 0.022 from the prevalence obtained from the Diabetes Association of Nigeria [10] we have

$$N = \frac{1.96^2 \times 0.022(1 - 0.022)}{(0.05)^2} = 33$$

After calculating the sample size, a response rate of 80% was assumed therefore the actual sample size was 33/0.8 = 41 cases.

2.2 Sample Collection

Fasting blood samples were collected between 8.00am and 9.00am owing to diurnal variation of cortisol secretion. With minimal constriction and stasis, 6 milliliters of venous blood was aseptically collected by venepuncture from each subject. Two milliliters of blood from each subject was dispensed into a tri-potassium ethylene diamine tetra-acetic acid (EDTA) bottle for glycated hemoglobin estimation and 2 ml into a fluoride oxalate bottle for glucose estimation. The remaining 2 milliliters of blood was collected into plain containers for serum extraction, which was used for cortisol assay. The saliva samples were collected into sterile wide mouth containers between 7.30 -9.00 am after an overnight fast prior to the day of the collection. The subjects were instructed to rinse their mouth with clean water severally and discard the water. Then the subjects spat in the container several times until about 1 ml of saliva was obtained. It was then spun at 3000 rpm for 5 minutes and supernatant obtained. Serum and saliva not used immediately was kept frozen till used.

2.3 Glucose Analysis

Fasting plasma glucose was estimated using a test kit of glucose-oxidase-peroxidase method produced by Giesse Diagnostics Inc., Italy. Normal range 3.5-5.5 mmol/L.

2.4 Glycated Hemoglobin Estimation

Glycated hemoglobin was estimated using kits by Teco diagnostics, Anaheim, USA. The kit employed a weak binding cation-exchange resin for the rapid separation of glycohemoglobin (fast fraction) from non-glycated hemoglobin (HbA_o). Normal range: <6%.

2.5 Serum cortisol Estimation

Serum and salivary cortisol was determined by the DRG cortisol enzyme immunoassay kit. It was obtained from DRG international Inc. USA. The DRG cortisol ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. Absorbance was read at 450nm with an ELISA microtiter plate reader. Normal range of serum cortisol – 138 nmol/L–635 nmol/L.

2.6 Statistical Analysis

This was done using the PAWstatistic 18, a statistical package from SPSS Inc, California, USA. The results were expressed as Mean ± SD. The data was analyzed by Student's t-test and Analysis of variance (ANOVA) followed by a post hoc test using least significant difference (LSD). The level of significance was set at 95% confidence interval, where *P*-value less than .05 ($P < .05$) was considered as statistically significant. Correlation was done using Pearson's correlation. Graphs were done using Microsoft excel 2007 version.

2.7 Definition of Terms

Good glycaemic control: was defined as HbA1c value of < 7% in the diabetic patients.

Poor glycaemic control: was defined as HbA1c value of ≥7% in the diabetic patients [11].

3. RESULTS AND DISCUSSION

3.1 Results

A comparison of the mean values of fasting plasma glucose, glycated haemoglobin and serum and salivary cortisol levels in diabetics and control subjects showed that the mean fasting plasma glucose, glycated haemoglobin and serum and salivary cortisol levels were significantly (*P* = .000) higher in diabetics when compared to the control subjects. There was no significant difference between the percentage difference between the serum and salivary cortisol levels in both the diabetics and controls Table 1.

Table 2 shows a comparison of fasting plasma glucose, glycated haemoglobin, serum and salivary cortisol in diabetics and controls based on glycaemic control. The mean fasting plasma glucose, glycated haemoglobin and serum cortisol levels were significantly lower (*P* = .000) in controls when compared with the two groups of diabetics. A similar pattern was also observed in the salivary cortisol (*P* = .006). The diabetics with poor glycemic control had the highest levels of mean fasting plasma glucose (FPG) and glycated haemoglobin. However, serum and salivary cortisol levels between the diabetics with poor glycaemic control and those with good glycaemic control were similar. There was a significant positive correlation between serum cortisol and salivary cortisol in both the diabetic subjects (r = 0.362, P = .007) and controls (r =0.406, P = .019) (Figs. 1,2).

Table 1. Comparison of age, fasting plasma glucose, glycated haemoglobin, serum and salivary cortisol in diabetics and non diabetics

Parameter	Diabetics	Non diabetics	Calc. t-value	Crit. t-value	p-value
Age (year)	47.2±10.10	44.8±10.65	1.076	2.00	0.285
Fasting plasma glucose (mmol/L)	8.42±3.30	4.25±0.53	7.187	2.00	0.000
HbA1c (%)	7.96±1.78	5.04±0.62	9.082	2.00	0.000
Serum cortisol (nmol/L)	442.9±179.68	296.3±88.36	5.110	2.00	0.000
Salivary cortisol (nmol/L)	108.6±32.32	88.4±17.59	3.800	2.00	0.000
% difference between serum and salivary cortisol	72.6±11.70	68.8±12.71	1.397	2.00	0.167
n	55	33			

Mean ± SD

Table 2. Comparison of fasting plasma glucose, glycated haemoglobin and serum and salivary cortisol in diabetics and non diabetics

Parameter	Diabetics with poor glycaemic control	Diabetics with good glycaemic control	Non-diabetics	Calc. F value	Crit. F value	p-value
Fasting plasma glucose (mmol/L)	9.59±3.45*#	6.18±1.18	4.25±0.53	47.251	3.103	0.000
HbA1c (%)	8.91±1.38*#	6.15±0.70	5.04±0.62	130.427	3.103	0.000
Serum cortisol (nmol/L)	438.9±172.10#	450.5±197.91#	296.3±88.36	9.500	3.103	0.000
Salivary cortisol (nmol/L)	107.7±31.52#	109.1±33.16#	88.4±17.59	5.433	3.103	0.006
n	36	19	33			

Mean ± SD

*Key: *- higher than that of diabetics with good glycaemic control;*
 # - higher than that of controls

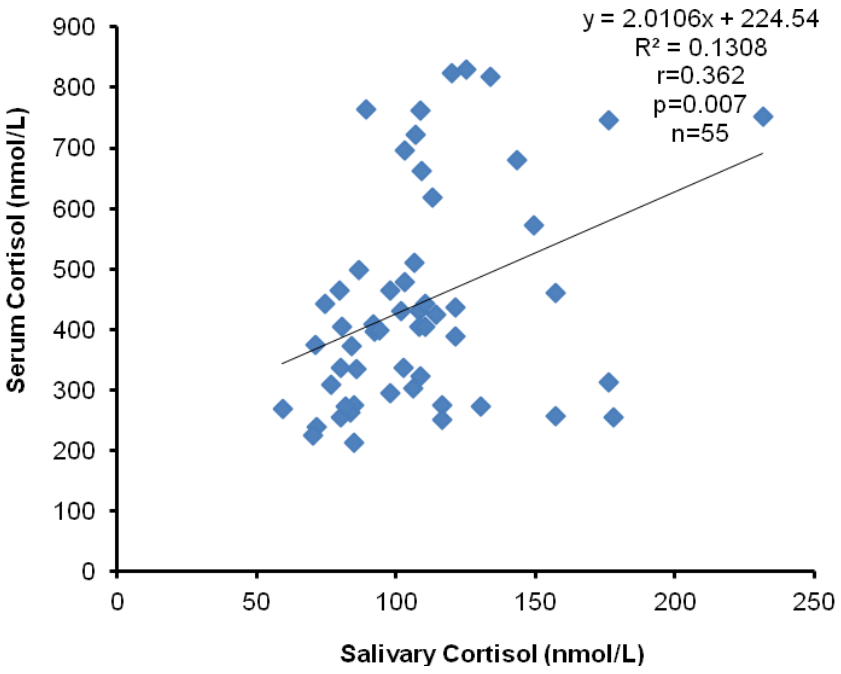

Fig. 1. Correlation plot of serum cortisol against salivary cortisol in diabetics

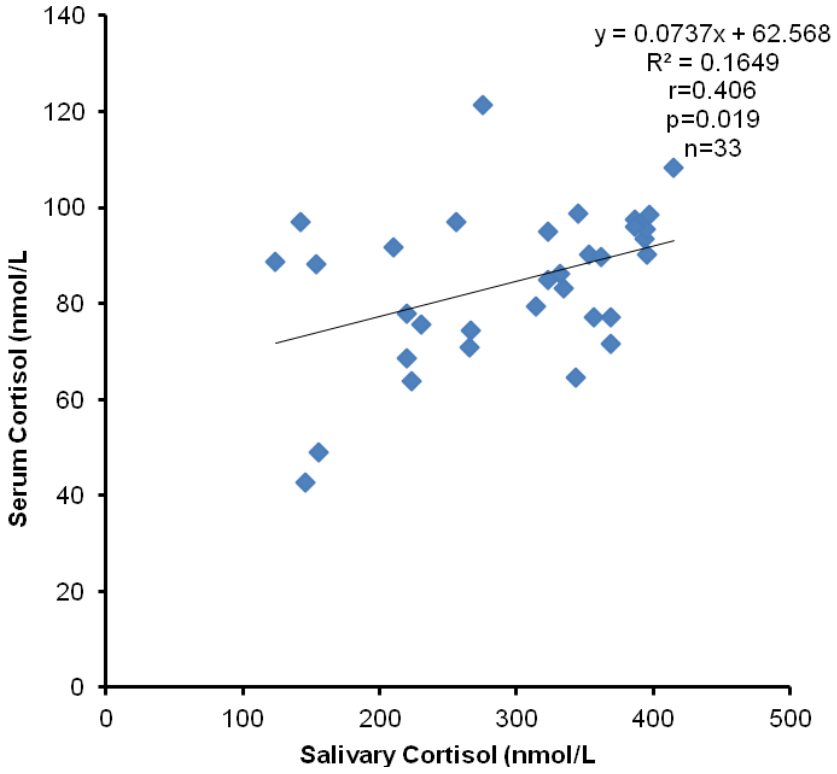

Fig. 2. Correlation plot of serum cortisol against salivary cortisol in non-diabetics

3.2 Discussion

In our study, the mean levels of both serum and salivary cortisol were significantly higher in type 2 diabetics when compared with controls. Cortisol is a glucocorticoid therefore increase in cortisol levels induces hyperglycemia. Its role in diabetes mellitus may however be adverse as it tends to sustain hyperglycemia leading to diabetes-related complications. The values found in this study indicate that cortisol may be a contributor to the diabetic condition. This agrees with observations by Chiodini et al. [12] who reported that the degree of cortisol secretion as reflected by F24 was directly associated with both the type 2 diabetes and number of complications. The increased levels of cortisol in saliva of the diabetics is reflective of the increased levels of serum cortisol concentration in these patients.

Surprising however was the observation that there was no significant difference in the mean serum or salivary cortisol levels between poorly controlled diabetics and diabetics with good glycaemic control. This may imply that in people with diabetes there is altered cortisol secretion and/or metabolism irrespective of their glycaemic status. Findings by Oltmanns et al. [13] differ from those in this study. They reported that HbA1C was directly associated with cortisol secretion in type 2 diabetic subjects with normal HPA activity.

The salivary cortisol was about 70% lower than the serum cortisol in each group, this may be because salivary cortisol reflects or estimates free cortisol levels [14] rather total serum cortisol which was what was estimated in this study. Despite, this difference, the concentration patterns for serum and salivary cortisol in both diabetic groups and controls were similar. The percentage difference between serum and salivary cortisol in both groups did not vary significantly. There were also significant positive correlations between serum cortisol and salivary cortisol in both groups. This shows a consistency in the patterns of similarity observed in the groups with normal cortisol levels as well as those with high cortisol levels. This finding is similar to the observations made by Trilck et al [15] in the use of salivary cortisol in diagnosis of Cushing's syndrome and Marcus-Perlman et al [16] in the use of salivary cortisol in low dose ACTH test. The limitation of this research is that we did not estimate free serum cortisol. This might have given a clearer picture.

4. CONCLUSION

Cortisol levels in saliva reflected those in serum in both diabetics and controls therefore salivary cortisol may be used in place of serum cortisol in the management of diabetes.

CONSENT

All authors declare that written informed consent was obtained from the patient to participate in the study and publish the results.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Catargi B, Rigalleau V, Poussin A, Ronci-Chaix N, Bex V, Vergnot V, Gin H, Roger P, Tabarin A. Occult Cushing's syndrome in Type-2 Diabetes. J Clin Endocrinol Metab. 2003;88:5808-5813.
2. Bassey IE, Ogbolu IA, Gali RM, Essien OE, Usoro CAO. The effect of glycemic control on cortisol levels in Type II diabetics in University of Calabar Teaching Hospital. Bo Med J. 2015;12(1):9–15.
3. Liebman T. The role of cortisol and abdominal obesity in the epidemic of type 2 diabetes; 2010 Assessed: 2011 Jul 21. Available:http://www.kon.org/urc/v9/liebman.html
4. Constanti A, Bartke A, Khardori R, editors. Basic Endocrinology, 4th ed. Amsterdam: Gordons and Breach Publishing Group; 2005.
5. Cartmell JW. Cortisol and diabetes. BMJ. 2006;177:505-510.
6. Shlomowitz A, Feher MD. Anxiety associated with self monitoring of capillary blood glucose. Br J Diabetes Vasc Dis. 2014;14:60-63.
7. Lewis JG. Steroid Analysis in Saliva: An overview. Clin Biochem Rev. 2006;27(3): 139-146.

8. Vining RF, McGinley RA. The measurement of hormones in saliva: Possibilities and pitfalls. J Steroid Biochem. 1987;27(1-3):81-94.
9. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic--pituitary--adrenal axis activity. Clin Endocrinol (Oxf). 2005; 63(3):336-41.
10. Diabetes association of Nigeria. National clinical guideline for diabetes management in Nigeria. Abuja: DAN; 2011.
11. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care. 2011; 34:e61-e99.
12. Chiodini I, Adda G, Scillitani A, Coletti F, Morelli V, Di Lembo S, et al. Cortisol secretion in patients with type 2 diabetes: Relationship with chronic complications. Diabetes Care. 2007;30:83-88.
13. Oltmanns KM, Dodt B, Raspe HH, Schweiger U, Born J, Fehm H, et al. Cortisol correlates with metabolic disturbances in a population study of type 2 diabetic patients. Eur J Endocrinol. 2006; 154:325-331.
14. Duplessis C, Rascona D, Cullum M, Yeung E. Salivary and free serum cortisol evaluation. Mil Med. 2010;175(5):340-6.
15. Trilck M, Flitsch J, Lüdecke DK, Jung R, Petersenn S. Salivary cortisol measurement--a reliable method for the diagnosis of Cushing's syndrome. Exp Clin Endocrinol Diabetes. 2005;113(4):225-30.
16. Marcus-Perlman Y, Tordjman K, Greenman Y, Limor R, Shenkerman G, Osher E, et al. Low-dose ACTH (1 microg) salivary test: a potential alternative to the classical blood test. Clin Endocrinol (Oxf). 2006;64(2):215-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10092