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Is Saliva an Alternative Non-Invasive Sample for the Estimation of Protein Profile amongst Diabetics and Gender-based Diagnostics?

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Abstract

Introduction

Protein profile is an important investigation utilized for the diagnosis and management of arrays of diseases such as diabetes mellitus. The measurement of protein profile is basically carried out through invasive sample blood which inflicts pain and is financially cumbersome. The study is aimed at generating an alternative non-invasive sample such as saliva for proteins analysis.

Materials and Methods

A total of 100 subjects were recruited for the study; 50 subjects diagnosed with type 2 diabetes mellitus and 50 apparently healthy control subjects. Protein profile incudes total protein, albumin, and globulin and albumin/globulin ratio. Total protein and albumin were estimated using biuret and bromocresol green methods respectively. Globulin and albumin/globulin were estimated mathematically.

Results

The study showed that there was no correlation ($P>0.05$) between saliva and serum protein profiles amongst controls and diabetes subjects. However, significant correlation ($P<0.05$) was observed for globulin and albumin/globulin ratio for diabetic subject with glucose level above threshold level. A comparison between diabetics and controls showed a significant decrease ($P<0.05$) in serum total protein and globulin, while an increase for serum albumin/globulin ratio. Salivary protein profile and gender influence on serum and salivary protein profiles exhibited a non-significant difference ($P<0.05$).

Conclusion

The findings showed that serum protein profiles is an important panel of laboratory investigation for the management of diabetes mellitus. Also, gender difference in customized diagnostics is of no clinical importance.

Keywords: Protein Profiles; Saliva; Diabetics; Threshold

Background Studies

Medical Laboratory gives direction to proper diagnosis and management of most diseased conditions utilizing various body fluids and tissues. This depends strongly on the choice of sample. Invasive method of sample collection involves the active puncture of the body cells or tissues, which in turn inflict pain on the subjects. Non-invasive method involves passive form of sample collection that does not involve any form puncture and pain. Examples of invasive samples are blood, vagina swabs, CSF, whereas those non-invasive samples are urine, stool, saliva etc. Blood collection is accompanied with painful sensation and sometime leads to injury and infection such as HIV/AIDS. Also, the tools used in blood collection are a multimillion naira industry involving massive investment and monetary implications. Unlike blood, saliva collection is simple, timely and very cost effective. Other advantages of saliva are the non-invasive nature, availability and the avoidance of pain or injury.

Blood is the fluid that the heart circulates through the body's arteries, capillaries, and veins [1]. It is mainly composed of plasma, and cells [2]. Saliva is a clear, alkaline, viscous fluid secreted from the parotid, sub maxillary, sublingual, and smaller mucous glands of the mouth. Approximately 0.8 to 1.2 L of saliva is produced daily. Constituents such as viruses, bacteria, proteins, hormones, therapeutic drugs, and drugs of abuse have been measured in saliva.

Protein profile is a panel of tests used to access the concentrations of its various components for clinical decision making. Protein profiles include total protein, albumin, globulin and albumin/globulin ratio. Protein profile is essential for the determination of the liver status, immune status, buffering capacity, water status, molecule transport status and nutritional status.

Albumin is a globular protein with a molecular weight of about 65000 and is synthesized by the liver [3]. Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. It is composed of four major groups that can be identified: gamma globulins, beta globulins, alpha-2 globulins, and alpha-1 globulins. Globulin to an extent reflectas the body's defense status [4,5].

The World Health Organization (WHO) defined Diabetes Mellitus on the basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140 mg/dl) or greater than 11.1 mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion, even if the fasting concentration is normal. The definition stands if only the laboratory investigation carried out more than twice still give diabetic range values [6].

A study on the correlation between plasma and salivary protein profile is still nascent with paucity of literatures. The aim of the present study is to explore the possible use of saliva as choice sample in protein profile analysis and the need to integrate protein profiles in the growing field of customized diagnostics.

Materials and Methods

Study area

The subjects of the study were recruited from Federal Medical Centre, Yenagoa and its outreach post at Otuoke and the General Hospital Sagbama. The three locations represent the three senatorial districts of Bayelsa State. Bayelsa was created out from the old Rivers State in 1996 during the military administration of Gen. Sani Abacha of blessed memory. Bayelsa state is located within Latitude 40 151 North and Latitude 50 and 231 South. It is also within longitude 50 221 West and 60 451 East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts. According to the 2006 census figures, Bayelsa has a population of about 1.7 million people [7].

Study population

A total of one hundred (100) subjects were utilized for the study. The study was carried out in two sets of samples. Group 1 was constituted of fifty diabetic subjects and group 2 fifty healthy non-diabetic subjects. All the subjects were between the ages of 18 and 60 years. Fasting and postprandial blood and saliva were collected from group 1. Fasting blood and saliva were collected from subjects categorized under group 2.

Ethical approval

The experimental protocol was approved by the ethics committee of the Federal Medical Center, Yenagoa, Bayelsa State. Also individual subjects were educated of the essentials of the research before samples collection. Samples were collected only from those that gave oral consent.

Selection criteria for subjects

Subjects with diabetes mellitus and those without diabetes mellitus were recruited for the study. The status of diabetes mellitus was determined as per the criteria by The Expert Committee on Diagnosis and classification of Diabetes mellitus, 1998 [8]. Patients with severe diabetic complications were excluded. Study participants with any other systemic illness or on medications other than for diabetes were excluded.

Collection of samples

Blood: Standard for phlebotomy was stringently adhered. 2 ml of blood was emptied into fluoride oxalate containers and centrifuged for 300 revolutions per minute (rpm) using the Vanguard V 6000 Centrifuge. The samples were analyzed immediately. Postprandial samples were collected from only the diabetic group two hours after normal meals.

Saliva: Salivary sample collection was done in the morning between 8.00-10.00 a.m. with study subjects sitting upright. Study subjects were instructed not to smoke or brush or eat or drink two hours prior to the time of saliva collection. In the beginning, subjects were asked to spit out initial saliva within the first thirty seconds before main collection commenced. Salivary samples were collected into a fluoride oxalate containers by spitting method. The unstimulated whole saliva in the fluoride oxalate test tubes were then spun [9-11] followed by the analysis.

Laboratory analysis

Salivary and serum total protein were estimated quantitatively using Biuret Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet), whereas salivary and serum albumin were estimated quantitatively using Bromocresol Green Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet). The procedure followed the leaflet standard operating procedure.

Salivary and serum globulin concentration was derived by subtracting vitreous albumin from vitreous total protein. The value is an estimate of serum globulin [12].

Globulin=Total Protein-Albumin

Salivary or serum A/G ratio was gotten mathematically by dividing blood or salivary albumin by blood or salivary globulin. The value is an estimate of blood or salivary albumin/globulin ratio [13].

Statistical analysis

Data were analyzed with SPSS program (SPSS Inc., Chicago, IL, USA; Version 15-21) and expressed as mean ± SE. Student t-test was used for comparing values of the measured biochemical parameters between non diabetic and diabetic subjects, p values less than 0.05 were considered significant. Correlational analysis was also used to establish an existence or non-existence of relationship between the protein profiles of the study samples Tables 1-7.

Discussion

Diabetes mellitus is a massive, growing, silent epidemic that has the potential to cripple health services in all parts of the world if appropriate caution is not taking. Diabetes mellitus is associated with serious complications of the eyes, kidneys, heart and blood vessels, and

other organ systems, which may markedly impair quality of life and shorten the patient's lifespan [14]. Protein profile is an essential investigation in the management of diabetes mellitus due to its essential roles in buffering, transportation and liver status. The study focused on the possibility of utilizing saliva as a substitute to blood for the analysis of protein profile. It went further to look at gender as a possible factor in protein profile analysis so as to enhance customized diagnostics.

Serum and salivary protein profile in diabetes mellitus

The research showed a significant decrease ($P<0.05$) in the fasting serum total protein and globulin, but an increase in A/G ratio levels in diabetics as compared with control subjects. However, the result of salivary protein of diabetics showed no significant difference with that of the controls. Also, the pearson's correlational studies utilized showed no relationship between serum and salivary protein profile of controls and that of diabetics group. Contrarily, Pearson correlation established a relationship between serum and salivary globulin and albumin/globulin ratio for group with glucose level above threshold value. In nutshell, the work established that plasma total protein, globulin and albumin/globulin ratio is affected as a result of diabetes mellitus in fasting state.

The work partly agreed with Hathama and Aymen, [15] which revealed presence of highly significant decrease in serum and highly significant increase in saliva total protein of patients with both types of diabetes mellitus. It also contrasted Ekin et al., [16] which showed no significant effect of diabetes mellitus on serum total protein. However, reports regarding salivary total protein levels in diabetics are controversial, as they have shown higher levels [11,17-20], lower levels [20], or comparable levels between diabetics and healthy non-diabetics [21-25].

The significant alteration observed in serum protein concentration can be due to any of following three changes: in the rate of their catabolism, rate of their anabolism and in the volume of distribution [26]. On the other hand it is well known that each protein has characteristic half-life in the circulation for example the half-life of albumin in normal healthy adult is approximately 20 day and in certain diseases, the half-life of the protein may be markedly altered [27]. The decrease in serum total protein in diabetes mellitus could be traced to hypervolaemia experienced in the ECF as a result of the increased osmolality of a diabetic blood. The diabetic blood is known to contain high concentration of glucose as compared to the ICF. The imbalance generates an osmotic movement of water from the region of high concentration to that of low concentration. This creates a simple gradient that enables water to move from the ICF to the ECF with ease, hence reducing the plasma total protein. This is the mechanism that results to increase thirst among diabetics.

The decrease in concentration of serum globulin could also be attributed to the increase in volume of fluid in the ECF, hence causing a great "dilutional effect". On the other hand, diabetes mellitus has the preponderance of immune-depression which creates ease acquisition of infection. Globulins as a marker of infection and inflammatory could be altered by diabetes mellitus as a proof of immune systemic compromise. Decrease in globulin levels could be a sign of several serious health conditions; renal disease, hepatic dysfunction, celiac disease, inflammatory bowel disease (IBD), acute haemolytic anaemia, agammagloblinemia and hypogammaglobulinemia. Diabetes mellitus is inseparable with immune compromise and depression [4] and decrease in globulin is a direct consequence of immune compromise [5]. Hence, diabetes mellitus is an immune problem that needs immune building therapy as part and parcel of the treatment regime.

The increase in A/G ratio is a product of decreased globulin which is a pointer to infection or inflammation [4]. The proper globulin to albumin ratio is 1:2, though 1.7-2.2 range still remains healthy. If this ratio changes to an extreme level it can cause a number of health concerns.

Effects of gender on serum and salivary protein profile in diabetes mellitus

The fact that diabetes mellitus affect both males and females is not disputable. However, the need for a biochemical profile with respect to gender is apt as gender based or customized medical regime is on the increase. Profiling biochemical parameters based on gender can help in the redesign of reference range and also facilitate evidence-based medicine. The search for customized diagnostics drove the research to profiling of Protein Profile based on gender in the saliva and plasma. The comparative analysis carried out to discriminate plasma and salivary protein profiles based on gender revealed no significant difference ($P>0.05$). This had satisfied that there is no need for a separate reference range for protein profiles in diagnosis and management of diabetes mellitus.

Results

Parameters	Salivary	Plasma
Measured	(Mean ± SD)	(Mean ± SD)
Control TP	5 ± 4	84 ± 3
FBTP (g/l)	5 ± 2	69 ± 6
2HPPTP	5 ± 3	74 ± 5
Control Albumin	2± 1	42 ± 2
Fasting Albumin (g/l)	2 ± 1	43 ± 3
2HPP Albumin (g/l)	2± 2	42 ± 4
Control Globulin	3 ± 2	42 ± 2
Fasting Globulin (g/l)	3 ± 2	26 ± 3
2HPP Globulin(g/l)	3 ± 1	34 ± 4
Control A/G Ratio	0.7 ± 0.4	1.0 ± 0.1
Fasting A/G Ratio	0.7 ± 0.3	1.7 ± 0.2
2HPP A/G Ratio	0.7 ± 0.5	1.3 ± 0.2
2HPP-2 Hour Postprandial, TP-Total Protein, FBTP-Fasting Blood Total Protein, A/G-Albumin/Globulin		

Table 1: The mean concentrations of serum and salivary protein profiles for controls and diabetes mellitus subjects.

Parameters	r	p-value	Interpretation
Total Protein	0.26	>0.05	NS
Albumin	0.32	>0.05	NS

Globulin	0.34	>0.05	NS
A/G	0.29	>0.05	NS
Ratio			
NS-NOT-SIGNIFICANT, S-SIGNIFICANT			

Table 2: The pearson's correlation between salivary and serum protein profiles of diabetic subjects n=50. showed no significant difference (p>0.05) as shown above.

Parameters	r	p-value	Interpretation
Total Protein	0.2	>0.05	NS
Albumin	0.21	>0.05	NS
Globulin	0.5	<0.05	S
Albumin/Globulin Ratio	0.41	<0.05	S
showed a significant correlation (P<0.05) for globulin and albumin/globulin ratio when serum was compared with saliva			

Table 3: the pearson's correlation between salivary and serum protein profiles of diabetic subjects with glucose level above threshold value n=19. showed a significant correlation (p<0.05) for globulin and albumin/globulin ratio when serum was compared with saliva.

Parameters Measured	Controls (Mean ± SD)	Diabetic Subjects (Mean ± SD)	P-Value	Comment
FBTP (g/l)	84 ± 3	79 ± 6	<	s
2HPPTP	79 ± 6	74 ± 5	>	ns
Albumin (g/l)	42 ± 2	43 ± 3	>	ns
2HPP Albumin (g/l)	40 ± 3	42 ± 4	>	ns
Globulin (g/l)	42 ± 2	32 ± 3	>	s
2HPP Globulin(g/l)	39 ± 5	34 ± 4	>	ns
A/G Ratio	±	1.4 ± 0.2	>	s
2HPP A/G	1.1 ± 0.3	1.3± 0.2	>	ns

Table 4: Comparison of serum protein profiles between controls and diabetes mellitus patients.

Parameters Measured Comment	Controls (Mean ± SD)	Diabetic Subjects (Mean ± SD)	P-Value
FBTP (g/l) ns	5 ± 4	5 ± 4	P>0.05
2HPPTP ns	6 ± 4	5 ± 3	P>0.05
Albumin (g/l) ns	2± 1	2± 1	P>0.05
2HPP Albumin (g/l) ns	3± 1	2± 2	P>0.05
Globulin (g/l) ns	4 ± 3	4 ± 3	P>0.05
2HPP Globulin (g/l) ns	3 ± 2	3 ± 3	P>0.05
A/G Ratio ns	0.7 ± 0.4	0.4 ± 0.3	P>0.05
2HPP A/G ns	1.0 ± 0.3	0.7 ± 0.7	P>0.05

Table 5: Comparison of salivary protein profiles between controls and diabetes mellitus subjects showed no significant difference (p>0.05) between saliva protein profiles when compared between controls and study group using student t-test.

Parameters measured	Female	Male	Female	Male
	Salivary (Mean ± SD)		Plasma (Mean ± SD)	
FBTP (g/l)	5 ± 4			78 ± 4
2HPPTP	6 ± 3	6 ± 5	72 ± 10	74 ± 3
2HPP Albumin (g/l)	4 ± 2	3 ± 2	39 ± 7	42 ± 2
Globulin (g/l)	2 ± 1	3 ± 2	32 ± 3	33 ± 2
2HPP Globulin(g/l)	2 ± 1	3 ± 2	33 ± 3	32 ± 2
A/G Ratio	1.5 ± 0.7	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.2
2HPP A/G	2.0 ± 1.0	1.0 ± 0.7	1.2 ± 0.2	1.3 ± 0.3

Table 6: The gender mean±sd concentrations of salivary and serum protein profiles of diabetic subjects showed no significant difference (p >0.05) when saliva and serum protein profiles were compared individually for the diabetes mellitus group based on gender difference.

Parameters measured	Female	Male	Female	Male
	Salivary (Mean ± SD)		Plasma (Mean ± SD)	
FBTP (g/l)	4 ± 2	6 ± 3	72 ± 7	74 ± 4
2HPPTP	5 ± 3	6 ± 5	70 ± 10	76 ± 3
Albumin (g/l)	2 ± 1	3 ± 1	42 ± 4	45± 2
2HPP Albumin (g/l)	3 ± 2	3± 1	39 ± 7	42 ± 2
Globulin (g/l)	2 ± 1	3 ± 2	30 ± 4	29 ± 3
2HPP Globulin(g/l)	2 ± 1	3 ± 1	31 ± 5	34 ± 4
A/G Ratio	1.0 ± 0.7	1.0 ± 0.3	1.4 ± 0.6	1.6 ± 0.6
2HPP A/G	1.5 ± 1.0	1.0 ± 0.7	1.3 ± 0.3	1.2 ± 0.2

Table 7: The observed gender mean ± sd concentrations of salivary and protein profiles of control subjects showed no significant difference (p>0.05) when saliva and serum protein profiles were compared individually for the controls based on gender difference.

Conclusion

The core of the research has clearly showed that non-invasive sample such as saliva is not yet feasible as an alternative to blood for the estimation of protein profile. The research also revealed that serum protein profile is an important panel of investigation for the management of diabetes mellitus. It further showed that salivary protein profile is of no clinical value for the management of diabetes

mellitus. However, the research revealed that healthcare professions should introduce serum protein profile as an integral investigation for the management of diabetes mellitus. Finally, it proffered that gender is not a factor in the assay of serum protein profile, hence not valid for customized diagnostics.

References

1. Alberts B (2012) Blood Cells Molecular Biology of the Cell.
2. Ochei J, Kolhatkar A (2003) Haematology In: Medical Laboratory Science Theory and practice Tata McGraw-Hill.
3. Mayne DP (2003) "Albumin" In Clinical Chemistry in Diagnosis and Treatment ELST pp: 31.
4. Solomon AEY, Wankasi M, Ndubisi A (2013) Studies on Serum Proteins Concentrations in Pregnant women in Bayelsa State. Journal of Medical Laboratory Science 22: 49-57.
5. Agoro ES, Ogbotobo R, Ombor J, Thomas C, Mac'odo Y, et al. (2012) Relationships between Serum Globulins Albumin/Globulin Ratio and C-Reactive Proteins in Pregnancy Trimesters. Journal of Medical Laboratory Science 21: 49-57.
6. Agoro ES, Wankasi MM, Ileimokumo O (2015) Relationship between Serum Anion Gap and Diabetes Mellitus. JDM 5: 199-205.
7. Alagoa E J (2009) The land and people of Bayelsa State: Central Niger Delta Onyoma Research Pub.
8. Manfredi M, McCullough, MJ Vescovi P, Kaarawi ZM, Porter SR (2004) Update on diabetes mellitus and related oral diseases Oral Dis 10: 187-200.
9. Darwazeh AM, MacFarlane TW, McCuish A, Lamey PJ (1991) Mixed salivary glucose levels and candidal carriage in patients with diabetes mellitus. J Oral Pathol Med 20: 280-283.
10. Chávez EM, Borell LN, Taylor GW, Ship JA (2001) A longitudinal analysis of salivary flow in control subjects and old adults with type 2 diabetes. OralSurg Oral Med Oral Pathol Oral Radiol Endod 91: 166-173.
11. Pal P, Desai NT, Kannan N, Masur VN, Daniel MJ, et al. (2003) Estimation of salivary glucose salivary amylase salivary total protein and periodontal microflora in diabetes mellitus. J Indian Dent Assoc 74: 143-149.
12. Michael LB, Janet LD, Edward PF (1992) Amino Acids and Proteins.
13. Jeremy EK (2010) Serum protein Medical Board of California. 800: 633-2322.
14. Little RR, Goldstein DE (1995) Endocrine (standardization of glycohemoglobin measurement) Anal chem 67: 393-397.
15. Hathama RH, Aymen A (2015) Influence of diabetes disease on concentration of total protein albumin and globulins in saliva and serum: A comparative study. Iraqi National Journal of Chemistry 15.
16. Ekin S, Meral I, Gunduz H, Mert N (2003) Comparative study of total protein and total and lipid-associated serum sialic acid levels in patients with type 2 diabetes mellitus. J Clin Lab Anal 17: 124-126.
17. Lopez ME, Colloca ME, Paez RG, Schallmach JN, Koss MA, et al. (2003) Salivary characteristics of diabetic children. Braz Dent J 14: 26-31.
18. Dodds MW, Dodds AP (1997) Effects of glycemic control on saliva flow rates and protein composition in non-insulin-dependent diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 83: 465-470.
19. Meurman JH, Collin HL, Niskanen L, Toyry J, Alakuijala P, et al. (1998) Saliva in non-insulin-dependent diabetic patients and control subjects: the role of autonomic nervous system. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 86: 69-76.
20. Yavuzyilmaz E, Yumak O, Akdoganli T, Yamalik N, Ozer N, et al. (1996) The alterations of whole saliva constituents in patients with diabetes mellitus. Aust Dent J 41: 193-197.
21. Tenovuo J, Lehtonen OP, Viikari J, Larjava H, Vilja P, et al. (1986) Immunoglobulins and innate antimicrobial factors in whole saliva of patients with insulin-dependent diabetes mellitus. J Dent Res 65: 62-66.
22. Sharon A, Aryeh BH, Itzhak B, Yoram K, Szargel R, et al. (1985) Salivary composition in diabetic patients. J Oral Med 40: 23-26.
23. Reuterving CO, Reuterving G, Hägg E, Ericson T (1987) Salivary flow rate and salivary glucose concentration in patients with diabetes mellitus influence of severity of diabetes. Diabete Metab 13 :457-462.
24. Aryeh BH, Cohen M, Kanter Y, Szargel R, Laufer D (1988) Salivary composition in diabetic patients. J Diabet Complications 2: 96-99.
25. Twetman S, Nederfors T, Stahl B, Aronson S (1992) Two-year longitudinal observations of salivary status and dental caries in children with insulin- dependent diabetes mellitus. Pediatr Dent 14: 184-188.
26. Marshall S, Biddle S, Gorely T, Cameron N, Murdey I (2004) Relationships between media use body fatness and physical activity in children and youth: a metanalysis. Internation Association for the study of Obesity 28: 1238-46.
27. Murry R (2012) Porphyrins and Bile pigment Haper's Illustrative Biochemistry.