How to Cite: Razzaq A, Sajid M, Iqbal N. 2017. Investigation of Biochemical Changes in Saliva and Blood of Diabetic Patients. International Journal of Applied Biology and Forensics 1(3): 72-77

RESEARCH ARTICLE

Investigation of Biochemical Changes in Saliva and Blood of Diabetic Patients

*Abdul Razzaq[1], Muhammad Sajid[2] and Nasir Iqbal[3]

[1, 2, 3] Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan.
*Correspondence: *arazzaq1122@gmail.com*

Abstract

Diabetes mellitus is group of metabolic disorders characterized by an inability to produce sufficient insulin or to responds insulin that results high blood glucose level. Type-1 diabetes mellitus considered as an autoimmune disease and required life-long treatment with exogenous insulin. Blood glucose levels along with serum insulin level required for diagnosis. In present study serum and salivary biochemical changes in type-1 diabetes mellitus patients were analyzed to evaluate non-invasive diagnostic markers. For this purpose, serum calcium, phosphate, total protein, alkaline phosphatase and salivary calcium, phosphate and alpha amylase were analyzed. Overall high serum calcium levels were found in diabetic patients. Serum phosphate levels were found lower in diabetic patients as compare to normal individuals. Serum alkaline phosphatase and total protein levels were also significantly higher in diabetic patients. Salivary calcium levels increased significantly in diabetic patients as compare to normal individuals. While the salivary phosphate and salivary alpha amylase levels significantly decreased in type-1 diabetic patients. It is suggested that saliva is absolute surrogate of blood that can be used to diagnose and evaluate the progression of type-1 diabetes mellitus and as non-invasive diagnostic body fluid.

Keywords: Diabetes Mellitus, Exogenous Insulin, Autoimmune, Non-Invasive Diagnostic Markers, Surrogate.

Introduction

Glucose is the body's primary energy source. After a meal, carbohydrates are broken down into glucose and simple sugars. This causes blood glucose levels to rise and stimulates the pancreas to release insulin into blood stream. Insulin is a hormone produced by beta cells in the pancreas. It regulates the transport of glucose into the most of the body's cells. If someone is unable to produce enough insulin, or if the body's cells are resistant to its affects (insulin resistance), then less glucose is transported from blood into cells (Alberti, 1998).
Diabetes mellitus includes two types, type-1 and type-2 diabetes. Type-1 diabetes is caused by a deficiency in insulin secretion due to the loss of pancreatic β cells, and the disease requires life-long treatment with exogenous insulin. Without the body's own insulin production, the body loses its ability to utilize carbohydrates as an energy source. Type-1 diabetes is considered as an autoimmune disease, which is developed due to the T-cell-

mediated destruction of β cells in the islets of Langerhans of the pancreas. In children with an active β-cell destruction process, auto antibodies against β-cell structures appear in the circulation. Type-1 diabetes is the most common form of diabetes in children worldwide. Type-1 diabetes has a strong heritable component, genetic risk explains only a fraction of the overall risk, but environmental factors contribute to the risk of developing type-1 diabetes mellitus (Gepts, 1965).

Protein is essential as it is a part of every organ and tissue in the body. It is however, continually broken down and therefore, we need to ingest more to replace what is lost. Protein helps maintain and repair muscle mass and normal body functioning (Gannon, 2004). In uncontrolled diabetes, muscle protein is broken down into amino acids to be converted into glucose by the liver. Proteins have to supply enough energy to substitute for carbohydrates; proteins are broken down faster than they are made. The body ends up with a protein deficit, a situation with subtle, yet far-reaching effects on normal body functions. Importantly, for diabetics, a protein deficit has been shown to impair resistance to infections (Gannon, 1986).

Alkaline Phosphatase is a membrane-bound metalloenzyme comprising a group of isoenzymes encoded by at least four different gene loci. They are tissue specific, placental, intestinal and germ cell alkaline Phosphatase. The two major and clinically most relevant isoenzymes in human serum are bone and liver alkaline Phosphatase. Significant amount of alkaline phosphatase is found in the liver, placenta, intestine, kidney, bone and platelets in decreasing order. Increased serum levels are seen in liver disease associated with metabolic disorders hepatic obstruction, obstructive jaundice, diabetes mellitus, infectious mononucleosis, biliary cirrhosis and cholestasis (Afonja, 1974).

Calcium is a mineral that is vital for a number of body functions, such as muscle contraction, hormone secretion and second messenger. Most of the body's calcium present in bones, but a small amount circulates in the blood for these important functions (Vestergaard, 2007). The calcium ion is an almost ubiquitous intracellular second messenger and direct correlation has been found between the blood sugar levels and the excretion of calcium in the urine and the loss of calcium from the forearm may account to as much as 10% within the first 5-years after the diagnosis of diabetes. The effects of high blood glucose on calcium excretion in the urine increased as the blood pressure also affected (Shi, 2001).

Diabetic ketoacidosis is associated with intracellular phosphate depletion, because of a shift of phosphorus from the intracellular to the extracellular compartments and prolonged and excessive hyper phosphaturia. The plasma inorganic phosphate (Pi) may be normal or even elevated despite intracellular phosphate depletion. Initial treatment with intravenous fluid and insulin allows entry of plasma glucose and phosphate into the insulin sensitive tissues for cellular phosphorylation. When insufficient phosphate and oxygen are available for adenosine triphosphate (ATP) synthesis, cell homeostasis cannot be maintained and may result in cell lyses (Yadav, 2000).

Saliva is complex and dynamic biological fluid used for detection of physiological and chemical changes in human body. The biochemical and physical properties of these salivary components also affects the oral health. Saliva can use for the various compounds like drugs, pollutants and hormones but also with microbial and systemic diseases. Exogenous insulin is able to stimulate salivation in diabetes mellitus patients. Medications used in these patients can also be responsible for the decreased salivary flow rate. Patients with diabetes mellitus express higher levels of alpha amylase and secretary IgA in whole saliva constituents. It can be speculated that variations in salivary amylase levels will simultaneously affect salivary total protein levels (Dawes, 1987).

Materials and Methods

Total 35 patients of diabetes mellitus were included in group A that were under treatment in "Jinnah Hospital Lahore". 35 normal healthy individuals were selected as control in group B. In group A only diabetic patients were included. Blood and saliva samples were collected from the diabetic patients and normal individuals. All the biochemical analysis was done at "Institute of Molecular Biology and Biotechnology" The University of Lahore.

Total protein, calcium, phosphate and alkaline phosphatase were estimated in blood of diabetic patients and normal individuals by enzymatic kit method.

In alkaline medium the copper reacts with the peptide bonds of proteins to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation, and potassium iodide prevents the auto reduction of copper. The absorbance of standard and sample was taken at 546nm (Spectrum, Germany) as described by Gornall AG.

Calcium ions form a violet complex with ortho-cresolphthalein complex in alkaline medium. The intensity of the color complex is directly proportional to the concentration of calcium in the sample. The absorbance was measured at 578nm spectrophotometrically.

Phosphorus in serum reacts with ammonium molybdate to form phosphorus molybdate, which is then reduced by stannous chloride and hydrazine sulphate to molybdenum blue. The intensity of the color complex is directly proportional to the concentration of phosphate in the sample. Measure the absorbance at 640nm spectrophotometrically.

In the presence of alkaline phosphatase, p-nitro phenol and phosphate was formed by the reaction of p-nitro phenyl phosphate and water. The absorbance of sample (As) and standard (Astd) was taken at 405 nm spectrophotometrically.

Salivary alpha amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-1-galacto-pyranosyl-maltoside (GALG2-CNP) to glucose polymers and p-nitro phenyl oligosaccharide at short chain producing 2-chloro-4-nitrophenol. The increased destruction of glucose can be measured by spectrophotometrically at 405nm.

The statistical analysis was done by T-Test using SPSS (version 16) software. The difference in values was indicated in the form of probability ($P \leq 0.05$) values.

Results

Following tables shows different values calculated for all the parameters.

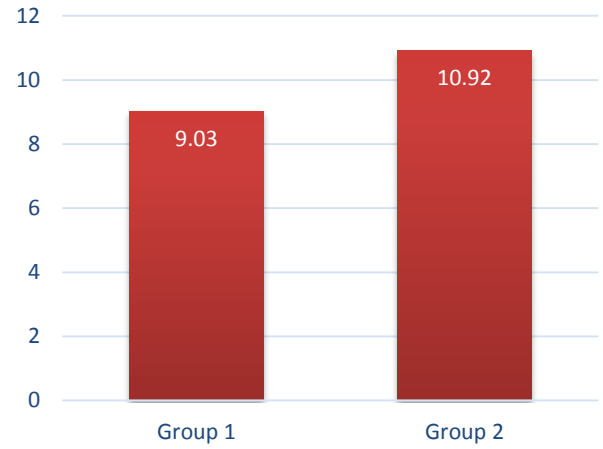

Table 1: Serum Calcium Levels
Group-1 (Control), Group-2 (Diabetic)

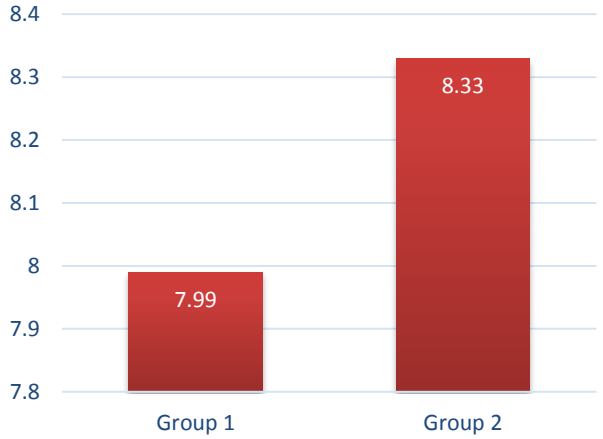

Table 2: Salivary Calcium Levels
Group-1 (Control), Group-2 (Diabetic)

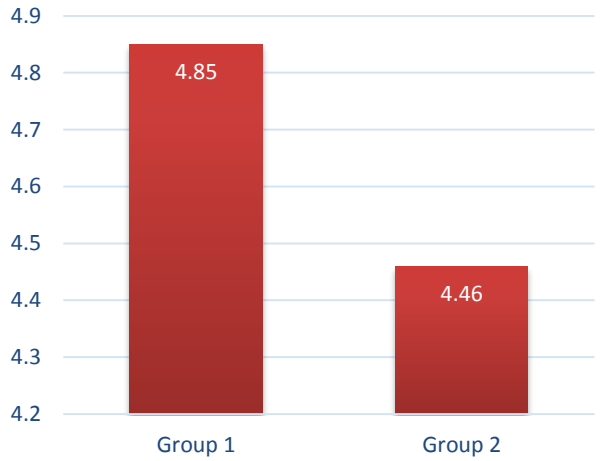

Table 3: Serum Phosphate Levels.
Group-1 (Control), Group-2 (Diabetic)

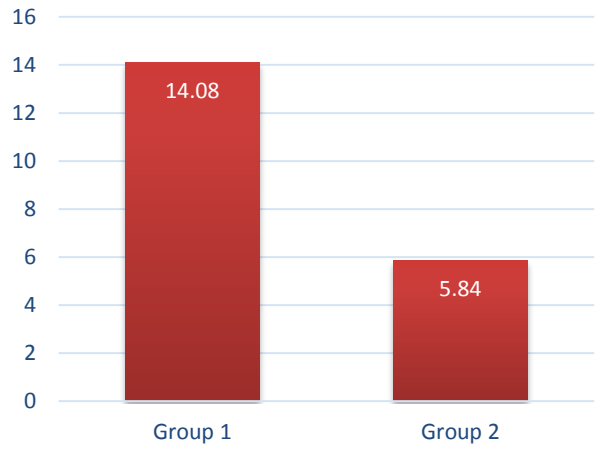

Table 4: Salivary Phosphate Levels
Group-1 (Control), Group-2 (Diabetic)

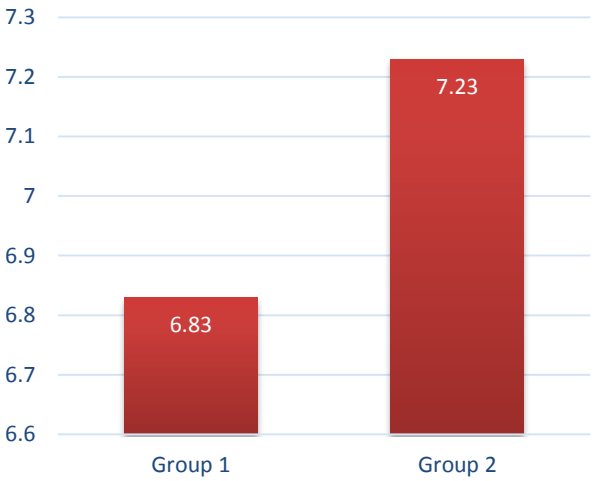

Table 5: Serum Total Protein Levels.
Group-1 (Control), Group-2 (Diabetic)

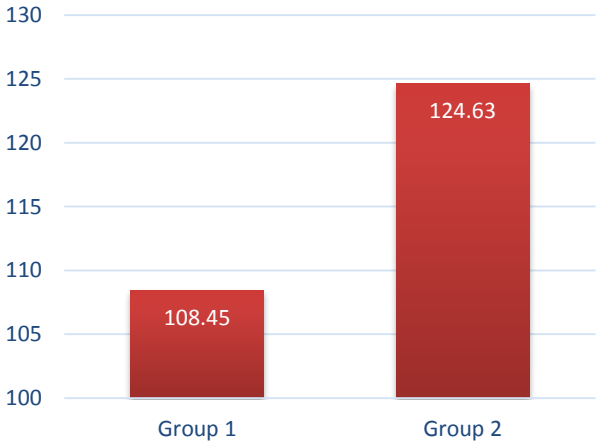

Table 6: Serum Alkaline Phosphatase Levels.
Group-1 (Control), Group-2 (Diabetic)

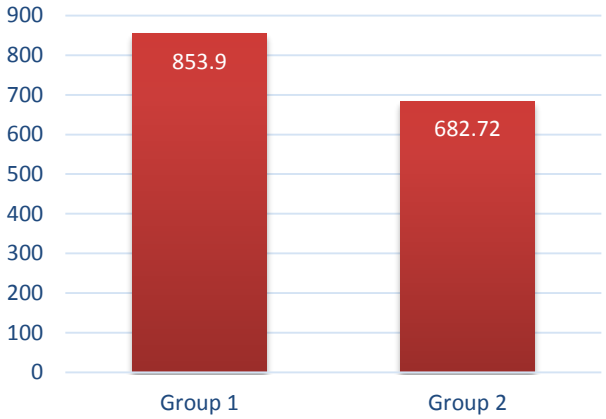

Table 7: Salivary Alpha Amylase Levels
Group-1 (Control), Group-2 (Diabetic)

Discussion

The present study was designed to investigate the biochemical changes in saliva and blood serum of diabetic patients. Overall calcium levels in saliva and serum of both groups were studied and significantly ($p<0.05$) high serum calcium levels were observed in diabetic patients. Outcomes were in line with the work of Jawed et al, (2010). Where they observed increased serum calcium levels in diabetic patients. Salivary calcium levels were increased in the present study but there was insignificant ($p > 0.05$) increase in salivary calcium levels in diabetic patients as compare to normal individuals. The outcomes were correlated with the study of Belazi et al, (1998).

Phosphate levels in serum and saliva of diabetic patients were measured. Insignificantly ($p<0.05$) decreased serum phosphate levels were observed in diabetic patients. The outcomes were correlated with the work of Gayoum et al, (2008) where they studied the decreased levels of serum phosphate in diabetic patients. Significantly ($p<0.05$) decreased levels of salivary phosphate were observed in diabetic patients and the results were in line with the study of Jawed et al. Where they observed low phosphate levels in saliva of diabetic patients during the study of oral health in diabetic patients.

Significantly ($p >0.05$) increased levels of serum total protein were observed in diabetic patients as compare to normal individuals. The outcomes were in line with the findings of Francisco et al,

(2009) where they studied the hyper-proteinemia in diabetic patients. Peritoneal protein loss was seen in diabetic patients and it was related to high membrane transport in these patients. The condition of high transport in diabetic patients could be a result of diabetic micro vascular lesions that cause permeability in the peritoneal and glomerular membrane. Increasing the protein content of the diet with a corresponding decrease in the carbohydrate content potentially was a patient empowering way of reducing the hyperglycemia present with type-2 diabetes mellitus Gannon et al, (1986).

Alkaline phosphatase levels were measured in diabetic patients. Significantly ($p>0.05$) increased levels of serum alkaline phosphatase were observed in diabetic patients as compare to normal individuals. The outcomes were in line with the findings of Jehle et al, (1998). Alkaline phosphatase levels were deceased after treatment with rosiglitazone in diabetic patients Zehra et al, (2007). They investigated that an alteration in alkaline phosphatase activity in patients suffering from diabetes and liver cirrhosis.

Overall decreased levels of alpha amylase were observed in diabetic patients significantly ($p<0.05$). The outcomes were correlated with the work of Arati et al, (2010). Where they observed low salivary alpha amylase levels in diabetic patients.

Conclusion

It was concluded from the outcomes of the present study that significant biochemical changes observed in saliva as well as in serum of diabetic patients. It was suggested that salivary biochemical changes can be used to observe progression of diabetes mellitus and used as non- invasive diagnostic markers.

References

Afonja JP, Baron, DN. 1974. Plasma alkaline phosphatase isoenzymes in hepatobiliary disease. J Clin Path 27: 916-920.

Alberti, KG, Zimmet PZ. 1998. For the WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. Provisional report of a WHO Consultation. Diabetic Medicine 15: 539–553.

Arati S, Panchibahi, Shirish S, Degwekar, Rahul R. 2010. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetes in India. J Oral science 52: 359-368.

Belazi, MA, Galli TA, Drakoulakos D, Fleva A. 1998. Salivary alterations in insulin-dependent diabetes mellitus. J Paediatr Dent 8: 29-33.

Dawes C. 1987. Physiological factors affecting salivary flow rate, oral sugar clearance and the sensation of dry mouth in man. J Dent Res 66: 648-653.

Francisco, C, Secundino C, Jose AH, Jesus D, Fernanda R, Antonio G. 2009. Peritoneal protein losses in diabetic patients starting peritoneal dialysis. Adv in peritoneal dialysis 25.

Gannon MC, Nuttall FQ. 2004. Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. Diabetes 53: 2375-2382.

Gannon MC, Krezowski PA, Nuttall FQ, Bartosh NH. 1986. The effect of protein ingestion on the metabolic response to oral glucose in normal individuals. Am J Clin Nutr 44: 847-56.

Gayoum A, Abdulla S, Musa. 2008. The affect glycemic control on serum lipid and calcium phosphate profiles in patient with type 2 diabetes mellitus. Egyptian J Biochem and Mol Bio 27: 89-92.

Gepts W. 1965. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes, 14: 619-633.

Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of biuret reagent. J Biol Chem 177: 751.

Jawed, Shahid S, Shah AQ, Abid A. 2010. Dental caries in diabetes mellitus: role of salivary flow rate and minerals. J diabetes and complications 25(3): 6-183.

Jehle PM, Jehle, DR, Mohan S, Bohm BO. 1998. Serum levels of insulin-like growth factor system components and relationship to bone metabolism in Type 1 and Type 2 diabetes mellitus patients. J Endocrinol 159: 297-306.

Shi H, Dirienzo D, Zemel MB. 2001. Effects of Dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. FASEBJ 15: 291–293.

Vestergaard P. 2007. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes a meta-analysis. Osteoporoses Int. 18: 427-444.

Yadav D, Nair S, Norkus EP, and Pitchumoni CS. 2000. Non-specific hyper amylasemia and hyperlipasemia in diabetic keto acidosis, incidence and correlation with biochemical abnormalities. Am J Gastroenterol 95(11): 3123-3128

Zehra B, Alptekin G, Nilufer B, Ayse CY, Neslihan T, Nilgun GD. 2007. Rosiglitazone decreases serum bone-specific alkaline phosphatase activity in postmenopausal diabetic women. J Clin Endocrin Metab 1: 431.