GLUCOSE ESTIMATION IN THE SALIVARY SECRETION OF DIABETES MELLITUS PATIENTS

DISSERTATION

Submitted to

BABU BANARASI DAS UNIVERSITY,

LUCKNOW, UTTAR PRADESH

In partial fulfilment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In

ORAL MEDICINE AND RADIOLOGY

By

Dr. Siddharth Jaiswal

Under the guidance of

Dr. Neeta Misra

Professor & Head

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

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ENROLLMENT NO: 11603242124 ACADEMIC BATCH: 2016-2019

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Glucose Estimation In The Salivary Secretion Of Diabetes Mellitus Patients"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Neeta Misra**, Professor & Head, Department of Oral Medicine and Radiology, Babu Banarasi Das College Of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date:

Place: Lucknow

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CERTIFICATE BY THE GUIDE/CO-GUIDE

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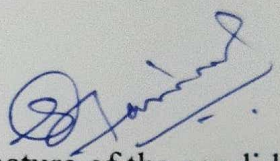

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"A TEACHER IS SOMEONE WHO SHOWS YOU THE SMOOTH ROADS TO WALK ON, IN LIFE."

Words cannot describe my emotions for my beloved **Parents, Mr. Satya Narayan Jaiswal** and **Mrs. Shobha Jaiswal, my dear wife Dr. Namrata Jaiswal, my sister Samriddhi Jaiswal and brother in law Dr. Vishwajeet Jaiswal** who always stood by me in times of joy and distress and have given me the strength to face the world.

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--Dr. SIDDHARTH JAISWAL

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ABBREVIATIONS

- DM :: Diabetes mellitus
- DM2 :: Diabetes mellitus Type 2
- IDDM :: Insulin dependent diabetes mellitus
- NIDDM :: Non insulin dependent diabetes mellitus
- BBDCODS :: Babu Banarasi Das College of Dental Sciences
- IEC :: Institutional Ethical committee
- OPD :: Out patient department
- EDTA :: Ethylene diaminetetracetic acid
- HBA1C :: Glycatedhaemoglobin
- RPM :: Revolutions per minute
- Mg/dl :: Milligrams per decilitre
- GOD-POD :: Glucose oxidase -peroxidase
- P –VALUE :: Calculated probability
- GDM :: Gestational diabetes mellitus
- MODY :: Maturity onset diabetes of the young
- UTP :: Uridine triphosphate
- UDP :: Uridinediphosphate
- ATP :: Adenosine triphosphate
- ADP :: Adenosine diphosphate
- NAD :: Nicotinamide adenine dinucleotide
- NADH :: Nicotinamide adenine dinucleotide –hydrogen
- c-AMP :: Cyclic adenosine monophosphate
- H_2O :: Dihydrogen oxide (water)
- AGEs :: Advanced glycosylation end products
- AOP :: Advanced oxidation protein products
- SOD :: Superoxide dismutase
- CAT :: Catalase
- Px :: Peroxidise
- Cp :: Ceruloplasmin
- GSH-Px :: Glutathione peroxidise

ABBREVIATIONS

- GSSG :: Glutathione disulfide
- CHOL :: Hypercholesterolemia
- TG :: Hypertriglyceridemia
- LDLc :: Low Density Lipoprotein -cholesterol
- HDLc :: High Density Lipoprotein -cholesterol
- NO :: Nitric oxide
- O2S :: Superoxide anion
- PD :: Periodontal disease
- IL-6 :: Interleukin 6
- CRP :: C- reactive protein
- BMS :: Burning mouth syndrome
- Cu+ :: Cupric cation
- YSI :: Yellow spring instrument
- Mmol/kg :: Millimoles per kilogram
- NI :: Noninvasive
- BG :: Blood glucose
- ISF :: Interstitial fluid
- BMI :: Body mass index
- UV :: Ultra violet
- IV :: Intravenous
- PAS :: Periodic acid Schiff
- BGL :: Blood glucose level
- T1DM :: Type 1 diabetes mellitus
- D :: Diabetic
- ND :: Non diabetic
- GOD –PAP assay :: Glucose oxidase-phenol and 4 -aminophenazone
- FPG :: Fasting plasma glucose
- FSG :: Fasting saliva glucose
- SG :: Salivary glucose
- SpH :: Salivary pH

- MDH :: Malic dehydrogenase
- MDA :: Malonaldehyde
- IgA :: Immunogloblin A
- WHO :: World health organization

ABSTRACT

Introduction: Diabetes mellitus is a group of chronic diseases characterized by insulin deficiency, cellular resistance to insulin action, or both, resulting in hyperglycemia and other related metabolic disturbances. Early screening of type 2 diabetes mellitus is essential for improved prognosis and effective delay of clinical complications associated with diabetes, and has been suggested as an important strategy to lower the incidence of this disease worldwide. Saliva testing potentially bypasses the issues associated with both urine and blood tests: it is non-invasive and painless, and can be performed with ease at any time. Hence, the present study was conducted to estimate glucose levels in diabetes mellitus patients and to determine the role of saliva as a diagnostic tool.

Objectives – the present study was done with the following objectives

1. To estimate salivary glucose in diabetes mellitus (controlled and uncontrolled) patients and to compare with healthy non diabetic control group.
2. To Compare serum and salivary glucose levels in patients with diabetes mellitus (controlled and uncontrolled).

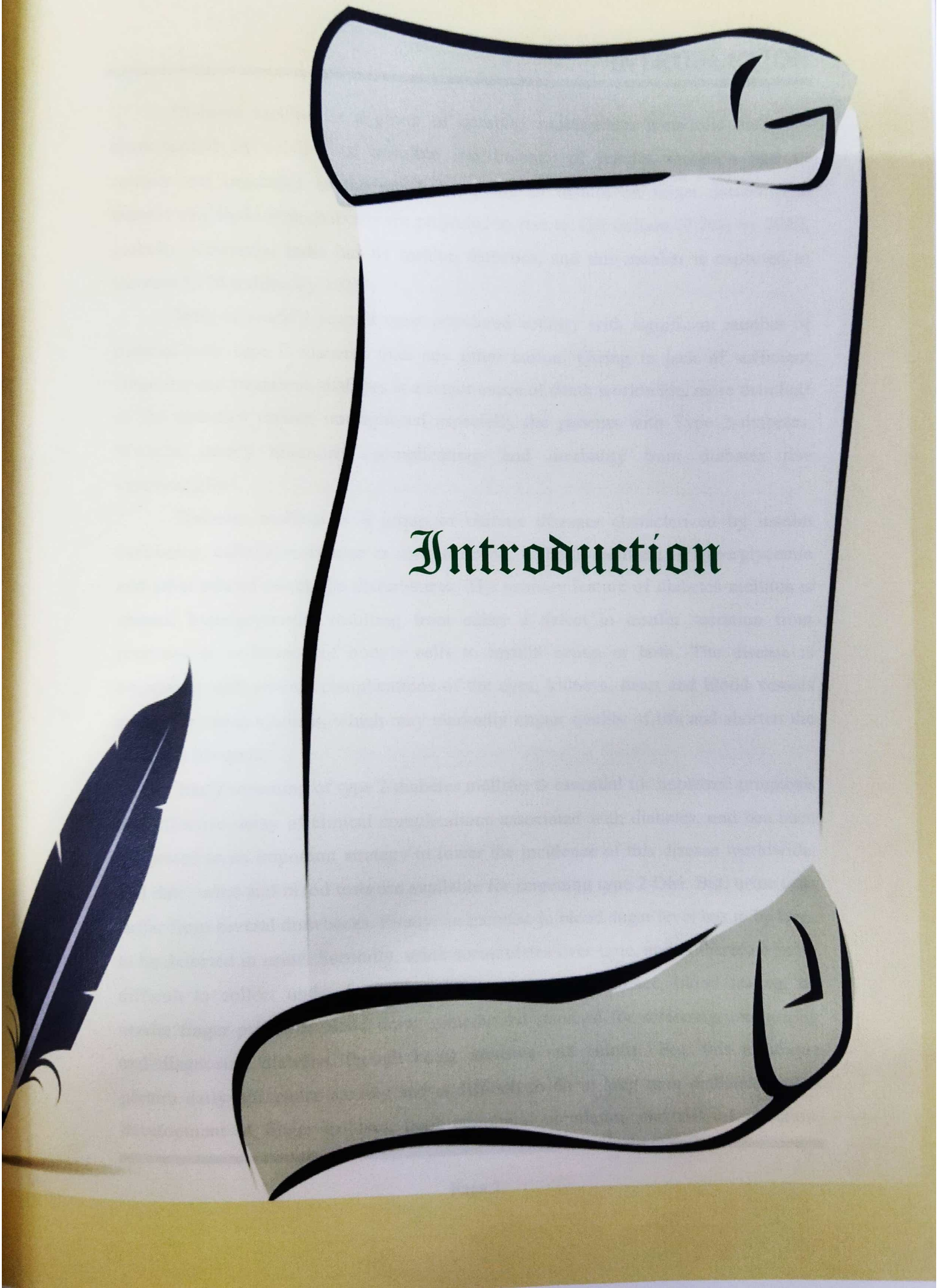
Methodology: 120 patients aged between 35 – 65 years who were age and gender matched was grouped into three categories. Group I had 40 patients of controlled diabetes mellitus, Group II had 40 patients with uncontrolled diabetes mellitus and Group III had 40 patients as healthy controls. Permission to conduct the study was obtained from Institutional Ethical committee (IEC) of BBDCODS, Lucknow. 5 ml of venous blood was collected for blood glucose estimation. Glucose was estimated in the serum and supernatant saliva by the glucose oxidizes method in a semi automated analyzer. Statistical package for Social Sciences 19.0 version was used for data analysis. Comparison of blood glucose levels and salivary glucose levels was done by Kruskal Wallis test.

Results: The mean salivary glucose level in uncontrolled diabetics in the present study was 9.55±2.00 and controlled diabetics was 4.05 ± 1.00.The mean fasting blood sugar level for uncontrolled diabetics was 163.55 ± 18.44 and 134.90 ± 29.38

for controlled diabetics. The mean salivary glucose level in control group was obtained at 1.30 ± 0.41 and mean fasting blood sugar was 8.61 ± 12.78. Correlation coefficient between fasting blood glucose and salivary glucose in uncontrolled diabetics was found to be 0.345 and p-value was 0.029 which was statistically significant.

Conclusions: The present study showed a positive correlation between salivary glucose and blood glucose. So from the observations of the present study, it can be inferred that saliva could act as a potential noninvasive adjunct to monitor glycemic control in diabetic patients.

Keywords: Saliva, salivary glucose, blood glucose, GOD - POD



Introduction

INTRODUCTION

Diabetes mellitus is a group of complex multisystem metabolic disorders characterized by relative or absolute insufficiency of insulin secretion and or concomitant resistance to the metabolic action of insulin on target tissues. The number of people with diabetes are projected to rise to 439 million (7.7%) by 2030, globally. Currently, India has 41 million diabetics, and this number is expected to increase to 70 million by 2025[1].

India is world's second most populated country with significant number of patients with type 2 diabetes than any other nation. Owing to lack of sufficient diagnosis and treatment, diabetes is a major cause of death worldwide, more than half of the diabetics remain undiagnosed especially the patients with Type 2 diabetes. Without timely diagnosis, complications and morbidity from diabetes rise exponentially [2].

Diabetes mellitus is a group of chronic diseases characterized by insulin deficiency, cellular resistance to insulin action, or both, resulting in hyperglycemia and other related metabolic disturbances. The primary feature of diabetes mellitus is chronic hyperglycemia, resulting from either a defect in insulin secretion from pancreas or resistance of body's cells to insulin action or both. The disease 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. [3]

Early screening of type 2 diabetes mellitus is essential for improved prognosis and effective delay of clinical complications associated with diabetes, and has been suggested as an important strategy to lower the incidence of this disease worldwide. Till date, urine and blood tests are available for screening type 2 DM. But, urine tests suffer from several drawbacks. Firstly, an increase in blood sugar level has to be large to be detected in urine. Secondly, urine accumulates over time, and is therefore more difficult to collect under fasting conditions than blood. Hence, blood testing, by needle finger pricks or blood draw, remains the standard for screening, monitoring and diagnosing diabetes, though being invasive and painful. But, this technique perturb daily life, cause anxiety and is difficult to do in long term diabetics due to development of finger calluses, poor peripheral circulation and risk of infection.

Recent studies have focused on the development of saliva based tests for screening or monitoring systemic diseases, including diabetes mellitus.

Saliva testing potentially bypasses the issues associated with both urine and blood tests: it is non-invasive and painless, and can be performed with ease at any time.[4]

Saliva is a complex fluid, whose important role is to maintain the well being of oral cavity. Saliva can be gland specific saliva and whole saliva. The average daily flow of saliva varies between 1 and 1.5 l. about 99% of saliva is water. The remaining 1% consists of most part of the large organic molecules (proteins, glycoprotein and lipids), small organic molecules (glucose and urea) and electrolytes (sodium, calcium, chlorides and phosphates). Parotid glands produce a watery secretion. Submandibular gland and sublingual gland produces more viscous fluid than parotid gland.

The importance of well functioning salivary glands for oral health is well known. Impaired flow rate, altered composition of saliva and increased oral microbial counts may increase the susceptibility to caries, periodontal disease and oral mucosal lesions. [5]

Saliva offers some distinctive advantages. Whole saliva can be collected non-invasively and by individuals with limited training. No special equipment is needed for collection of the fluid. Diagnosis of disease via the analysis of saliva is potentially valuable for children and older adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Further analysis of saliva provides a cost effective approach for the screening of large populations. The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland specific pathology. The possibility of using saliva to reflect the glucose concentration in blood makes it an important diagnostic tool in the understanding and management.[6]

Among all salivary parameters, glucose appears to be most closely related to the oral environment in patient with Type 2 diabetes. Glucose is a small molecule which diffuses through the membranes of blood vessels, passing from the blood plasma to the gingival fluid, through the gingival sulcus and reaches the saliva. The increase in blood glucose in Type 2 diabetes patients can cause higher levels of

salivary glucose with the consequent loss of homeostasis and greater susceptibility to disease in the oral cavity. [7]

Saliva is the principal defensive factor in the mouth which contains informative components that can be used as diagnostic markers for human disease. A growing number of health professionals are also finding that saliva provides an easily available, non-invasive diagnostic medium for a rapidly widening range of diseases and clinical situations. The multifarious components within saliva not only protect the integrity of the oral tissues, but also provide clues to local and systemic diseases and conditions. [8]

Studies performed on the salivary composition of diabetic individuals are scanty in India and the results reported so far are contradictory in several aspects. Hence, the present study was conducted to estimate glucose levels in diabetes mellitus patients and to determine the role of saliva as a diagnostic tool.

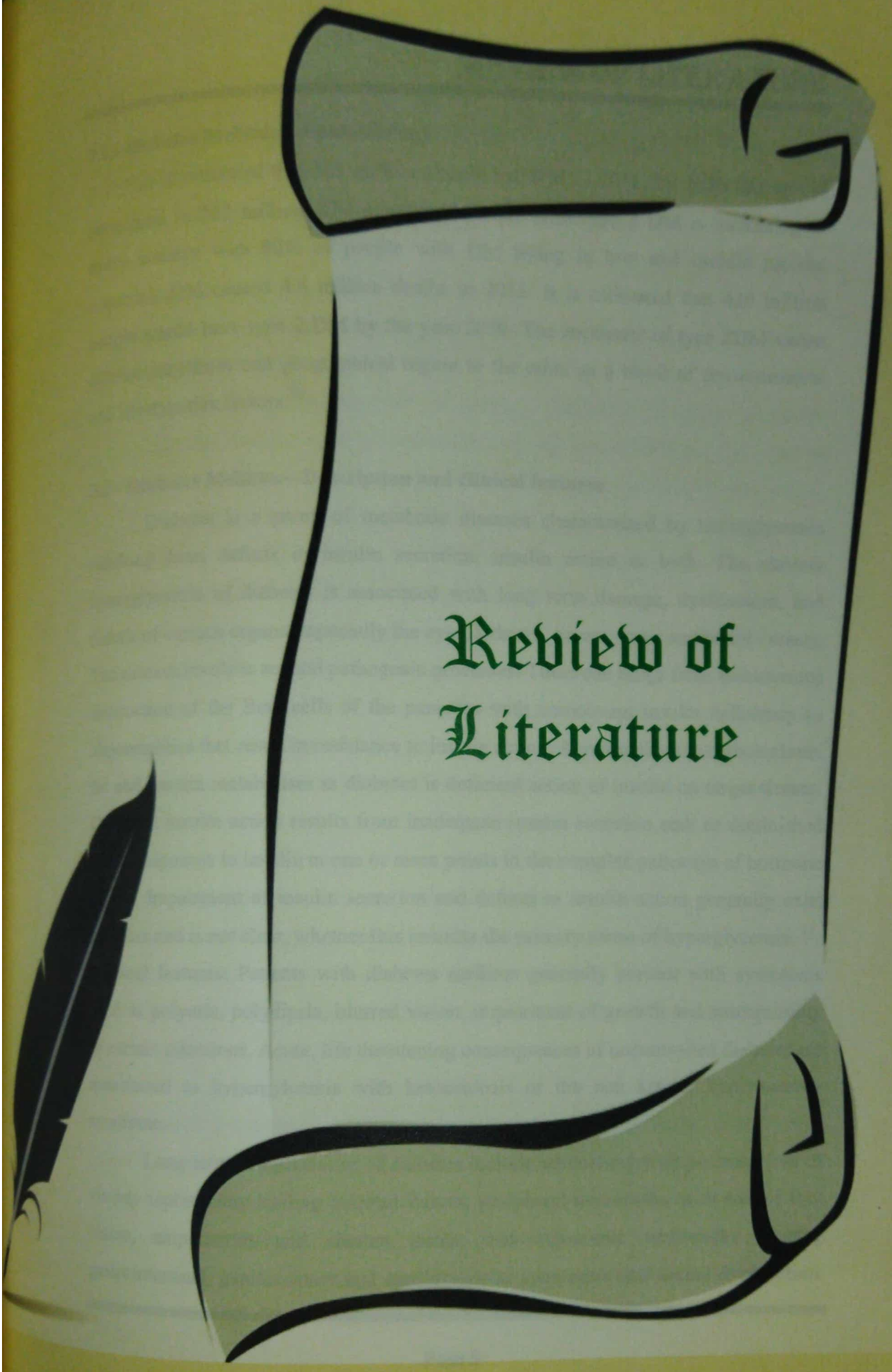
Aim & Objectives

AIM

- To estimate the salivary glucose level in diabetes mellitus (controlled and uncontrolled) patients and normal individual by GOD-POD (Glucose oxidase-peroxidase) method.

OBJECTIVES

1. To estimate salivary glucose in diabetes mellitus (controlled and uncontrolled) patients and to compare with healthy non diabetic control group.
2. To Compare serum and salivary glucose levels in patients with diabetes mellitus (controlled and uncontrolled).



Review of Literature

3.1 - Diabetes Mellitus – Epidemiology

It is estimated that 366 million people had DM in 2011; by 2030 this would have risen to 552 million. The number of people with type 2 DM is increasing in every country with 80% of people with DM living in low and middle income countries. DM caused 4.6 million deaths in 2011. It is estimated that 439 million people would have type 2 DM by the year 2030. The incidence of type 2DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors.[10]

3.2 - Diabetes Mellitus – Description and clinical features

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The disease involves several pathogenic processes. These can range from autoimmune destruction of the Beta cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/ or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action generally exist together and is not clear, whether this remains the primary cause of hyperglycemia. [11]
Clinical features: Patients with diabetes mellitus generally present with symptoms such as polyuria, polydipsia, blurred vision, impairment of growth and susceptibility to certain infections. Acute, life threatening consequences of uncontrolled diabetes are manifested as hypergylcemia with ketoacidosis or the non ketotic hyperosmolar syndrome.

Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations and charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction.

Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular diseases. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes. [12]

3.2.1 - Classification:

Diabetes falls into two broad etiopathogenetic categories, namely type I diabetes and type II diabetes.

a. Type I diabetes results from an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. This form of diabetes accounts for only 5 – 10 % of diabetic cases, previously called as insulin dependent diabetes or juvenile onset diabetes.

Type 1 diabetes mellitus (juvenile diabetes) is characterized by beta cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency. Type 1 is usually characterized by the presence of anti–glutamic acid decarboxylase, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta cell destruction. Eventually, all type1 diabetic patients will require insulin therapy to maintain normal glycemia.

b. Type II diabetes is caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. It might present without clinical symptoms and may be present for along period of time before diabetes is detected. This form of diabetes accounts for 90 – 95% of diabetic cases and was previously referred to as non insulin diabetes or adult onset diabetes.

The relative importance of defects in insulin secretion or in the peripheral action of the hormone in the occurrence of DM2 has been and will continue to be cause for discussion. DM2 comprises 80% to 90% of all cases of DM. Most individuals with Type 2 diabetes exhibit intra-abdominal (visceral) obesity, which is closely related to the presence of insulin resistance. In addition, hypertension and dyslipidemia (high

triglyceride and low HDL-cholesterol levels; postprandial hyperlipidemia) often are present in these individuals. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. It is more common in women, especially women with a history of gestational diabetes, and in Blacks, Hispanics and Native Americans.

c. Gestational Diabetes Mellitus is an operational classification identifying women who develop diabetes mellitus during gestation. Women who develop Type I diabetes mellitus during pregnancy and women with undiagnosed asymptomatic Type 2 diabetes mellitus which is discovered during pregnancy. In most women who develop GDM, the disorder has its onset in the third trimester of pregnancy.

d. Other specific type (Monogenic diabetes) - Types of diabetes mellitus of various known etiologies are grouped together to form the classification called "Other Specific Types". This group includes persons with genetic defects of beta-cell function (this type of diabetes was formerly called MODY or maturity-onset diabetes in youth) or with defects of insulin action; persons with diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis; persons with dysfunction associated with other endocrinopathies (e.g. acromegaly); and persons with pancreatic dysfunction caused by drugs, chemicals or infections and they comprise less than 10% of DM cases.[13,14,15]

3.2.2 - Factors affecting blood glucose[16]

Factors rising blood glucose

- Too much food, like a meal or snack with more carbohydrates than usual
- Not being active
- Not enough insulin or oral diabetes medications
- Side effects from other medications, such as steroids, anti-psychotic medications
- Illness – your body releases hormones to fight the illness, and those hormones raise blood glucose levels
- Stress, which can produce hormones that raise blood glucose levels

- Short- or long-term pain, like pain from a sunburn – your body releases hormones that raise blood glucose levels
- Menstrual periods, which cause changes in hormone levels
- Dehydration

Factors decreasing blood glucose

- Not enough food, like a meal or snack with fewer carbohydrates than usual, missing a meal or snack
- Alcohol, especially on an empty stomach
- Too much insulin or oral diabetes medications
- Side effects from other medications
- More physical activity or exercise than usual – physical activity makes body more sensitive to insulin and can lower blood glucose.

3.3 - Glycogenesis[17]

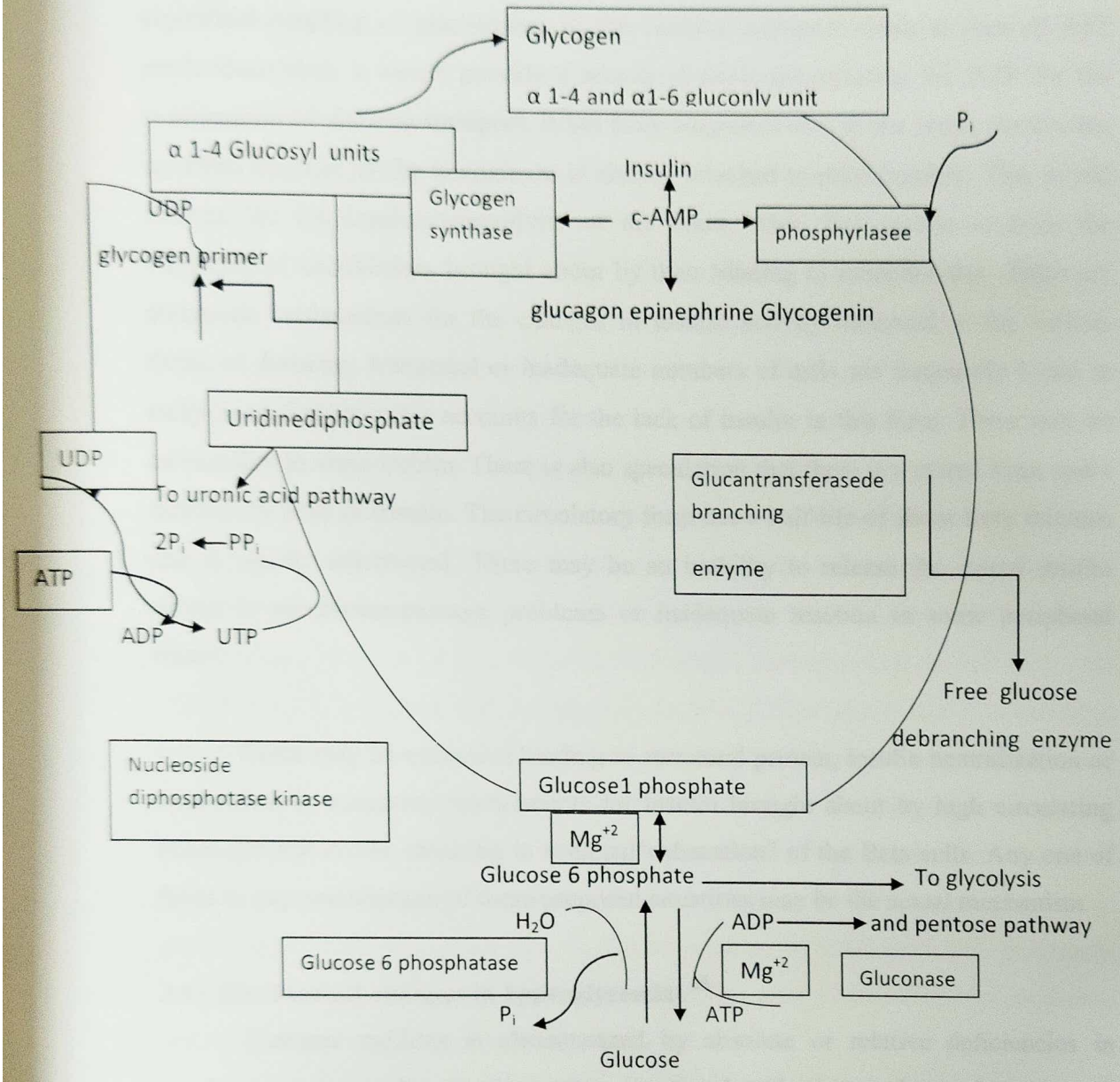
Glycogenesis is the biosynthesis of glycogen, the major storage form of carbohydrate. Glucose is the major source of energy to the cells. Our body has a built in mechanism which stores the excess carbohydrates we consume, in the form of glycogen which could be broken down to glucose when needed.

3.4 - Glycogenolysis: [18]

Glycogenolysis is the process of degradation of glycogen to glucose 1 phosphate and glucose in liver and muscle. Glycogenolysis happens in the major storage organs of glycogen – liver and muscle, when the blood glucose is low. Glycogenolysis is not the reversal of Glycogenesis.

The glycogen in the liver is used to increase the blood glucose level when needed. The glycogen in muscle is used to supply energy during muscle contraction as in physical exercise and not to increase blood glucose. The end products – glucose and glucose 1 phosphate are formed by the combined action of the two enzymes – debranching enzyme and glycogen phosphorylase.

Figure: 1 Pathway of glycogenesis and glycogenolysis in liver[18]



3.5 - The Role of Insulin[19]

Insulin activity occupies a central position in any study on diabetes. It is a regulator which promotes glucose metabolism, protein anabolism, fat disposition (increased lipogenesis) and, in general, it will reverse most of the basic changes of diabetes. It acts in opposition to many of the adrenal and pituitary hormones. How much of its effect is related to preferential glucose uptake in the various tissues, how much is due to a direct effect on enzyme synthesis and how much is just a "pulling"effect through the interrelated metabolic pathways is not well understood.

It is proposed that one of the primary actions of insulin may be on the Mg++ dependent coupling of glucokinase to the electron transport chain at sites of ATP production. Here it would provide a means of dephosphorylating the ATP for the continuation of electron transport. It has been suggested that in the brain, insulin has no effect because all the hexokinase is already attached to mitochondria. This would account for the insulin-insensitivity of the brain. Also, that insulin reverses the inhibition of hexokinases brought about by their binding to mitochondria. There are numerous explanations for the changes in insulin activity observed in the various forms of diabetes; Abnormal or inadequate numbers of cells are frequently found in early-onset diabetes; this accounts for the lack of insulin in this form. There may be an inability to store insulin. There is also speculation that there is a stored form and a circulatory form of insulin. The circulatory form has a half-life of about forty minutes and is rapidly inactivated. There may be an inability to release the stored insulin owing to membrane-passage problems or inadequate reaction in some peripheral tissue.

There may be excessive binding to structural protein, insulin neutralization or destruction or excessive requirements for insulin brought about by high circulating blood glucose levels, resulting in eventual"exhaustion" of the Beta-cells. Any one of these or any combination of these proposed situations may be the actual mechanism.

3.6 - Biochemical changes in hyperglycemia: [20]

Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Long-term vascular complications represent a major cause of morbidity and mortality in patients with diabetes mellitus. In addition, various biochemical disorders associated with vascular complications, such as hyperlipidemia and oxidative stress which frequently co-exist with diabetes mellitus, appear inadequate to explain the increased risk of vascular diseases. The observations suggest that additional factors may be involved in the acceleration of diabetic vascular disease.

Metal ions are known to play an essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients. It has been reported that the urinary excretion of calcium, zinc and magnesium is increased in two types of diabetes mellitus causing a decrease in blood levels of these elements from these patients. It is also reported that the levels of zinc and magnesium were significantly lower while the level of copper was significantly higher in serum of patients with IDDM .

Advanced glycation end products (AGEs) are complex components formed from the non enzymatic glycosylation of proteins, i.e. binding of monosaccharides to amino groups of proteins, which alter protein structure and functions. In addition, advance oxidation protein products (AOPP) are formed during oxidative stress by the action of chloramines (produced by myeloperoxidase in activated neutrophils).These compounds (AGEs and AOPP) accumulate in biological systems and thus take part in the diabetic long-term complications by causing damage to biological membranes and endothelium. A recent study reported that AGEs were elevated in NIDDM only whereas AOPP were elevated significantly in both IDDM and NIDDM. It has been reported that diabetic patients have significant defects of antioxidant protections and generation of reactive oxygen species (oxidative stress) which may play an important role in the etiology of diabetic complications. Decrease of superoxide dismutase (SOD), catalase (CAT), peroxidise(Px), ceruloplasmin (Cp) and glutathione peroxidise(GSH-Px) activities as well as a decrease in the GSH level and an increase in the concentration of glutathione disulfide (GSSG) were observed in erythrocytes of diabetic patients and in tissues from diabetic animals .

In diabetes mellitus (DM), the disorders of carbohydrates, lipids and proteins metabolism play predominant role in diabetic complications. Hypercholesterolemia (CHOL) and hypertriglyceridemia(TG) are mostly observed and related largely to the degree of diabetic control. Serum HDLc was reported to be low in diabetic patients of both types of DM . Hyperglycemia may alter lipoproteins to a form that promotes atherogenesis. Low-density

Lipoprotein-cholesterol (LDLc) levels are frequently altered in diabetic patients. Lipid peroxidation products, which increase in clinical and experimental diabetes, are important results of oxygen-derived free radicals stress. These products may be important in the pathogenesis of vascular complications in DM.

Nitric oxide (NO) is considered as a potent endothelium-derived vasodilator that participates in the general homeostasis of the vasculature. Studies have demonstrated that the development of diabetic complications in diabetes is closely related to the increased generation of superoxide anion (O2S_)and nitric oxide (NO).The exocrine pancreatic function and secretion of amylase in particular are altered in diabetes . It has been reported that the activity of amylase was elevated in poorly controlled diabetes. Alterations in the plasma concentrations of several trace elements have been suspected in diabetic patients and may be involved in some of the metabolic dysfunctions in diabetes mellitus. Interconnecting systems of antioxidant micronutrients (minerals) and enzymes also accomplish the body's defense against oxidative stress. In addition, diabetes mellitus is frequently associated with pancreatic enzyme abnormalities and oxidative stress. The role of trace element status including Cu2 +, Zn2 +, Mg2 +and Ca2 +, NO levels, antioxidants, AGEs and AOPP formation, lipid profiles and serum amylase activity in the pathogenesis and progression of two types of diabetes mellitus (DM).

3.7 - Hypoglycemia and changes in the body[21]

Hypoglycemia puts patients at risk for injury and death. Consequently the workgroup defines iatrogenic hypoglycemia in patients with diabetes as all episodes of an abnormally low plasma glucose concentration that expose the individual to potential harm. A single threshold value for plasma glucose concentration that defines hypoglycemia in diabetes cannot be assigned because glycemic thresholds for symptoms of hypoglycaemia (among other responses) shift to lower plasma glucose concentrations after recent antecedent hypoglycemia and to higher plasma glucose

concentrations in patients with poorly controlled diabetes and infrequent hypoglycaemia.

Nonetheless, an alert value can be defined that draws the attention of both patients and caregivers to the potential harm associated with hypoglycemia. The workgroup suggests that patients at risk for hypoglycemia (i.e., those treated with a sulfonylurea, glinide, or insulin)should be alert to the possibility of developing hypoglycemia at a self-monitored plasma glucose or continuous glucose monitoring subcutaneous glucose concentration of 70 mg/dL (3.9mmol/L). This alert value is data driven and pragmatic .Given the limited accuracy of the monitoring devices, it approximates the lower limit of the normal postabsorptive plasma glucose concentration, the glycemic thresholds for activation of glucose counter regulatory systems in non diabetic individuals ,and the upper limit of plasma glucose level reported to reduce counter regulatory responses to subsequent hypoglycemia. Because it is higher than the glycemic threshold for symptoms in both non diabetic individuals and those with well controlled diabetes, it generally allows time to prevent a clinical hypoglycaemic episode and provides some margin for the limited accuracy of monitoring devices at low-glucose levels. People with diabetes need not always self-treat at an estimated glucose concentration of 70 mg/dL(3.9 mmol/L). Options other than carbohydrate ingestion include repeating the test in the short term, changing behavior(e.g., avoiding driving or elective exercise until the glucose level is higher), and adjusting the treatment regimen. Although this alert value has been debated, a plasma concentration of 70 mg/dL (3.9mmol/L) can be used as a cut-off value in the classification of hypoglycemia in diabetes.

Consistent with past recommendations, the workgroup suggests the following classification of hypoglycemia indiabetes:[22]

1) Severe hypoglycemia. Severe hypoglycaemia is an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event, but neurological recovery following the return of plasma glucose to normal

is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

2) Documented symptomatic hypoglycemia.

Documented symptomatic hypoglycaemia is an event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration70 mg/dL(3.9 mmol/L).

3) Asymptomatic hypoglycemia. Asymptomatic hypoglycemia is an event not accompanied by typical symptoms of hypoglycaemia but with a measured plasma glucose concentration 70 mg/dL(3.9mmol/L).

4) Probable symptomatic hypoglycemia. Probable symptomatic hypoglycaemia is an event during which symptoms typical of hypoglycemia are not accompanied by a plasma glucose determination but that was presumably caused by a plasma glucoseconcentration70mg/dL (3.9mmol/L).

5) Pseudo-hypoglycemia. Pseudo hypoglycemia is an event during which the person with diabetes reports any of the typical symptoms of hypoglycaemia with a measured plasma glucose concentration.70 mg/dL (3.9mmol/L) but approaching that level.

3.7.1 - Implications of hypoglycemia on both short- and long-term outcomes in people with diabetes[23]

Iatrogenic hypoglycaemia is more frequent in patients with profound endogenous insulin deficiency type 1 diabetes and advanced type 2 diabetes and its incidence increases with the duration of diabetes. It is caused by treatment with a sulfonylurea, glinide, or insulin and occurs about two to three times more frequently in type 1 diabetes than in type 2 diabetes. Event rates for severe hypoglycemia for patients with type 1 diabetes range from 115 to 320 per 100 patient-years. Severe hypoglycaemia in patients with type 2 diabetes has been shown to occur at rates of 35 to 70 per 100 patient-years. However, because type 2 diabetes is much more prevalent

than type 1 diabetes, most episodes of hypoglycemia, including severe hypoglycemia, occur in people with type 2 diabetes.

There is no doubt that hypoglycaemia can be fatal. In addition to case reports of hypoglycemic deaths in patients with type 1 and type 2 diabetes, four recent reports of mortality rates in series of patients indicate that 4% , 6% , 7% , and 10% of deaths of patients with type 1 diabetes were caused by hypoglycemia. A temporal relationship between extremely low subcutaneous glucose concentrations and death in a patient with type 1 diabetes who was wearing a CGM device and was found dead in bed has been reported. Although profound and prolonged hypoglycaemia can cause brain death, most episodes of fatal hypoglycemia are probably the result of other mechanisms, such as ventricular arrhythmias.

3.8 - Oral manifestations of Diabetes Mellitus[24]

For years, research into diabetes has explored the many clinical implications of this highly prevalent disease. These include the need for periodontal control as tissue destruction may be accelerated among diabetics, and early management of oral infection will avoid exacerbating the existing metabolic imbalance. It has been found that an individual with uncontrolled diabetes presents a higher risk of infection, as well as abnormal prolonged healing time that will endanger the health of the oral cavity. Research has established that patients with DM may present a variety of oral manifestations.

3.9 - Methods in determination of glucose:[25,26,27]

Broadly, two methods are used in determining glucose:

I. *Invasive methods:* These methods utilize the presence of glucose in the blood.

II. *Non invasive methods:* These methods utilize the presence of glucose in body fluids like saliva, which makes it easily available and accessible.

3.9.1 - Invasive methods used for determination of glucose includes:[27]

1. Enzymatic methods –: Glucose is **oxidized** by glucose oxidase →gluconic acid + H_2O_2→**red dye**. It is more specific and gives precise results.

a. Hexokinase methods – Done either with pre deproteinization of a sample
b. Glucose oxidase methods – done by Trinder's or Kinetic or Polarigraphic method.
c. Glucose dehydrogenase methods

2. Reduction methods – which is based on the ability of glucose to **reduce** Cu^{++} to Cu^{+} →less sensitive, →substances that could reduce Cu^{++} : fructose, galactose, vitamin C, uric acid, etc.
   a. Smogi Nelson methods
   b. Hoffman methods

Chemical methods for blood glucose assay invariably rely upon stages involving enzymes (e.g. glucose oxidase, glucose dehydrogenase, hexokinase, etc.) linked to chromogenic reactions or to reactions featuring changes in electron flow that can be measured by suitable electronic meters. There are also techniques, less widely available, that employ physical methods for glucose detection, such as differences in infrared spectra.

At first it seems strange that laboratory techniques should be the simplest, most straightforward types of blood glucose assay in use, whereas some of the near-patient or point-of-care methods are really quite complicated in principle, but this is largely due to the constraints placed upon the latter by the requirement to be able to measure the glucose in a non-homogeneous matrix, i.e. whole blood.

All blood glucose assays does not measure the same variable. Laboratory methods using plasma, essentially a homogeneous matrix, do generally agree quite well because the assay responds to the glucose dissolved in the entire volume of the sample and results are usually expressed in terms of concentration of glucose per unit volume of plasma, e.g. in mmol/L. For methods using whole blood, the situation is very different and partly depends on whether the blood sample is first haemolyzed or diluted in some way before the measurement is performed.

Understanding this depends on the knowledge that red cells and plasma contain different amounts of dissolved solids such as proteins and, hence, have different proportions of water per unit volume, the water content of a volume of red cells being lower than that of an equal volume of plasma.

Glucose is dissolved in the water of the specimen (ignoring any bound to proteins) and equilibrates freely between the red cells and plasma of a whole-blood specimen, so the concentrations in plasma water and red-cell water are the same, but the concentration in total red-cell contents is lower than in plasma and the concentration in a volume of whole blood lies somewhere in between, varying with the hematocrit of the specimen.

Consequently, the glucose concentration influencing the assay system will be determined by whether the assay responds to the concentration in the water, in the plasma, in the red cells, in a mixture of plasma and red cells, or in a dilution of the blood specimen. The sophisticated principles involved in many point-of-care glucose methods make it difficult to know exactly which fraction of the sample is influencing the method's response, but in those cases where a filtering process takes place, retaining the red cells and allowing the plasma to seep through into the reagent area, it appears safe to assume that it is only the plasma glucose that is being measured.

However, the situation may not be quite so simple in that blood samples with different proportions of red cells may influence the flow and volume of plasma entering the reagent layers, and thus may affect the result.

In methods where the whole-blood sample is in contact with the reagents, there can still be interference from hematocrit, and even those techniques where the red cells are lyzed, releasing red-cell contents into the reaction milieu, are potentially subject to influence by the different glucose concentrations of plasma and red cells.

Some laboratory instruments capable of accepting a whole-blood sample (e.g. YSI) involve diluting it without lysis of red cells before the measurement takes place, using a glucose oxidase-linked electrode system.

Even the direct-reading electrode systems usually factorize the results to express them in conventional concentration terms, rather than as the activity actually measured, and it has been proposed that all methods should report as plasma glucose concentration to avoid confusion, no matter what sample type or measurement method has been used.

While this may be satisfactory for direct-reading electrode systems, it was not found to be entirely reliable for ear-prick capillary blood hemolysates assayed by a glucose dehydrogenase method

Problems with blood glucose estimation: [28,29]

Although blood glucose measurement is commonly performed, the use of a whole-blood sample introduces complications and compromise in terms of the assay principle, the method of calibration and the expression of results. Most point-of-care systems are calibrated against a method chosen by the manufacturer for reference purposes and assumptions are made, not necessarily valid ones that blood samples from different individuals will behave similarly in both the reference and point-of-care methods. While most conventional laboratory techniques measure blood glucose as concentration in plasma or whole blood, direct-reading electrode systems measure it as molality in mmol/kg water, which is numerically greater, but results are often factorized and expressed, e.g. as plasma glucose concentration. However, there is inconsistency and the variety of techniques and principles leads to some difficulty in comparing results of blood glucose measurements by different methods.

3.9.2 - Noninvasive (NI) methods used for the determination of glucose fall into two categories.[30]

i. The first is based on the measurement of glucose using one or more of its intrinsic molecular properties, such as near-infrared or mid-infrared absorption coefficient, optical rotation, Raman shifts, and photoacoustic absorption, as well as others. These methods assume the ability to detect glucose in tissue or blood independently of other body components or physiological state.

ii. The second category measures the effects of glucose on the physical properties of blood and tissue. This category is based on an assumption that glucose is a dominant (highly fluctuating) blood analyte and, as such, contributes significantly to the change in the relevant physical parameters of the tissue. Hence, measurement of such parameters can lead indirectly to evaluation of the blood glucose (BG) level. The measured parameters are evaluated relatively to calibration, performed through correlation of the NI signal to a reference BG value. Therefore, the relative change of glucose in blood or interstitial fluid (ISF) plays the major role, as other blood analyses, which are less fluctuating, are fully or at least partially eliminated through calibration.

However, glucose determination in indirect and nonspecific NI measurement faces several obstacles. Depending on the particular method used, readings may vary with changes in glucose level, but may also be affected by sensor–skin interface variations, changes in microcirculation and blood supply, medications that affect fluid distribution, comorbidities, a person's metabolic rate, and so on. The main concern, consequently, is to achieve high accuracy results, despite the fact that no direct blood or ISF glucose measurement is performed.

3.10 - Saliva as a diagnostic marker for diabetes:[31]

Various diagnostic devices are available in the market to measure the blood glucose level. There is a necessity to establish a noninvasive procedure to determine the blood glucose level without taking blood. Agarwal et al used GOD technique of analyzing glucose level in saliva.

Shreya Gupta et al[32] (2017)aimed to study correlation of blood glucose level and salivary glucose level in DM patients. A cross sectional study was conducted in 120 patients who were categorized as 40 controlled diabetics, 40 uncontrolled diabetics and 40 healthy age and sex matched individuals constituted the controls. The blood and unstimulated saliva samples were collected from the patients at the different intervals for fasting, random and postprandial levels. These samples were then

subjected for analysis of glucose in blood and saliva using glucose oxidase / peroxidise reagent in HITACHI 902 Automatic analyzer and the results were recorded. The mean SGLs were higher in uncontrolled and controlled diabetic groups than in non-diabetic group. A highly statistically significant correlation was found between fasting saliva glucose and fasting blood glucose in all the groups.

Cho Naing and JoonWah Naik[33] (2017) performed a systematic review to establish the relationship between salivary glucose level and blood glucose level in monitoring glycemia in patients with type I diabetes mellitus. Electronic databases were searched to evaluate studies on salivary glucose levels and serum glucose levels. Due to heterogeneity of studies, qualitative synthesis of studies was conducted. Ten observational studies were included in the review. A total of 321 cases and 323 controls with ages between 3 and 61 years were taken. Two studies were done exclusively on children below 17 years old. The significant difference between salivary glucose levels in type 1 diabetes mellitus and controls were reported in 6 studies with 8 data sets. Five studies with 7 data sets reported the correlation coefficient between salivary glucose and blood glucose in patients with diabetes. Findings suggested that salivary glucose concentrations may be helpful in monitoring glycaemia in type 1 diabetes mellitus.

Monica Virginia Viegas Lima – Aragao et al[34](2016) aimed to evaluate the biochemical and immunological characteristics of saliva from diabetic patients compared to non – diabetic adults. 88 diabetic adults and 39 non diabetic patients acting as controls were included in the study. Colorimetric method was used to determine glucose, urea, calcium, total protein and amylase. Secretory Ig A and Ig A anti – streptococcus mutans and anti-insulin antibodies were measured by enzyme linked immunosorbent assay. The levels of glucose, urea, calcium, anti-S.mutans IgA, total Ig A and anti-insulin Ig A were significantly higher in diabetic patients, whereas total protein and amylase levels were lower. No positive correlation was seen between blood and salivary glucose levels in either group. Saliva can be a useful tool to follow the systemic health status in these patients.

Dhanya S Hegde[8](2016) undertook a study to evaluate the relationship of blood glucose level with salivary glucose in diabetic and non – diabetic patients. The study sample included 100 diabetic patients and 100 non diabetic patients aged above 35 years of age. Fasting blood and salivary glucose levels were measured in two groups. Pearson's correlation coefficient was used to assess the correlation of blood glucose with salivary glucose in two groups. The results of the study revealed an increase in the level of fasting salivary glucose in diabetics compared to that of non diabetic patients. It was concluded that fasting salivary glucose level can be used as a noninvasive diagnostic, as well as monitoring tool to assess the glycemic status of Type II diabetes mellitus patients.

Wenjun Zhang et al [35](2015)evaluated a non-invasive glucose monitoring using saliva nano-biosensor. To provide accurate, low cost, and continuous glucose monitoring, they developed a unique, disposable saliva nano-biosensor. More than eight clinical trials on real time noninvasive salivary glucose monitoring were carried out on two healthy individuals. Excellent clinical accuracy was revealed as compared to the UV Spectrophotometer. By measuring subjects' salivary glucose and blood glucose in parallel, they found two generated profiles sharing the same fluctuation trend, but the correlation between them was individual dependent. A good correlation of glucose levels in saliva and in blood before and 2 hours after glucose intake was observed. Thus, disposable biosensor can be an alternative for real time salivary glucose tracking at any time.

Shruti Gupta et al [1](2015) compared salivary and serum glucose levels in diabetic patients. Their objective was to assess if any significant correlation existed between the serum and salivary glucose levels and also to correlate salivary glucose levels with regard to duration of diabetes, age and gender. Serum and salivary glucose levels of 200 subjects were estimated by glucose oxidase method. Glycosylated haemoglobin levels were also measured in randomly selected 40 diabetic subjects. A significant correlation between salivary and serum glucose levels in both diabetic and non-diabetic subjects were found.

Deepa Lakshmi et al*[36] *(2015) planned a study to compare salivary glucose values with blood glucose values and the biochemical characteristics of saliva in IDDM children. Thirty IDDM children and 30 healthy children were selected for the study. Fasting blood sample and unstimulated salivary sample were collected from all the subjects and were subjected for analysis. A weak positive correlation was noticed between fasting blood glucose and salivary glucose values in IDDM children. But a mean average of salivary glucose was high in IDDM children when compared with healthy children. The biochemical parameters like acid phosphatase, total protein count, and alpha amylase were increased, whereas salivary urea did not show significant variation between the groups. The authors concluded that with presently used diagnostic armamentarium, estimation of salivary glucose cannot replace the standard method of estimation of glucose in diabetic mellitus children.

Indira M et al*[7] *(2015) did a study to evaluate to determine, if saliva can be used as a non-invasive tool to monitor glycemic control in Type 2 diabetes. 40 individuals, 20 with Type 2 diabetes and 20 controls of age group 40 – 60 years were selected for the study. Diabetic status was assessed by estimating random blood glucose levels. Unstimulated saliva was collected from each patient and salivary glucose estimation was performed using glucose oxidase method. Results showed that significantly higher salivary glucose, lower amylase and total proteins were observed in patients with Type 2 diabetes than controls. The authors suggested that diabetes influences the composition of saliva.

Rathy Ravindran et al*[37] *(2015) analyzed the possibility of using salivary glucose and glycogen content of buccal mucosal cells as a diagnostic marker in Type II Diabetes mellitus patients which can be considered as adjuvant diagnostic tool to the gold standard. 30 study and 30 control were taken as samples. Saliva was collected by passive drool method. IV blood samples were collected for glucose estimation. Exfoliated buccal mucosal cells were collected from apparently normal buccal mucosa, smeared on dry glass slide and stained with PAS. Blood and salivary glucose are estimated by Glucose oxidase endpoint method. Results revealed a significant

increase in the salivary glucose level and the number of PAS positive buccal mucosal cells in the diabetics than in the controls.

Bhumika J Patel [38] ***(2015)*** performed a study to detect and compare salivary glucose with plasma glucose level and post prandial blood sugar and fasting blood sugar in diabetic and non-diabetic subjects. A total of 100 patients were participated in this study. They were divided into two groups, each group consist of 50 patients. Unstimulated saliva and blood were collected and investigated for glucose levels. FBS, PPBS, plasma glucose levels and salivary glucose levels were higher in diabetic patients than healthy controls. Thus salivary glucose level can be used for monitoring tool to assess the glycemic status of diabetes mellitus patients as it is non-invasive and diagnostic method.

Vidya Kadashetti et al [6] ***(2015)*** aimed to estimate and correlate the plasma and salivary glucose levels in diabetic and non-diabetic subjects, with special reference to age. The study population consisted of three groups: Group I consisted of diabetes with BGL >200 mg/dl and Group 2 of diabetics with BGL 130-200 mg/dl based on their random plasma glucose levels. Group 3 was a control population with BGL <130 mg/dl. 2 ml of peripheral blood was collected for the estimation of random plasma glucose levels and unstimulated saliva was collected for the estimation of salivary glucose. The salivary glucose levels were significantly higher in group I and goup 2 diabetics when compared with controls.

BNVS Satish et al [39] ***(2014)*** undertook a study to correlate the glucose levels in saliva and blood of diabetic and healthy non diabetic individuals and to determine the efficacy of saliva as a diagnostic tool. A total of 30 individuals of which 20 patients were diabetic patients and on medication and 10 patients were healthy non diabetic individuals were included in the study. Blood and saliva were collected under resting conditions and were subjected to glucose estimation. A significant correlation was found between fasting blood glucose and fasting salivary glucose for diabetic group

and control respectively. A positive correlation was found between fasting salivary glucose and HbA1c for diabetic and control group respectively.

Azizi and A.Modaberi*[40] *(2014) conducted a study to find relationship between the blood glucose level and salivary glucose in diabetic patients. A case control study was conducted on 75 diabetic patients as the case and 75 healthy subjects as the control group. Blood and salivary glucose levels were measured in the two groups. The mean blood glucose and salivary glucose levels was 247+24.2 mg/dl and 1.4+0.2 mg/dl in the case group respectively. These rates were 84.97+15.8 and 1.09+0.12 mg/dl in the control group, respectively. The study showed a high correlation between blood glucose level and salivary glucose in diabetic patients.

Preethi Balanetal*[41] *(2014) conducted a case control study to analyze concentrations of salivary glucose and blood glucose in type 2 diabetes mellitus patients. The study assessed glucose levels using the glucose oxidase method in blood and unstimulated saliva in 90 subjects who were divided into 3 equal groups of controlled type 2 diabetes, uncontrolled type 2 diabetes and those without diabetes. Salivary glucose levels were significantly higher in patients with diabetes than controls. There was a significant positive correlation between salivary and plasma glucose levels in patients with diabetes.

Prathibha KM et al*[42] *(2013) evaluated salivary profile among adult type 2 diabetes mellitus patients in South India. Salivary flow rates and salivary physical and biochemical parameters of diabetic (D) and non diabetic (ND) subjects were compared. 30 non diabetic subjects and 30 diabetic volunteers who had Type 2 diabetes mellitus for a minimum of 2 years were included. Unstimulated whole saliva was collected in fasting state. Salivary pH, flow rate and organic and inorganic constituents were evaluated. Salivary pH, flow rate and salivary amylase were significantly lower in diabetics. The authors concluded that evaluation of salivary parameters can be a cost effective and a non invasive alternative for screening, diagnosis and monitoring of diabetes.

IrajMirzaii – Dizgah et al [43] *(2013)* performed a study to investigate the stimulated salivary glucose as a diagnostic specimen in clinical practice for detection of diabetes mellitus. A case control study was carried out in 30 patients with diabetes mellitus aged 25 – 71 years who were hospitalized with diabetes side effects, and 30 healthy control subjects aged 25 – 71 years. Serum and saliva samples were obtained. Glucose level was determined by an enzymatic colorimetric GOD – PAP assay. Statistical analysis of the Student's t test and Pearson correlation coefficient were used. The mean stimulated whole saliva glucose level was significantly higher in case than in control group. There was a significant positive correlation between serum and saliva glucose concentration.

Agrawal RP et al [44] ***(2013)*** aimed to estimate blood and salivary glucose level in diabetic and non-diabetic subjects. Forty diabetic and forty non- diabetic subjects were randomly selected. A detailed history of each patient was obtained. The quantitative estimation of blood and saliva glucose levels was performed by glucose oxidase method using enzymatic kit GOD – POD, glucose oxidase peroxidise. The values observed regarding blood and saliva glucose level were found distinctly different between normal and diabetic subjects suggesting that monitoring of saliva glucose level can be used as an index of diabetes mellitus.

L. Malathiet al [45] ***(2013)*** performed a study to estimate the salivary amylase levels in non-insulin dependent diabetes mellitus patients and to correlate these findings with those in normal individuals. 60 samples were chosen for the study. Thirty non-insulin dependent diabetes mellitus patients of age group 30 to 60 years and healthy individuals of same number and age group were included in the study. The study found that mean scores of age, fasting blood sugar, post prandial blood sugar, HbA1c and salivary amylase levels were greater in diabetic patients than in non-diabetic patients.

Seyyed Omid Mahdavi et al [46](2012) presented a new method to evaluate Fasting plasma glucose (FPG) by salivary glucose measurement. A cross sectional study was done on 52 diabetic patients (test group) and 47 non diabetic patients (control group). After collection of saliva and blood samples, the FPG level was measured by GOD – PAP method and FSG level was measured by Glucose oxidase / peroxidases method. The study showed a significant linear relationship between FPG and FSG. Hence, it was concluded that FSG amounts can be used as a non – invasive method to detect FPG.

Arati S Panchbhai[2](2012) conducted a study to estimate correlation of salivary glucose level with blood glucose level in diabetes mellitus. 2 sets of samples of people with diabetes and age and sex matched non-diabetic subjects were recruited for the study. The salivary glucose was analyzed in unstimulated whole saliva samples using glucose oxidase method. A significant ($p<0.05$) positive correlation of salivary glucose level and fasting blood glucose level was observed in people with uncontrolled diabetes in both the sets of sample. The authors are of the opinion that saliva offers some potential as a marker in monitoring of diabetes mellitus.

Panda Abikshyeet et al [3](2012) did a study to find a medium which can be used to diagnose and monitor diabetes. Saliva samples were compared with blood glucose and glycated haemoglobin (HBA1c) in healthy and diabetic subjects. 106 newly diagnosed patients with type 2 diabetes mellitus and 15 healthy control subjects were included. The saliva and sera from the blood samples were subjected to glucose estimation. The correlation coefficient between serum glucose and salivary glucose in the patient group was calculated and the r value was found to be 0.7866, which was highly significant ($P<0.01$).

V.Nagalaxmi et al [47](2011) conducted a study to estimate and correlate salivary glucose levels in type 1 diabetes mellitus patients and healthy controls. 50 type 1 diagnosed diabetes mellitus patients and 50 age and sex matched healthy controls were included in the study. The fasting whole saliva was collected over ice with 0.1%

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w/v sodium fluoride and fasting venous blood samples were collected with 20ml of sodium fluoride. The samples were analyzed for glucose by using autoanalyser. The results were analyzed statistically using unpaired t test, Chi square test and Pearson's correlation test. The results showed a significant correlation between salivary and serum glucose in type 1diabetic patients and in control group.

David A Scott et al [48] ***(2010)*** employed infra red spectroscopy as a novel diagnostic tool in the prediction of diabetic status by analyzing the molecular and sub – molecular spectral signatures of saliva collected from subjects with diabetes and healthy controls. Spectral analysis revealed differences in several major metabolic components, lipid, proteins, glucose, thiocyanate and carboxylate, which clearly demarcates healthy and diseased saliva. The overall accuracy for the diagnosis of diabetes based on infrared spectroscopy was 100% on the training set and 88.2% on the validation set. Therefore, it was established that infrared spectroscopy can be used to generate complex biochemical profiles in saliva and identify several potential diabetes associated spectral features.

AnupamaHegde et al [49] ***(2010)*** evaluated the potential of saliva as a diagnostic tool in diabetes mellitus. 26 diabetes patients were compared with 21 age matched non – diabetic healthy controls for Fasting plasma glucose (FPG), salivary glucose (SG), salivary pH, oral health status and markers of oxidative stress in saliva namely Thiobarbituric acid reactive substance or Malondialdehyde and total antioxidant activity. Significantly high FPG along with high salivary AOA levels, markedly lesser SpH and MDH were found in the diabetic group. FPG showed positive correlation to SG and even better correlation with salivary MDA only in diabetes. Since SG levels did not differ between the two groups, the study concludes that conventional marker like FPG is a better indicator of glycemic status.

P.BakianianVaziri et al [50] ***(2010)*** evaluated differences between salivary IgA, glucose and flow rate in diabetic patients compared with healthy controls. 40 patients with type 1 diabetes, 40 patients with type 2 diabetes and 40 healthy controls were

selected. Whole unstimulated saliva samples were collected by the standard method and the salivary flow rate was determined. Nephelometric and Pars method were used to measure salivary IgA and salivary glucose concentrations, respectively. There were no significant differences in salivary IgA and glucose concentrations between type 1and type 2 diabetic patients and their matched control subjects. Salivary flow rate was significantly lower in diabetic patients.

Cedric Jurysta*[51] *(2009) evaluated salivary glucose concentration and excretion in unstimulated and mechanically stimulated saliva in both normal and diabetic subjects. In normal subjects, a decrease in saliva glucose concentration, an increase in salivary flow, but an unchanged glucose excretion rate was recorded when comparing stimulated saliva to unstimulated saliva. In diabetic patients, an increase in salivary flow with unchanged salivary glucose concentration and glucose excretion rate were observed under the same experimental conditions. Salivary glucose concentration and excretion were much higher in diabetic patients than in control subjects, whether in stimulated or unstimulated saliva. Results showed no significant correlation between glycemia and either glucose concentration or glucose excretion rate was found in the diabetic patients.

Sreedevi M.C et al*[5] *(2008) estimated and correlated salivary glucose concentration and serum glucose concentration in diabetics and healthy controls. 60 newly diagnosed diabetic patients and 60 age and sex matched control subjects were included in the study. Blood and saliva samples from both the groups were collected at least two hours after the breakfast. The samples were centrifuged and subjected to glucose analysis using Semiautoanalyser. For experimental group, the samples were collected again after the control of diabetes mellitus. A highly significant correlation was found between salivary glucose and serum glucose before the treatment and also after the control of diabetes. Hence salivary glucose holds the potential of being a marker in diabetes. It also has an added advantage of being non-invasive procedure with no need of special equipments and with fewer compliance problems as compared with collection of blood.

Jonathan A Ship*[52] *(2003) reviewed on diabetes and oral health. Diabetes is a common disease with concomitant oral manifestations impacting dental care. The author summarized the prevalence, signs, symptoms, diagnosis and treatment for diabetes, as well as dental treatment considerations for patients with diabetes. Safely managing the patient with diabetes requires effective communication among multiple health care providers. Dentists must be familiar with techniques to diagnose, treat and prevent stomatological disorder in patients with diabetes.

3.11 - Advantages of GOD POD: [53]

The GOD POD method is the most advantages of all, as it is linear, sensitive, simple as it requires only 10 ml of sample to be incubated for 30 minutes with a single reagent at room temperature and also requires simple instrumentation.

3.12 - Nanosensors for Glucose estimation[54]

Nanotechnology has been incorporated into glucose sensors using two primary approaches. First, sensors can be designed using macro- or microscale components (such as electrodes, membranes and supporting hardware) but incorporate either a nanostructured surface or a nanomaterial into this design. The nano scale properties of these modified systems have several advantages, including higher surface areas (yielding larger currents and faster responses) and improved catalytic activities. These sensors, owing to their size, would be implanted similar to current technology if used for continuous monitoring. Accordingly, these sensors could experience the same drawbacks as current sensors, including sensor fouling and decreased sensor life as a result of immune foreign body response.

Secondly, nanofabrication techniques can generate glucose sensors that are nano scale in all dimensions. These sensors offer some advantages over traditional sensors for continuous monitoring: these sensors would be injectable, which could lead to more facile administration of the sensing system than the current implantation approach. Additionally, because of the small size of these sensors, they could

potentially avoid the foreign body response of the immune system and, therefore, have longer useful lives.

3.13 - Insulin sensitivity and insulin resistance[55]

The acute metabolic action of insulin and its essential importance for survival are well recognized. Insulin directs the selection of metabolic fuels for energy production and, in doing so, it is the only hormone committed to the prevention of hyperglycemia. Insulin resistance is essentially a condition of reduced insulin sensitivity. Insulin sensitivity is commonly described as the ability of insulin to lower plasma glucose levels, which it does by suppressing hepatic glucose production and stimulating glucose uptake in skeletal muscle and adipose tissue. Insulin resistance describes an impaired biological response to insulin, but there is sufficient variability in normal sensitivity to insulin that there is no specific boundary at which sensitivity ends and resistance begins. The need for a flexible interpretation of insulin resistance is emphasized by evidence that insulin resistance affects different tissues and different actions of insulin to different extents.

There is no absolute definition of hyperinsulinemia, since an insulin concentration that is raised for an individual is usually still within the wide range of normality. While hyperinsulinemia may compensate for resistance to some actions of insulin, it can result in overexpression of actions that retain normal or nominally impaired reactivity to insulin. Also, high concentrations of insulin might act via receptors for insulin-like growth factors. This accentuation of some of the actions of insulin with simultaneous resistance to other actions gives rise to a diversity of clinical presentations and sequelae of insulin resistance.

3.14 - Pharmacologic Management[56]

An "ominous octet" that leads to hyperglycemia, which occurs in isolation or in combination, has been proposed for eight pathophysiological mechanisms underlying NIDDM. These include

(i) reduced insulin secretion from pancreatic β-cells,

(ii) elevated glucagon secretion from pancreatic α cells,

(iii) increased production of glucose in liver,

(iv) neurotransmitter dysfunction and insulin resistance in the brain,

(v) enhanced lipolysis,

(vi) increased renal glucose reabsorption,

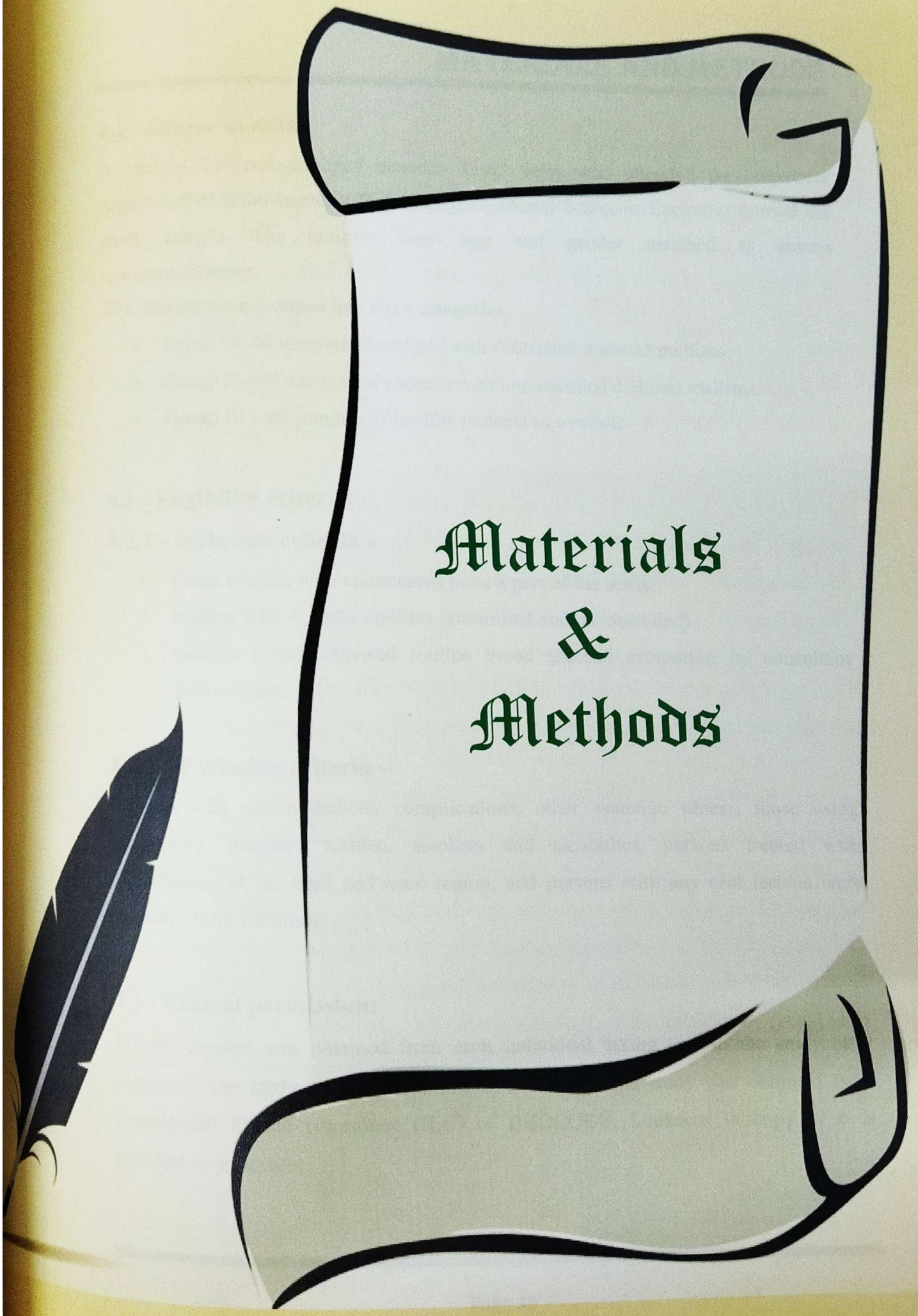
(vii) reduced incretin effect in the small intestine, and

(viii) impaired or diminished glucose uptake in peripheral tissues such as skeletal muscle, liver, and adipose tissue.

Currently available glucose-lowering therapies target one or more of these key pathways. Good glycemic control remains the main foundation of managing NIDDM. Such approaches play a vital role in preventing or delaying the onset and progression of diabetic complications. It is important that a patient-centered approach should be used to guide the choice of pharmacological agents. The factors to be considered include efficacy, cost, potential side effects, weight gain, comorbidities, hypoglycemia risk, and patient preferences.

Pharmacological treatment of NIDDM should be initiated when glycemic control is not achieved or if HbA1C rises to 6.5% after 2–3 months of lifestyle intervention. Not delaying treatment and motivating patients to initiate pharmacotherapy can considerably prevent the risk of the irreversible microvascular complications such as retinopathy and glomerular damage. Monotherapy with an oral medication should be started concomitantly with intensive lifestyle management.

The major classes of oral antidiabetic medications include biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, and α-glucosidase inhibitors. If the HbA1C level rises to 7.5% while on medication or if the initial HbA1C is ≥9%, combination therapy with two oral agents, or with insulin, may be considered. Though these medications may be used in all patients irrespective of their body weight, some medications like liraglutide may have distinct advantages in obese patients in comparison to lean diabetics.



Materials & Methods

4.1 - Source of data:

A total of 120 patients aged between 35-65 years who attended the outpatient department of Babu Banarasi Das College of Dental Sciences, Lucknow formed the study sample. The samples were age and gender matched to ensure representativeness.

The patients were grouped into three categories:

- Group I – 40 samples of patients with controlled diabetes mellitus.
- Group II – 40 samples of patients with uncontrolled diabetes mellitus.
- Group III – 40 samples of healthy patients as controls.

4.2 - Eligibility criteria:

4.2.1 - Inclusion criteria –

1. Those of them who volunteered to be a part of the study.
2. Patients with diabetes mellitus (controlled and uncontrolled)
3. Patients already advised routine blood glucose estimation by consultant diabetologist.

4.2.2 - Exclusion criteria –

Patients with severe diabetic complications, other systemic illness, those using medication, pregnant women, smokers and alcoholics, persons treated with radiotherapy of the head and neck region, and persons with any oral lesions were excluded from the study.

4.3 - Ethical permission:

Written consent was obtained from each individual taking part in the study after explaining the study protocol. Permission to conduct the study was obtained from Institutional Ethical committee (IEC) of BBDCODS, Lucknow (a copy of it is attached as annexure).

4.4 - Data collection:

A data sheet was taken of each patient detailing the patient's name, age, gender and relevant medical history. Detailed history was taken to find out the subject's type of medication.

All the sample collections were performed by the investigator only in the OPD of BBDCODS.

4.5 - Sample collection:

4.5.1 - Blood sample collection:

Both patients and controls were requested to report into the clinic in the morning, on an empty stomach, after 8 hours of fasting; 5 ml of venous blood was collected. The subjects were made to sit comfortably on a chair with arm extended straight from the shoulder. The antecubital fossa was exposed and a tourniquet was applied about 1.5 – 2 inch above the antecubital fossa. The area was rendered aspetic with cotton soaked in spirit. Using a 2ml sterile disposable plastic syringe and a 24 – guage needle, the vein was punctured and 2ml of blood was drawn. The tourniquet was relieved and cotton soaked with spirit was applied on the punctured site after the needle was removed.

Blood glucose estimation: 2 ml of it was collected in an ethylenediaminetetracetic acid (EDTA) containing blood collection tube and stored. The rest of the blood was collected in a sterilized glass test tube. The collected blood in the EDTA tubes was centrifuged and was then subjected to $HbA1_c$ level estimation using the glucose oxidase method. The sample was centrifuged at 3000 rpm for about 5 min. one millilitre of glucose reagent was added to 10 ml of test sample and glucose standard. Both were incubated at 37oc for about 10 min. the absorbance values were measured on a semiautomatic analyzer and the values were expressed as mg/dl.

4.5.2 - Saliva sample:

Saliva sample collection:

Patients were asked not to eat or drink for 2 hours before the time of saliva collection. Samples were collected 2 hours after the subject's breakfast. Spit technique was used to collect the unstimulated saliva. Salivary sample collection was performed in the morning between 9:00 am and 11:00 am. Patient was asked to sit in the dental chair with head tilted forward and instructed not to speak, swallow or do any head movements during collection of the sample. The patients were asked to wash their mouths with tap water and to spit two or three times, after which they were told to spit the saliva pooled in their mouths for the following 10 minutes into the sterile sample collection container. Saliva of about 2 ml was collected. The unstimulated saliva samples were centrifuged at 3000 rpm for 20 min and clear supernatants were processed immediately for estimation of glucose.

4.6 - Saliva glucose estimation:

Glucose was estimated in the serum and supernatant saliva by the glucose oxidase method in a semiautomated analyzer. The sample (100 ml) was mixed with the reagent in the ratio of 1:3 and incubated for 5 min at 37oc. The readings of absorbance values of standard and the sample against the reagent blank was noted. Standard was diluted 10 times for estimating salivary glucose levels. This method was standardized and could measure a minimal salivary glucose concentration of 0.2 mg/dL. [57]

4.7 - Equipments used for the study:

- Pair of sterile disposable gloves and mouth mask
- Stainless steel kidney tray, mouth mirror, straight probe, tweezers and explorer.
- Sterile gauze piece and cotton swab.
- Sterile disposable syringe– 5 ml
- Graduated test tube

4.8 - Principle of method:[58]

Glucose oxidase catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide is detected by a chromogenic oxygen acceptor phenol, 4 – AP, 4 – aminophenazone in the presence of peroxidise.

The intensity of the color formed is proportional to the glucose concentration in the sample.

Principle:

mutarotase

- α-D-glucose $\longrightarrow$ β-D-glucose

glucose oxidase

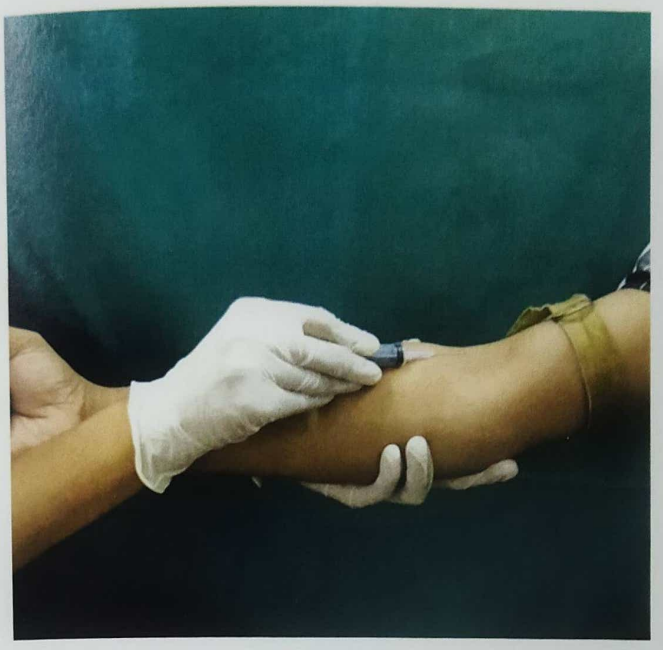
- β-D-glucose + H_2O + O_2 $\longrightarrow$ D-gluconic acid + H_2O_2

peroxidase

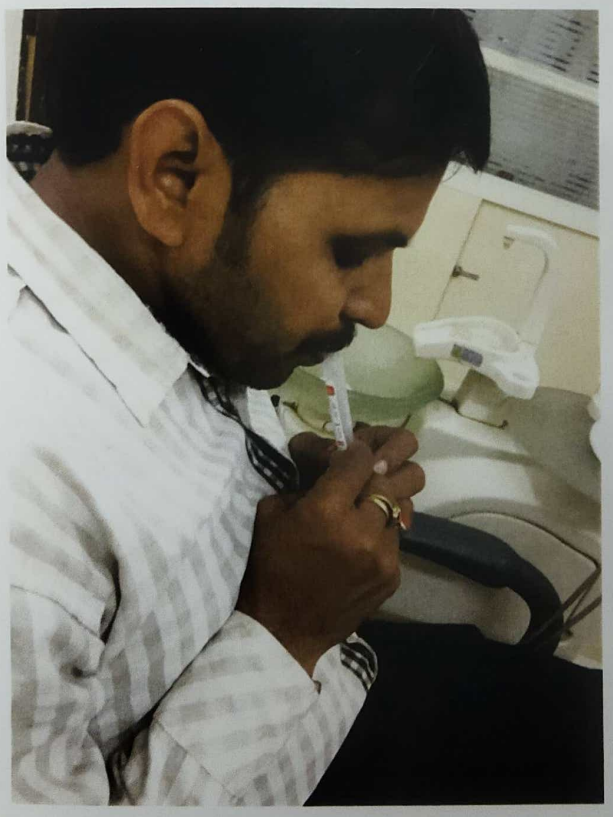
- H_2O_2 + 4-Aminophenazone + phenol $\longrightarrow$ Quinonemine + $4H_2O$

4.9 - Statistical analysis:

The data obtained was transferred to MS excel. Statistical package for Social Sciences 19.0 version was used for data analysis. Values were expressed as means + standard deviation and $p < 0.05$ was considered significant. Comparison of blood glucose levels and salivary glucose levels was done by unpaired t test.

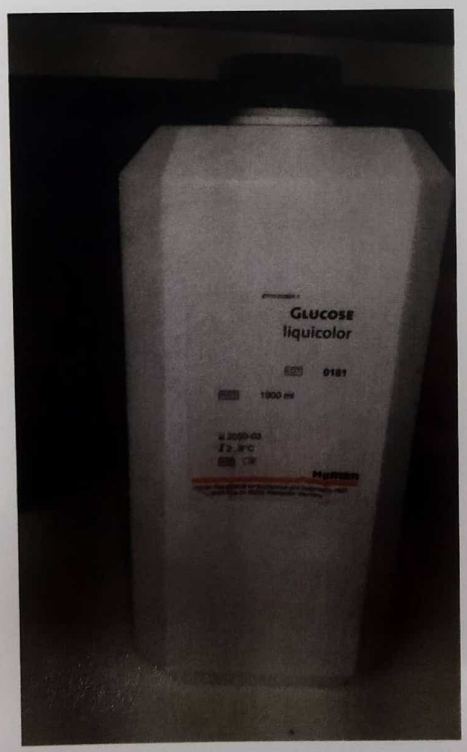


PHOTOGRAPH 1: BLOOD SAMPLE COLLECTION



PHOTOGRAPH 2: SALIVA SAMPLE COLLECTION

PHOTOGRAPH 3: CENTRIFUGE MACHINE

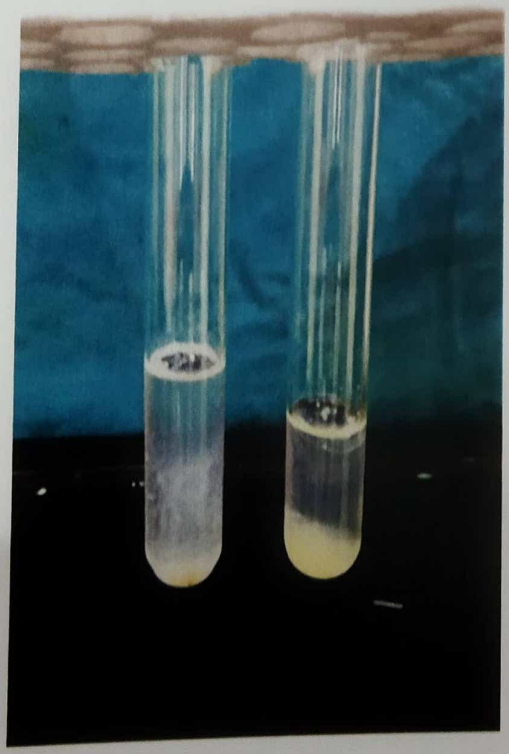


PHOTOGRAPH 4: REAGENT 1

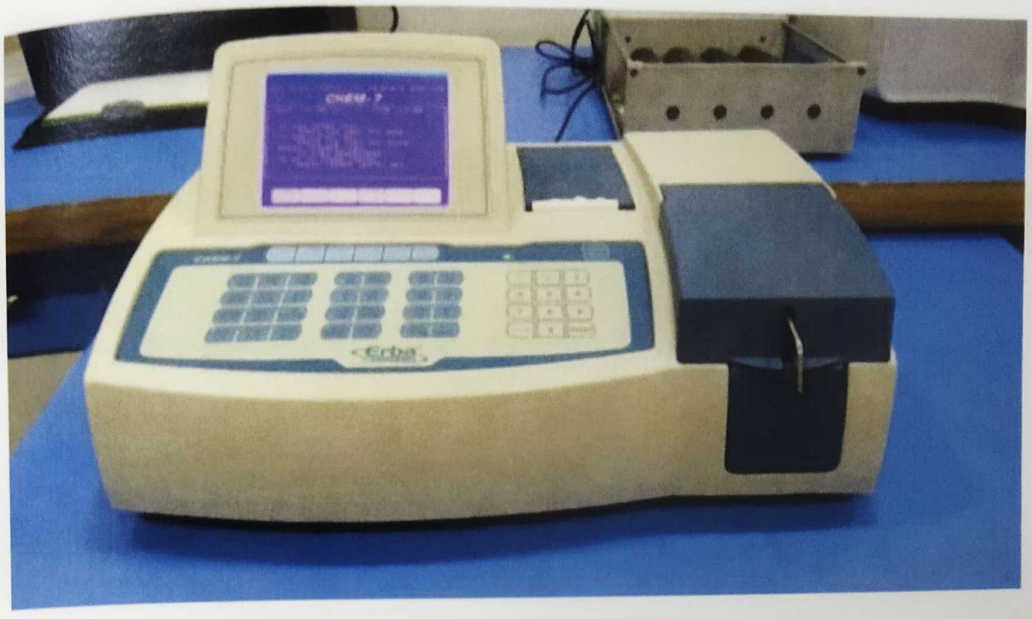
PHOTOGRAPH 5: REAGENT 2

PHOTOGRAPH 6: REAGENT 3

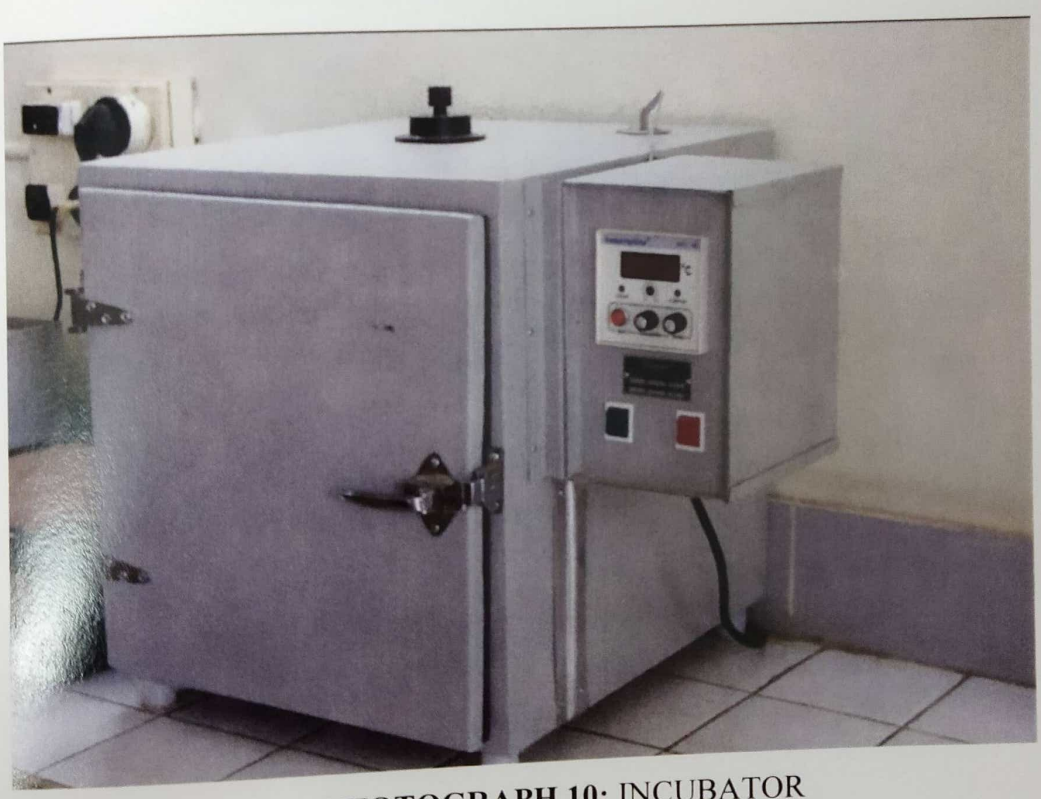
PHOTOGRAPH 7: SUPERNATANT OF BLOOD SAMPLE



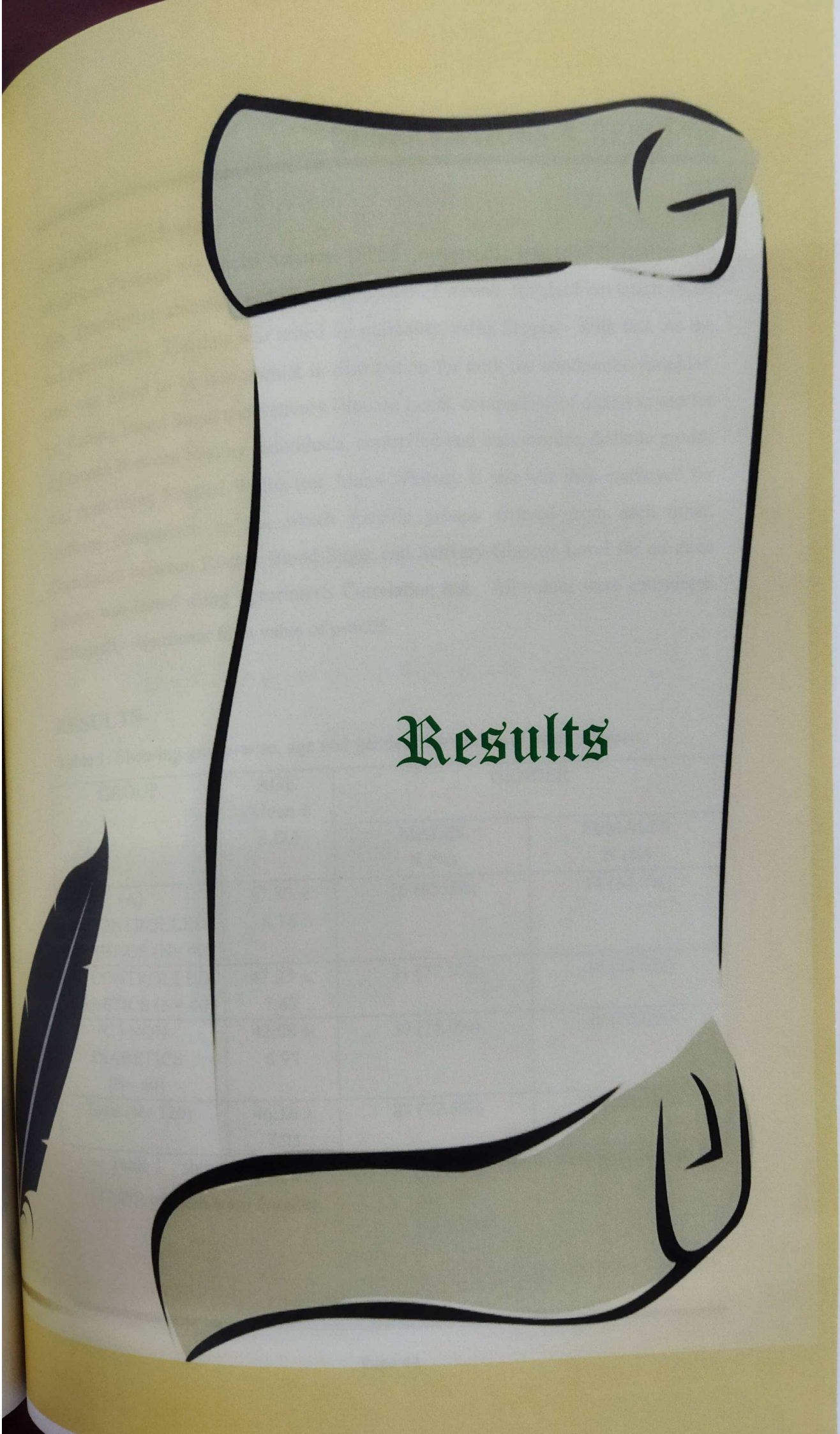
PHOTOGRAPH 8: SUPERNATANT OF SALIVA SAMPLE



PHOTOGRAPH 9: SEMI-AUTOANALYZER



PHOTOGRAPH 10: INCUBATOR



Results

Statistical analysis:

Statistical Package for Social Sciences (SPSS- version 21) was used to analyze the data. Descriptive statistics included calculation of means, standard deviation (S.D) and percentages. The data was tested for normality, using Shapiro- Wilk test. As the data was found to be non-normal in distribution for both the continuous variables, i.e. Fasting Blood Sugar and Salivary Glucose Level, comparison of means to test for difference between healthy individuals, controlled and uncontrolled diabetic groups was done using Kruskal Wallis test. Mann Whitney U test was then employed for pairwise comparison to test which specific groups differed from each other. Correlation between Fasting Blood Sugar and Salivary Glucose Level for all three groups was tested using Spearman's Correlation test. All values were considered statistically significant for a value of $p<0.05$.

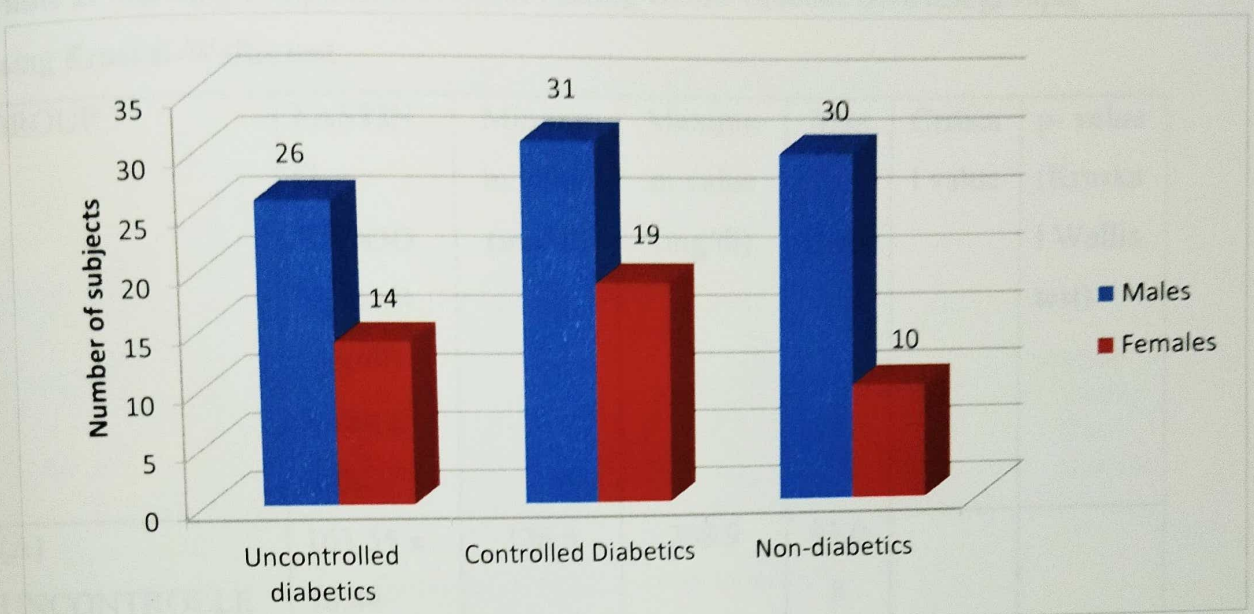
RESULTS-

Table 1: Showing group-wise, age and gender distribution of study subjects

GROUP	AGE (Mean ± S.D.)	GENDER	
		MALES N (%)	FEMALES N (%)
(A) UNCONTROLLED DIABETICS (N= 40)	47.80 ± 8.16	26 (65.0%)	14 (35.0%)
(B) CONTROLLED DIABETICS (N= 40)	47.25 ± 7.62	31 (77.5%)	19 (22.5%)
(C) NON-DIABETICS (N= 40)	42.08 ± 6.97	30 (75.0%)	10 (25.0%)
Total (N= 120)	46.16 ± 7.95	87 (72.5%)	33 (27.5%)

→ Table 1 shows that 87(72.5%) of the study populations were males while 33 (27.5%) of them were females.

Graph1: Group-wise gender distribution of study subjects



Graph2: Group-wise age distribution of study subjects

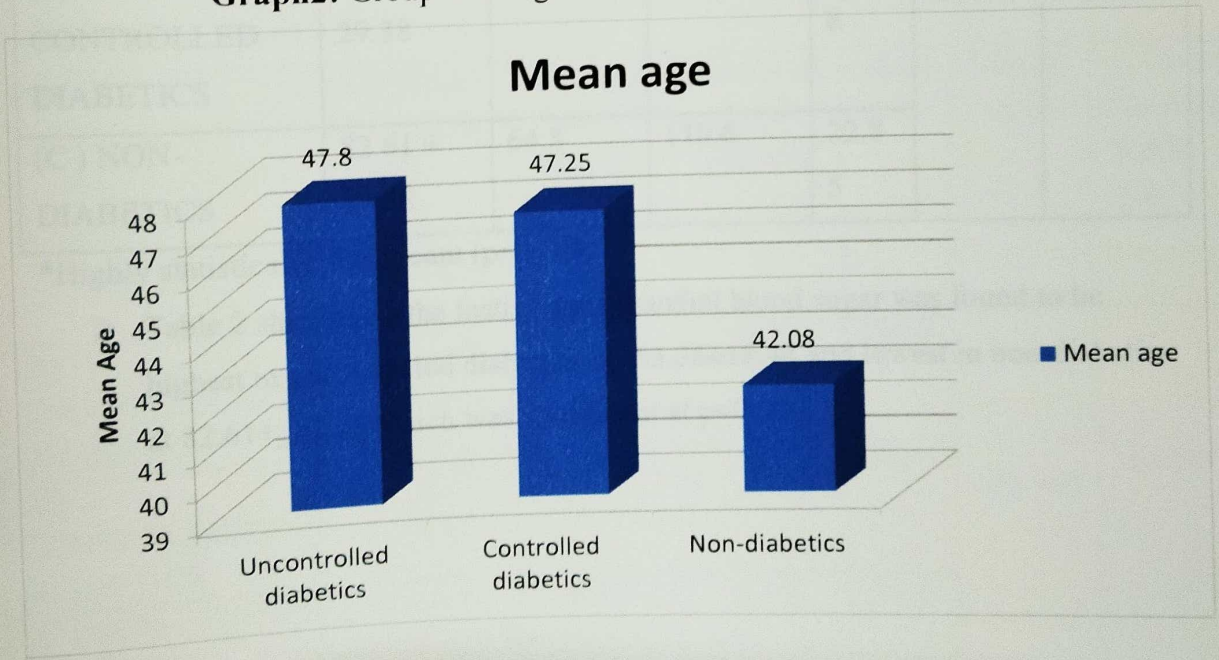


Table 2: Showing comparison of mean Fasting Blood Glucose between groups, using Kruskal-Wallis test

GROUP	FASTING BLOOD SUGAR (mg/dl) Mean ± S.D.	Minimum value (mg/dl)	Maximum value (mg/dl)	Mean Rank	Critical value	p- value (Kruskal Wallis test)
(A) UNCONTROLLED DIABETICS	163.55 ± 18.44	136.6	208.9	93.08	82.793	0.000*
(B) CONTROLLED DIABETICS	134.90 ± 29.38	77.3	213.5	65.58		
(C) NON-DIABETICS	82.61 ± 12.78	64.8	110.6	22.85		

*Highly statistically significant (p<0.01);

- Table 2 shows that the fasting pre parandial blood sugar was found to be highest in uncontrolled diabetics at 163.55±18.44 and lowest in non-diabetics at 82.61+_12.78 which was significant at p=0.000.

Graph3: Mean Fasting Blood Glucose of groups A, B and C

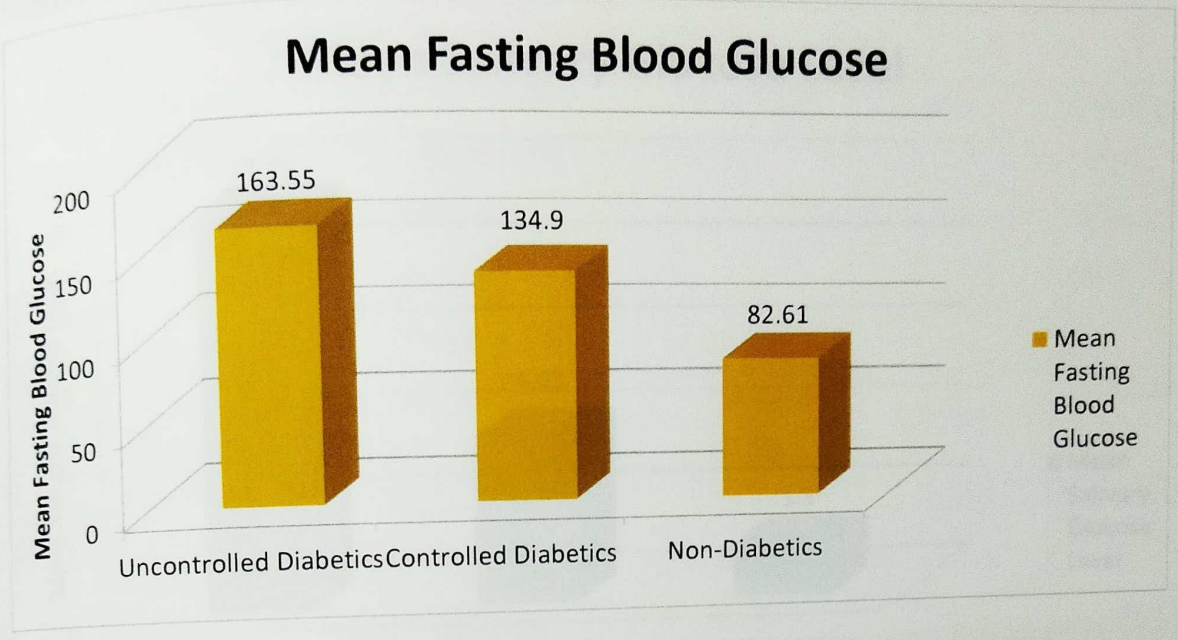


Table 3: Showing comparison of mean Salivary Glucose Level between groups, using Kruskal-Wallis test

GROUP	SALIVARY GLUCOSE LEVEL (mg/dl) Mean ± S.D.	Minimum value (mg/dl)	Maximum value (mg/dl)	Mean Rank	Critical value	p- value (Kruskal Wallis test)
(A) UNCONTROLLED DIABETICS	9.55 ± 2.00	6.82	13.9	100.5	105.792	0.000*
(B) CONTROLLED DIABETICS	4.05 ± 1.00	2.28	5.91	60.5		
(C) NON-DIABETICS	1.30 ± 0.41	0.62	2.20	20.5		

*Highly statistically significant (p<0.01)

- Table 3 shows that the salivary mean glucose level was 9.55+_ 2.00 , 4.05+_1.00 and 1.30+ _0.41 in the uncontrolled diabetics , controlled diabetics and healthy controls respectively and was highly significant at p =0.000.

Graph4: Mean Salivary Glucose Level of groups A, B and C

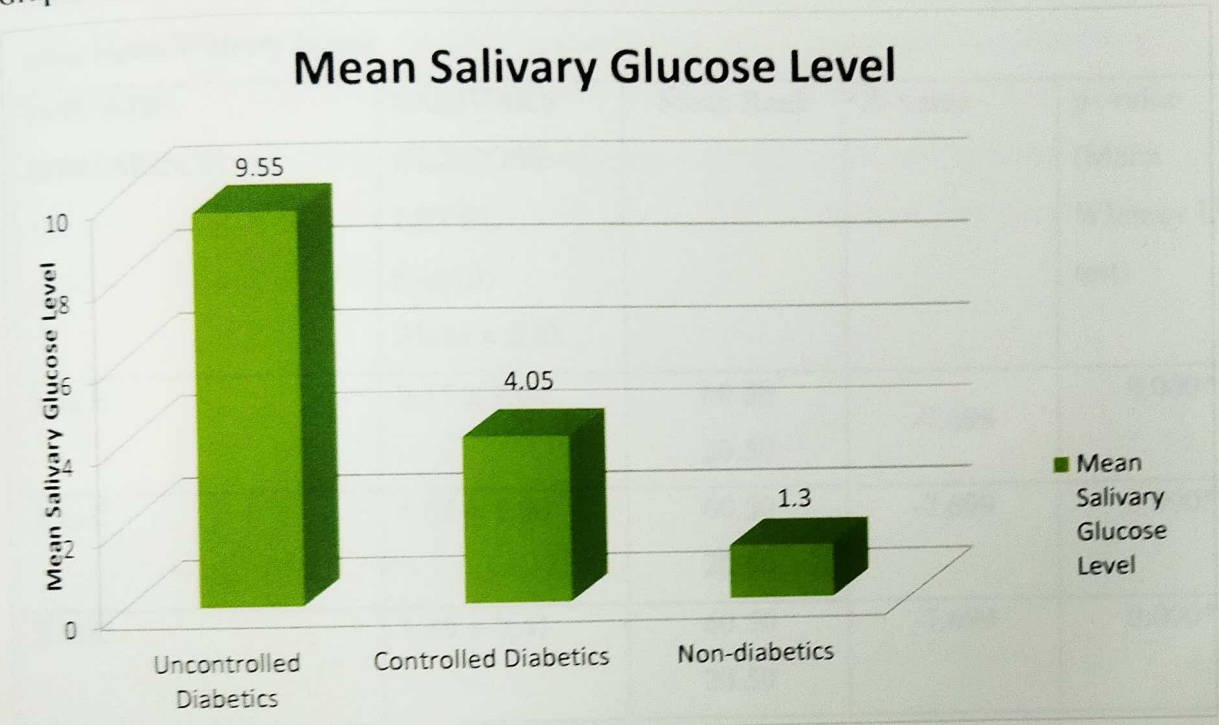


Table 4: Pair-wise comparison of mean Fasting Blood Glucose between groups, using Mann Whitney U test

PAIR-WISE COMPARISON	FASTING BLOOD SUGAR (mg/dl) Mean ± S.D.	Mean Rank	Z- value	p- value (Mann Whitney U test)
A vs B	163.55 ± 18.44	53.08 27.93	-4.84	0.000*
A vs C	134.90 ± 29.38	60.50 20.50	-7.698	0.000*
B vs C	82.61 ± 12.78	58.15 22.85	-6.794	0.000*

*Highly statistically significant ($p<0.01$)

- Table 4 shows that a mean fasting blood glucose between the groups ; it was found to be significantly presented at p=0.000.

Table 5: Pair-wise comparison of mean Salivary Glucose Level between groups, using Mann Whitney U test

PAIR-WISE COMPARISON	SALIVARY GLUCOSE LEVEL (mg/dl) Mean ± S.D.	Mean Rank	Z- value	p- value (Mann Whitney U test)
A vs B	9.55 ± 2.00	60.50 20.50	-7.698	0.000*
A vs C	4.05 ± 1.00	60.50 20.50	-7.699	0.000*
B vs C	1.30 ± 0.41	60.50 20.50	-7.699	0.000*

*Highly statistically significant ($p<0.01$)

- Table 5 shows that when the study findings were compared between A and B , A and C & B and C ;it was found to be significant with uncontrolled diabetics exhibiting highest salivary glucose levels and healthy controls having value of 1.30+_0.41 salivary glucose levels.

Table 6: Correlation between Fasting Blood Glucose and Salivary Glucose Levels for Groups A, B and C, using Spearman's correlation test

GROUP	A	B	C
Correlation Co-efficient (r)	0.345	0.047	0.504
p-value	0.029*	0.772^{NS}	0.001*

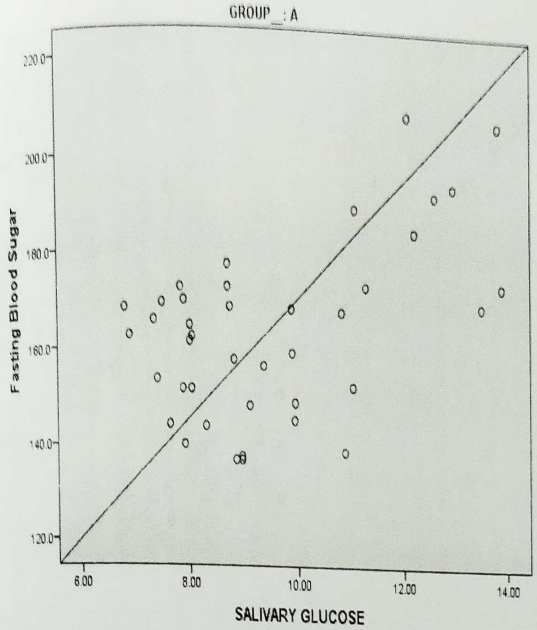
*Statistically significant ($p<0.05$); NS: Non-Significant ($p>0.05$)

Table 5 shows the Correlation between Fasting Blood Glucose and Salivary Glucose Levels for Groups A, B and C, using Spearman's correlation test. The correlation coefficient is 0.345 for uncontrolled diabetics, which signifies a slightly positive correlation between mean fasting blood sugar of 163.55 mg/dl and mean salivary glucose level of 9.55 mg/dl, which was statistically significant at p value of 0.029.

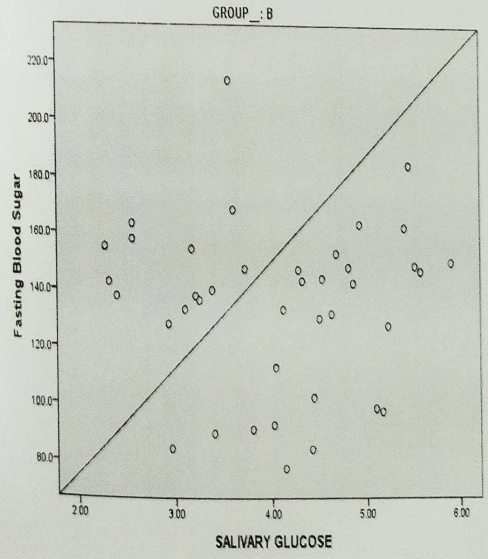
OBSERVATIONS & RESULTS

The correlation coefficient is 0.047 for controlled diabetics, which signifies no positive linear relationship between mean fasting blood sugar and mean salivary glucose level for this group. For group C, correlation coefficient is 0.504, which signifies a moderate positive linear relationship between mean fasting blood sugar and mean salivary glucose level for this group. This correlation was statistically significant at a p value of 0.001.

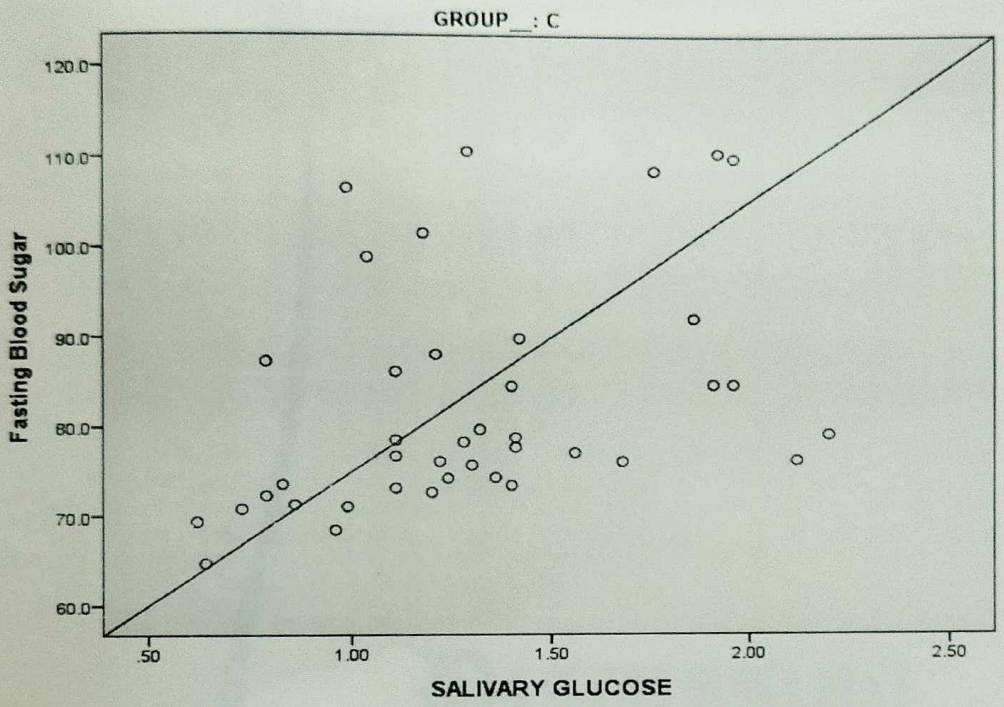
Graph5: Correlation between fasting blood glucose and salivary glucose level for group A

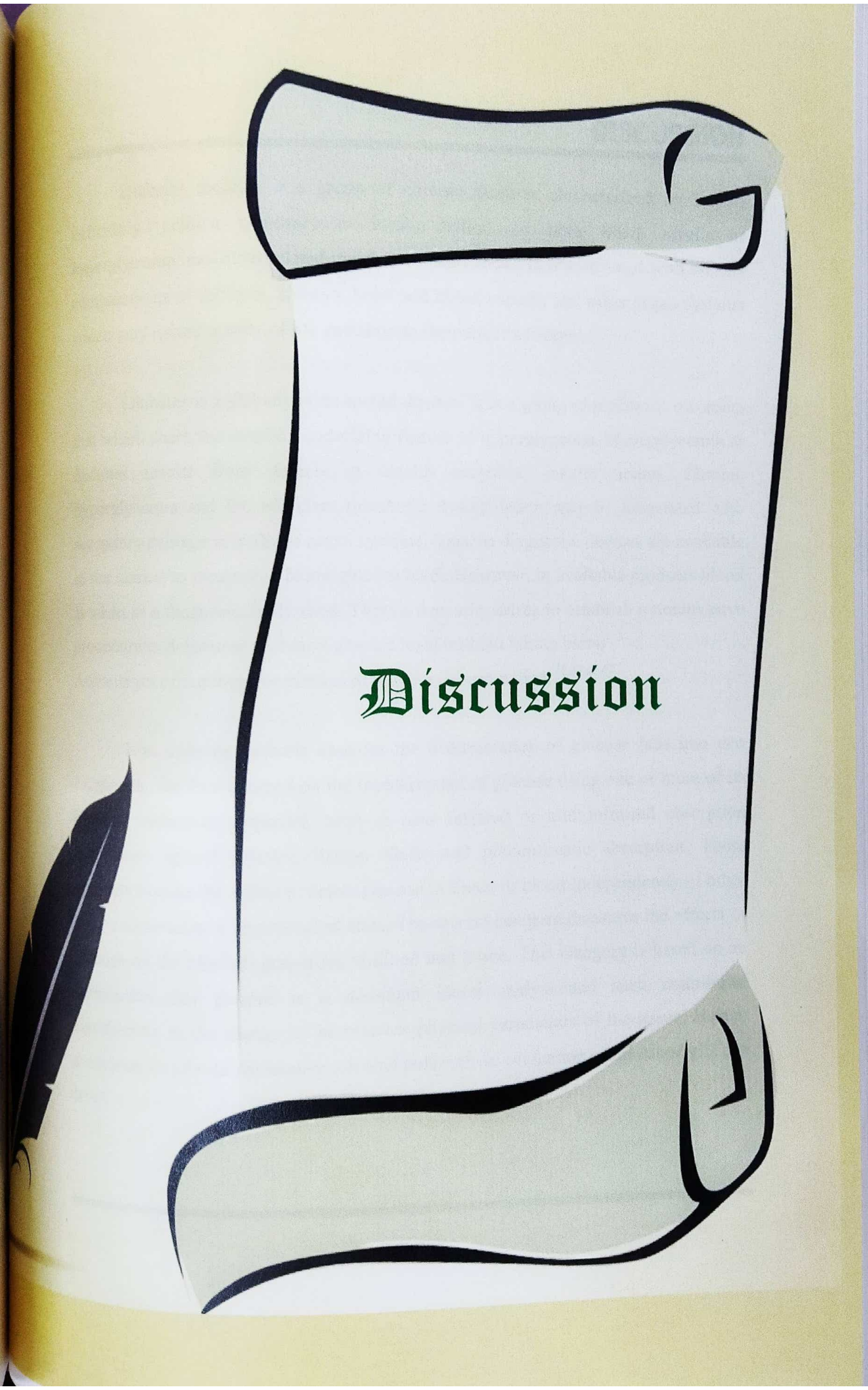


Graph6: Correlation between fasting blood glucose and salivary glucose level for group B



Graph7: Correlation between fasting blood glucose and salivary glucose level for group C





Discussion

Diabetes mellitus is a group of chronic diseases characterized by insulin deficiency, cellular resistance to insulin action, or both which results in hyperglycemia, and other related metabolic disturbances. It is associated with serious complications of the eyes, kidneys, heart and blood vessels, and other organ systems which may impair quality of life and shorten the patient's lifespan.

Diabetes is a globally wide spread disease. It is a group of metabolic disorders that which share the common underlying feature of hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action. Chronic hyperglycemia and the attendant metabolic dysregulation may be associated with secondary damage in multiple organ systems. Various diagnostic devices are available in the market to measure in blood glucose level. However, in available products blood is taken as a diagnostic body fluid. There a necessity arises to establish a noninvasive procedure to determine the blood glucose level without taking blood.
Advantages of noninvasive method of glucose determination:[59,60,61,62]

Non invasive methods used for the determination of glucose falls into two categories. The first is based on the measurement of glucose using one or more of its intrinsic molecular properties, such as near infrared or mid infra-red absorption coefficient, optical rotation, Raman Shifts and photoacoustic absorption. These methods assume the ability to detect glucose in tissue or blood independently of other body components or physiological state. The second category measures the effects of glucose on the physical properties of blood and tissue. This category is based on an assumption that glucose is a dominant blood analyte and such contributes significantly to the change in the relevant physical parameters of the tissue. Hence, measurement of such parameters can lead indirectly to evaluation of the blood glucose level.

The use of saliva for diagnosis is recently on increasing trend rather than blood. Saliva as a diagnostic tool has some distinct advantages. It can be collected non-invasively, and by individuals with limited training, no special equipment is needed for collection. Diagnosis of disease based on the salivary analysis is potentially valuable for children and older adults, since the collection of fluid is associated with fewer compliance problems when compared with a collection of blood [7]. Monitoring of markers in saliva instead of serum is advantageous because saliva collection is a more straight forward and inexpensive process posing no risk or infection or discomfort to the patient. It has been shown that diabetes mellitus affects the saliva composition, flow rate, buffering capacity, viscosity, electrolytic ionic composition, flow rate, buffering capacity, viscosity, electrolytic ionic composition and protein content quite significantly. Therefore, saliva is a well-established biofluid for classifying individuals into diabetics and non-diabetics. Keeping the above points in view, the present study was aimed to correlate blood and salivary glucose level in controlled and uncontrolled diabetics. It was designed to establish a non invasive procedure to measure glucose level using saliva which is most easily obtainable.

A comparative analysis between saliva and blood in the same individual can help us discover the importance of saliva as a diagnostic test. The advantage of using salivary assays over blood assays are, the sampling is very easy to do especially in non medical environment; multiple sample can be collected providing some information than that of single blood sample.

Gender: 87 (72.5%) of the study participants were males, making the majority of the sample. But the salivary glucose levels between males and females showed no significant difference. Several studies also were of the same opinion like Soares MS et al[63], Panda Aabikshyeet et al [3]Nagalaxmi V et al [47] and Shruti Gupta et al [1].

Age: No significant relationship between salivary glucose levels and age in diabetic subjects and non diabetic subjects were observed in this study, which was similar to the studies of Shruti Gupta et al[1], Agarwal et al[31] and Panda Abiskshyeet et al [3].

Fasting blood glucose: The mean fasting blood sugar level for uncontrolled diabetics was 163.55 ± 18.44 and 134.90 + 29.38 for controlled diabetics 82.61 ± 12.78 in the non – diabetics group in the present study. These findings were similar to studies conducted by Shruti Gupta et al [1]who reported mean random blood glucose level in Type 2 diabetes as 188.33 ±50.66. The values are also same when compared to Agarwal RP etal[31] whose study had a FBG level of 171.31 + 54.23 mg/dl in diabetics. In the control group, the FBG level was 82.61 + 12.78 which was in accordance with the study of Agarwal RP et al[31] with FBG of 92.11 + 9.39 mg / dl.

Qureshi et al[64]showed that there is increased leakage of glucose from the ductal cells of the salivary gland, so salivary glucose level is increased in diabetic patients. This is due to microvascular changes in blood vessels and change in the basement membrane in diabetic patients. Hyperglycemia leads to increased formation of advanced glycosylation end products. These AGEs products crosslink proteins such as collagen and extracellular matrix proteins, leading to basement membrane alteration and endothelial dysfunction, which makes them more permeable. This permeability is also increased by other products, such as sorbitol, diacylglycero and fructose 6 phophate which are formed because of chronic hyperglycemia, which explains the increased passage of glucose from the blood into the saliva in diabetes mellitus. This is supported by Belaziet al[65] who proposed that the increased permeability of basement membrane in diabetic patients may lead to enhanced leakage of smaller molecules like glucose into whole saliva via gingival crevices. Due to this increased glucose levels were reported in salivary secretion of patients with diabetes mellitus. The presence and increase of glucose levels in saliva is multifactorial and no single mechanism can be responsible in diabetic patients and

non diabetic patients. The mean fasting blood glucose ranged 163.55 ± 18.44 in the uncontrolled diabetics, 134.90 ±29.38 in the controlled diabetics and 82.61 ± 12.78 in the non – diabetics group which was found to be statistically significant at p = 0.000. The established glucose criteria for the diagnosis of diabetes remain valid. These include the Fasting plasma glucose and 2 hour PG. additionally, patients with severe hyperglycaemia such as those who present with severe classic hyperglycaemic symptoms or hyperglycaemic crisis can continue to be diagnosed when random plasma glucose of≥ 200 mg /dl is found. It is likely that in such cases the health professional would also measure an A1C test as a part of the initial assessment of the severity of the diabetes and that it would be above the diagnostic cut point for diabetes.

Salivary glucose: The mean salivary glucose level in uncontrolled diabetics in the present study was 9.55±2.00 and controlled diabetics were 4.05 + 1.00. This is in accordance with the study conducted by Shruti Gupta et al who found a mean salivary glucose level of 9.48±5.511. The levels were slightly higher when compared to study done by Panda et al[3] who reported salivary glucose level of 4.22 + 3.59 mg/dl. The control population in the present study had a salivary glucose level of 1.30 + 0.41 mg /dl which is lesser when compared to the study of Agarwal et al[31] who reported a FSG level of 6.08 + 1.16 mg /dl, but near to the study of Shreya Gupta et al who reported a level of 3.26 ± 1.66 mg/ dl.

Pair wise comparison of Fasting blood glucose: In the present study, the comparison between blood glucose levels in uncontrolled diabetics versus controlled diabetics showed a mean of 163. 55 + 18.44, uncontrolled diabetics versus control group obtained 134.90 + 29.38 and pair wise comparison between uncontrolled diabetics versus control group was obtained 82.61 + 12.78, which ensured almost identical entities were compared at p value of 0.000 making it statistically significant. Almost similar values were obtained by other studies like Vidya Kadashettiet al[6].

Pair wise comparison of salivary glucose: In the present study, the comparison between blood glucose level in uncontrolled diabetics versus controlled diabetics showed a mean of 9.55±2.00, uncontrolled diabetics versus control group obtained 4.05±1.00 and pair wise comparison between uncontrolled diabetics versus control group was obtained 1.30±0.41, which ensured almost identical entities were compared at p value of 0.000 making it statistically significant.

Correlation between salivary and plasma glucose: In the present study Spearman's correlation test was applied for evaluating the correlation coefficient (r) among the groups. This study recorded a mean fasting blood glucose of 163.55±18.44 and mean salivary glucose (9.55 ± 2.00) in uncontrolled diabetics with r value of 0.345 while in controlled diabetics , mean fasting blood glucose of 134.90±29.38 and mean salivary glucose (4.05 ± 1.00) with r value of 0.047 and in control group , mean fasting blood glucose of 82.61±12.78 and mean salivary glucose (1.30 ± 0.41) were found with r value of 0.504.

Correlation coefficient between fasting blood glucose and salivary glucose in uncontrolled diabetics was found to be 0.345 and p-value=0.029 which was significant. In controlled diabetics, the correlation co-efficient was 0.047 and p-value=0.772 which was non –significant. In non-diabetics, the correlation coefficient(r) was found to be 0.504 and p-value=0.001which was significant. This is in accordance with studies conducted by Arati S. Panchbhai[2] found pearson correlation in uncontrolled diabetics subjects of 0.32 and p-value=0.04 and controlled subjects, pearson correlation was -0.25 and p-value=0.11 while in non –diabetic subjects pearson correlation was 0.16 and p-value=0.51. in study conducted by Shreya Gupta et al[32] , it was found that fasting blood glucose in controlled diabetics , the pearson correlation was found to be 0.901and p-value=0.000 . In uncontrolled diabetics, pearson's correlation was 0.888 and p-value=0.000 while in non-diabetics pearson's correlation was 0.890 and p-value=0.001. M Dhanya et al[8] conducted a

study and found that in diabetics subjects the spearman's coeffient was 0.6342 and in non diabetics it was 0.8809 and both subjects has p-value≤ 0.01 which was highly statistically significant. Sreedevi et al[5] found in their study that in diabetics pretreatment and after treatment, the correlation =coefficient was +0.67 and+0.66 respectively while in controls, it was +0.74. BNVS Satishet al[39] found that correlation coefficient in control group and in diabetic group was 0.45 and 0.54 respectively. Bhumika J Patel et al[38] found in their study that pearson's correlation of fasting blood glucose and salivary glucose were 0.887 and 0.773 respectively. Rathy Ravindranet al[37] (found pearson correlation of 0.394 and p-value of 0.031). A. Aziziet al[40] found in pearson's correlation in diabetic and healthy groups were 0.9 and 0.18 respectively.

However, there is a difference in the mean salivary glucose levels, and this could be due to the differences in methods used for glucose estimation and saliva collection in all the studies. The elevated salivary glucose levels are due to diabetic membranopathy, which leads to leakage across the basement membrane and raised percolation of glucose from blood to saliva. (Fig 5, 6 and 7)

Saliva collection:The method of saliva collection varied between studies. The present study used unstimulated whole saliva samples because of ease of collection and higher patient compliance. The stimulated saliva sample may give an inaccurate measurement of saliva constituents because of increased dilution. Campbell and Mehrotra et al[66,67] also used whole saliva samples, while Forbe et al[68] collected specific parotid saliva.

Saliva indeed is a mirror of our blood as these bio fluids and their molecular components share many similarities. Realization of this fact and the possible utility of saliva as a diagnostic bio fluid using recent technological advances over the past decades has enabled many researchers to develop saliva based technology to detect

the transition between health and disease. It is a cost effective approach for the screening of a large population. but still the fact is that, it is the vital element that sustains life in the oral cavity. [6] Vidya et al

It was found in the present study that patients with serum glucose levels below 130 mg / dl showed a mean salivary glucose level below 2.18 mg /dl. Patients with serum glucose levels between 130 and 200 mg / dl showed a mean salivary glucose level below 4.05 mg /dl and patients with serum glucose levels above 200 mg / dl reflected a mean salivary glucose level of 8.74 mg / dl.

Lack of correlation between saliva glucose and plasma glucose was observed by Marchettiet al[69] in fasting state in both diabetics and non-diabetics and they indicated that degree of metabolic control doesn't affect the way in which salivary glands handle the glucose. This finding is in contradiction to present study result due to saliva they took from the parotid gland and the methodology used for estimation of salivary glucose was different. Nevertheless, majority of the studies supported the correlation between salivary glucose and blood glucose. Some concerns about salivary glucose replacing blood glucose are to be addressed. The raised glucose level is also recorded in gingival fluid, thus the glucose in saliva may not be exclusively of salivary gland origin.

In the research work carried so far, there is no uniformity in the methodology used for salivary glucose estimation. Salivary samples used were assorted as either whole saliva or saliva collected from the individual salivary glands; stimulated and unstimulated saliva was used. Accordingly, techniques for saliva collection also differed.

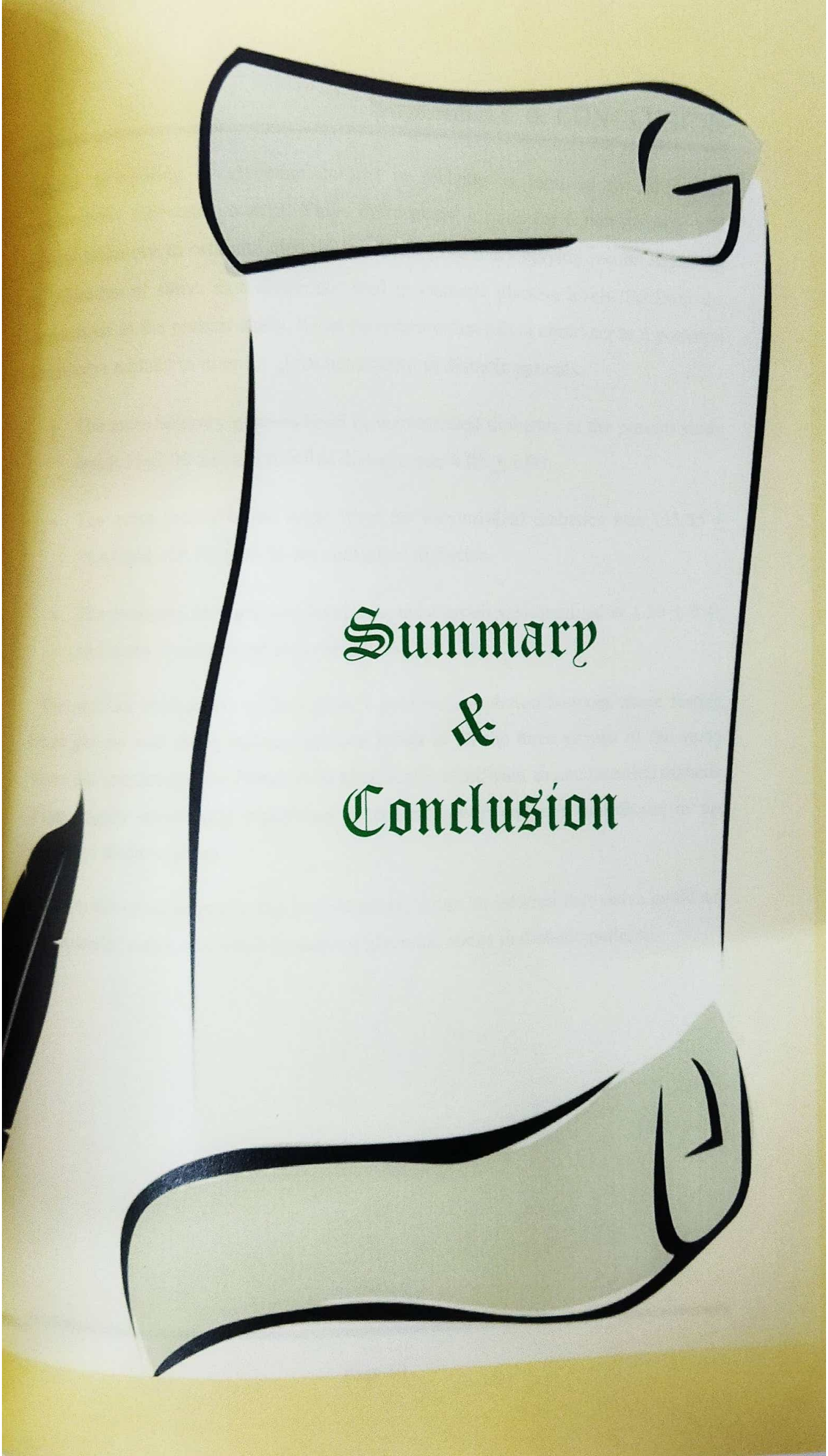
Literature findings suggest that glucose is present in saliva of normal individuals; however, the mechanism of its secretion is still obscure. Both paracellular

and intercellular pathways have been proposed, but this still is an hypothesis rather than an established theory. Many authors have tried to explain the increased glucose content in the salivary secretion of diabetic patients. Lopez et al[70] tried to show that the salivary glands act as filters of blood glucose that are altered by hormonal or neural regulation. Harrison commented that glucose is a small molecule that easily diffuses through semipermeable membranes. Thus large amounts of glucose become available to saliva when blood glucose levels are elevated, as in diabetes. Alterations in the permeability, occurring as a result of basement membrane changes in diabetes, may be an additional explanation for the increased concentration of glucose in saliva. It is well established that the complications of diabetes are due to microvascular changes. Many theories have been put forth to explain the microvascular alterations. To summarize, hyperglycemia leads to increased advanced glycosylation end products, commonly known as "AGEs". These AGEs crosslink proteins such as collagen and extracellular matrix proteins, leading to basement membrane alteration and hence endothelial dysfunction. This alters the microvasculature structure and makes it more permeable. Other products, such as sorbitol, diacylglycerol, and fructose – 6 – phosphate, which are formed because of chronic hyperglycemia also leads to basement membrane alteration by altering the extracellular matrix proteins. The end result is a leaky microvasculature and a leaky basement membrane, which explains the increased passage of glucose from the blood into the saliva in diabetes mellitus.

Also, the increased permeability of basement membrane in insulin dependent diabetes mellitus may lead to enhanced leakage of serum derived components into whole saliva via gingival crevices. The small glucose molecule can easily diffuse via the semipermeable basement membrane. The gingival crevicular fluid is blamed as the culprit for increased glucose levels in salivary secretion. This makes the presence of glucose in saliva is multifactorial and no single mechanism can be blamed.

This metabolic disease is a potential burden on both patients and society because of the high morbidity and mortality associated with infections and its renal, retinal and vascular complications. Thus it is essential to assess the magnitude of the problem and take steps for the early detection and control of DM with regular monitoring over glycemic control. Not only glucose level, there is also alteration of whole salivary constituents, such as salivary sodium, potassium, proteins, amylase, albumin and Ig A and a possible explanation was sought to the prevalence and severity of periodontal disease, dental caries in diabetes mellitus and role of saliva which bought these changes. The routinely employed investigative procedures for glucose monitoring are invasive, but saliva can best serve as a valuable non – invasive diagnostic aid.

However further studies on larger populations and in different geographic areas are required to establish salivary glucose estimation as a diagnostic tool in assessing glucose levels in diabetes mellitus.



Summary & Conclusion

SUMMARY & CONCLUSION

Frequent monitoring of glycemic control in diabetes is required to reduce the complications associated with it. Thus, there arises a need for a non-invasive and painless technique to estimate glucose levels. But there are varying results regarding the utilization of saliva as a diagnostic tool to evaluate glucose levels. So from the observations of the present study, it can be inferred that saliva could act as a potential noninvasive adjunct to monitor glycemic control in diabetic patients.

- The mean salivary glucose level in uncontrolled diabetics in the present study was 9.55±2.00 and a controlled diabetic was 4.05 ± 1.00.
- The mean fasting blood sugar level for uncontrolled diabetics was 163.55 ± 18.44 and 134.90 ± 29.38 for controlled diabetics.
- The mean salivary glucose level in control group was obtained at 1.30 ± 0.41 and mean fasting blood sugar was 82.61 ± 12.78.

The statistics applied to our data show a positive correlation between mean fasting blood glucose and mean salivary glucose levels in all the three groups of the study where the correlation was found to be statistically significant in uncontrolled diabetic group, highly statistically significant in control group and non significant in the controlled diabetic group.

So from the observations of the present study, it can be inferred that saliva could act as a potential noninvasive tool to monitor glycemic status in diabetic patients.

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Annexures

ANNEXURE 1

CASE HISTORY PROFORMA

GLUCOSE ESTIMATION IN THE SALIVARY SECRETION OF DIABETES MELLITUS PATIENTS

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY
BabuBanarasi Das College of Dental Sciences, Lucknow (U.P.)

Name:

Age:

Sex:

Medical History:

Diabetic Type-

Type I	Type II	Gestational Diabetes

Duration : Since which year :

Any systemic illness	Present	Absent
History of smoking	**Yes**	**No**
History of chewing tobacco	**Yes**	**No**
History of alcoholism	**Yes**	**No**
History of any prior radiotherapy of head & neck region	**Yes**	**No**

Vital Signs:

B.P	
Pulse	
Temperature	
Respiratory rate	

Family History :

Clinical Examination:

Oral lichen planus	Yes	No
Leukoplakia	Yes	No
Candidiasis	Yes	No
Oral Submucous fibrosis	Yes	No
Any other lesion	Yes	No

Clinical Reading:

HbA_1C	%
Salivary Glucose	mg/dl
Fasting blood sugar	mg/dl

Signature of Student **Signature of Guide**

ANNEXURE 2

CONSENT FORM

Title of the study.....

Study Number.....

Subject's Full Name.....

Date of Birth/Age.....

Address of the Subject.....

Phone No. and email address.....

Qualification.....

Occupation: Student/Self employed/Service/Housewife/Other

1. I confirm that I have read and understood the Participant Information Document dated ..... for the above study and have had the opportunity to ask questions

   OR

   I have been explained the nature of the study by the investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with the free will without any duress and that I am free to withdraw at any time, without given any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trail. However, I understand that my identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use any data or results that arise from this study provided such a use is only for scientific purpose(s).

   I agree to participate in the above study for the future research

   Yes [ ] No [ ] Not Applicable [ ]

5. I have been explained about the study, and have fully understood them. I have also read and understand the participant/volunteer's information document given to me.

Signature/Thumb impression of the subject/Legally acceptable Representative.....

Signatory's Name.....Date.....

Signature of Investigator's Name.....

Study Investigator's Name.....Date.....

Signature of the witness.....

Name of witness.....Date.....

Received a signed copy of the duly filled consent form

Signature/Thump Impression of the subject/Legally acceptable representative.....Date.....

सहमति पत्र

अध्ययन का शीर्षक.....

अध्ययन संख्या.....

विषय का पूरा नाम.....

जन्म / आयु की तिथि.....

विषय का पता.....

फ़ोन नंबर और ईमेल पता.....

योग्यता.....

व्यवसाय: छात्र / स्वयं नियोजित / सेवा / गृहिणी / अन्य

1. मैं पुष्टि करता हूं कि मैंने प्रतिभागी सूचना दस्तावेज को पढ़ और समझ लिया है .....उपर्युक्त अध्ययन के लिए और प्रश्न पूछने का अवसर मिला है

या

मुझे जांचकर्ता द्वारा अध्ययन की प्रकृति की व्याख्या की गई है और मुझे प्रश्न पूछने का अवसर मिला है।

2. मैं समझता हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और किसी भी दुविधा के बिना मुफ्त इच्छा के साथ दी गई है और मैं किसी भी समय बिना किसी कारण के और बिना चिकित्सा देखभाल या कानूनी अधिकारों के प्रभावित किए बिना वापस लेने के लिए स्वतंत्र हूं।

3. मैं समझता हूं कि परियोजना के प्रायोजकए प्रायोजक की तरफ से काम करने वाले अन्य लोगए नैतिकता समिति और नियामक प्राधिकरणों को वर्तमान अध्ययन के संबंध में और मेरे आगे के किसी भी शोध के संबंध में मेरे स्वास्थ्य रिकॉर्ड देखने की अनुमति की आवश्यकता नहीं होगी इसके संबंध में आयोजित किया गयाए भले ही मैं निशान से पीछे हट जाऊं। हालांकिए मैं समझता हूं कि मेरी पहचान तीसरे पक्ष को जारी या प्रकाशित किसी भी जानकारी में प्रकट नहीं होगी।

4. मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणामों के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं बशर्ते ऐसा उपयोग केवल वैज्ञानिक उद्देश्यों के लिए है।

5. मैं भविष्य के शोध के लिए उपर्युक्त अध्ययन में भाग लेने के लिए सहमत हूं

हां ☐ नहीं ☐ लागू नहीं ☐

6. मुझे अध्ययन के बारे में समझाया गया है, और उन्हें पूरी तरह से समझ लिया है। मैंने मुझे दिए गए प्रतिभागी / स्वयंसेवक के सूचना दस्तावेज को भी पढ़ और समझ लिया है।

विषय / कानूनी रूप से स्वीकार्य के हस्ताक्षर / अंगूठे की छाप

प्रतिनिधि.....

हस्ताक्षरकर्ता का नाम.....

जांचकर्ता के नाम का हस्ताक्षर.....

अध्ययन जांचकर्ता का नाम .... दिनांक.....

गवाह का हस्ताक्षर.....

गवाह का नाम ..... दिनांक .....

विधिवत भरे सहमति फॉर्म की एक हस्ताक्षरित प्रति प्राप्त की

विषय / कानूनी रूप से स्वीकार्य प्रतिनिधि के हस्ताक्षर / थंप इंप्रेशन

..... दिनांक .....

ANNEXURE 3

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **Glucose Estimation in the Salivary Secretion of Diabetes Mellitus Patients** submitted by **Dr. Siddharth Jaiswal** Post graduate student from the Department of **Oral Medicine and Radiology** as part of MDS Curriculum for the academic year 2016-2019 with the Accompanying proforma was reviewed by the institutional research committee present on **7th and 8th December 2016** at BBDCODS. The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the institutional ethics committee for granting ethical approval.

Prof. (Dr). Vivek Govila

Principal

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Faculty Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow 226028

Chairperson Institutional Research Committee

ANNEXURE 4

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the Vth Institutional Ethics Sub-Committee

BBDCODS/03/2017

IEC Code: 36

Title of the Project: Glucose Estimation in the Salivary Secretion of Diabetes Mellitus Patients.

Principal Investigator: Dr. Siddharth Jaiswal **Department:** Oral Medicine & Radiology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Siddharth Jaiswal

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 02nd March, 2017.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Neerja Singh Member	Prof. & Head, Department of Pedodontics, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics, BBDCODS, Lucknow
4.	Dr. Manu Narayan Member	Reader, Department of Public Health Dentistry, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The proposal was reviewed, comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 17/04/17.
(Dr. Lakshmi Bala)
Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028
Member Secretary
IEC

(Dr. Vivek Govila)
Principal
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028
BBDCODS

ANNEXURE 5

S.No	Name	Age	Sex	HbA1C	Fasting Blood Sugar	Duration	SALIVARY GLUCOSE_A	GROUP_	GROUP_
	UNCONTROLLED DIABETES MELLITUS								
1.	Surekha	55	F	8.4	162.5	10	6.91	2	A
2.	Ghanshyam	42	M	8.1	168.3	7	6.82	2	A
3.	V.K. Srivastava	60	M	8.2	165.7	14	7.36	2	A
4.	Dheerendra Singh	35	M	12.3	184.6	2	12.23	2	A
5.	Shailendra Singh	47	M	8.2	143.8	8	7.65	2	A
6.	Jagjeet	54	M	10.2	189.1	7	11.10	2	A
7.	Rajendra	40	M	9.6	144.9	1	9.95	2	A
8.	Sunita	57	F	9.1	156.1	11	9.38	2	A
9.	Shri Ram	45	M	10.2	158.9	2	9.91	2	A
10.	Saroj	40	M	14.6	192.4	2	12.62	2	A
11.	AbhaVerma	50	F	14.4	207.8	3	13.83	2	A
12.	Meenu	55	F	8.8	139.7	8	7.92	2	A
13.	Gyan	38	M	10.2	168.2	2	9.90	2	A
14.	Yunus Ali	58	M	8.1	153.2	4	7.42	2	A
15.	Pawan Kumar	40	M	8.6	143.5	2	8.31	2	A
16.	Renu	40	F	8.1	151.2	4	8.05	2	A
17.	Sachin	45	M	8.7	136.6	1	8.86	2	A
18.	P.K. Singh	36	M	13.6	174.1	3	13.90	2	A
19.	Meera	45	F	9.8	162.2	2	8.06	2	A
20.	Archana	41	F	11.1	167.8	3	10.84	2	A
21.	ShikhaSoni	52	F	9.1	157.4	7	8.83	2	A
22.	Dheeraj	35	M	8.8	164.6	7	8.02	2	A
23.	Shyam	40	M	8.7	169.8	1	7.91	2	A
24.	Ram	56	M	8.8	161.2	3	8.02	2	A
25.	Rama Nand	46	M	9.4	172.6	5	8.72	2	A
26.	Awadhesh	60	M	8.5	177.2	6	8.72	2	A
27.	Shakuntala	57	F	8.1	169.3	2	7.51	2	A
28.	Neena	60	F	8.4	172.5	9	7.85	2	A
29.	Sushil	53	M	8.8	137.3	6	8.97	2	A
30.	Anil	63	M	8.1	151.2	12	7.89	2	A
31.	Akhilesh	40	M	8.8	168.5	3	8.76	2	A
32.	PyareLal	50	M	10.2	152.1	3	11.05	2	A
33.	Sanjeev Singh	45	M	13.4	194.4	3	12.97	2	A
34.	Ganga Jal	50	F	8.8	147.8	2	9.12	2	A
35.	UditaVerma	40	F	9.1	138.6	6	10.89	2	A
36.	Mamta	44	F	9.4	148.5	4	9.96	2	A
37.	Rajesh Singh	55	M	12.1	173.2	8	11.30	2	A
38.	Mona	45	F	12.6	208.9	2	12.10	2	A
39.	Rahul Gaur	39	M	9.8	136.6	1	8.97	2	A
40.	P. Lal	59	M	13.8	169.8	5	13.51	2	A
	CONTROLLED DIABETES MELLITUS								
41.	Suraj Dixit	37	M	7.1	161.9	2	5.41	1	B
42.	Radhika	46	F	7.2	149.7	4	5.91	1	B
43.	S.S. Bhasin	50	M	6.3	213.5	5	3.56	1	B
44.	Wazid Khan	51	M	6.6	147.3	6	3.72	1	B
45.	Jag Prasad	40	M	6.0	128.0	2	2.93	1	B
46.	Jagat Pal	47	M	7.4	163.0	7	4.93	1	B
47.	Ram Nath	48	M	7.2	184.3	7	5.46	1	B
48.	Dinesh	51	M	7.4	131.6	9	4.63	1	B
49.	Pragya	37	F	6.0	157.6	2	2.56	1	B
50.	Kaushlendra	39	M	7.4	136.3	1	3.25	1	B
51.	Sunil	38	M	7.6	147.8	5	4.81	1	B
52.	Jagat Pal	40	M	7.4	143.9	2	4.53	1	B
53.	Saroj Gupta	65	F	6.8	154.2	2	3.17	1	B
54.	ChediLal	60	M	7.6	152.7	8	4.68	1	B
55.	Jai Ram	44	M	6.1	142.7	3	2.32	1	B
56.	Anupam	51	M	7.9	148.4	8	5.52	1	B
57.	Sumit	40	M	6.8	154.9	2	2.28	1	B

58.	Shankar	39	M	6.3	133.1	3	3.10	1	B
59.	Rajeev	50	M	7.2	142.3	6	4.86	1	B
60.	Arvind	52	M	7.8	146.5	8	5.58	1	B
61.	SunitaVerma	54	F	6.8	139.9	6	3.38	1	B
62.	Sangeeta	40	F	7.4	133.1	3	4.12	1	B
63.	Wazid	51	M	6.5	137.9	5	3.21	1	B
64.	Rameshwar	51	M	6.3	137.8	4	2.40	1	B
65.	Ghanshyam	56	M	7.4	168.2	3	3.60	1	B
66.	Amitabh	41	M	7.0	163.1	11	2.56	1	B
67.	Ajay Pratap	40	M	7.5	147.0	3	4.28	1	B
68.	Sarvan	36	M	7.0	143.1	2	4.32	1	B
69.	Anand	50	M	6.7	127.4	7	5.23	1	B
70.	Shanti	50	F	6.1	112.6	5	4.04	1	B
71.	HariMurti Singh	37	M	6.7	130.0	4	4.50	1	B
72.	Girish Gupta	60	M	6.7	102.0	10	4.44	1	B
73.	Deepanshi	40	F	6.2	92.6	1	4.02	1	B
74.	Seeta Singh	52	F	6.4	77.3	5	4.14	1	B
75.	B.B. Singh	55	M	6.8	98.3	4	5.10	1	B
76.	SatyaPrakash	46	M	6.7	84.1	2	4.42	1	B
77.	Umesh	55	M	6.2	89.6	6	3.40	1	B
78.	Ahmad Ali	60	M	6.7	91.0	5	3.80	1	B
79.	A.B. Singh	50	M	6.0	84.3	4	2.96	1	B
80.	Reema	41	F	7.1	97.2	2	5.17	1	B
					HEALTHY SUBJECTS				
81.	Sanjeevjaiswal	45	M	5.8	110.4		1.92	0	C
82.	Rajeev jaiswal	39	M	5.1	98.9		1.04	0	C
83.	Shobhajaswal	58	F	5.3	110.6		1.29	0	C
84.	Devyanisingh	38	F	4.9	101.5		1.18	0	C
85.	Manjulagupta	55	F	5.7	109.8		1.96	0	C
86.	sushantgupta	36	M	5.5	108.4		1.76	0	C
87.	gauravigupta	37	M	5.2	106.4		0.99	0	C
88.	Ahishek	38	M	5.6	79.8		1.32	0	C
89.	SakshiVerma	37	F	5.5	76.2		1.22	0	C
90.	Dev Brat	38	M	5.3	78.4		1.28	0	C
91.	Praveen	35	M	4.4	71.3		.86	0	C
92.	Mukesh Kumar	35	M	5.8	86.2		1.11	0	C
93.	Satya Narayan	45	M	5.1	76.3		2.12	0	C
94.	D.P. Yadav	40	M	5.7	88.1		1.21	0	C
95.	Rajveer	40	M	4.8	72.7		1.20	0	C
96.	Anita	36	F	5.9	89.9		1.42	0	C
97.	M.N. Gupta	50	M	5.7	74.4		1.36	0	C
98.	Rajendra Gupta	45	M	4.9	64.8		.64	0	C
99.	Mohan Chaddha	51	M	5.4	73.2		1.11	0	C
100.	Ghanshyam	40	M	5.2	74.3		1.24	0	C
101.	Ranjana Devi	60	F	5.3	77.8		1.41	0	C
102.	Harsh Yadav	38	M	4.0	70.8		.73	0	C
103.	K.K. Verma	39	M	4.1	73.6		.83	0	C
104.	V.K. Singh	40	M	5.5	77.2		1.56	0	C
105.	Vijay	37	M	4.1	72.3		.79	0	C
106.	Pushpa Devi	35	F	5.1	73.5		1.40	0	C
107.	AjeetChauhan	38	M	5.0	76.2		1.68	0	C
108.	SachidaNand	45	M	4.6	71.1		.99	0	C
109.	Anil Agrawal	55	M	5.6	84.7		1.96	0	C
110.	Sushil	40	M	5.2	78.9		1.41	0	C
111.	Jagriti	37	F	4.8	75.8		1.30	0	C
112.	GopeshTiwari	40	M	4.1	69.4		.62	0	C
113.	V.K. Gupta	59	M	5.2	79.2		2.20	0	C
114.	J.P. Maurya	43	M	4.7	78.6		1.11	0	C
115.	U.P. Singh	44	M	5.1	84.7		1.91	0	C
116.	ReenaJaiswal	36	F	4.3	68.5		.96	0	C
117.	R.K. Jaiswal	38	M	5.4	76.8		1.11	0	C
118.	UditaRawate	40	F	5.9	84.6		1.40	0	C
119.	Dhirendra Singh	39	M	5.1	92.1		1.86	0	C
120.	PawanPrakash	42	M	4.4	87.3		.79	0	C