ESTIMATION OF ADVANCED GLYCATION END PRODUCTS IN SALIVA OF DIABETIC PATIENTS

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH VI

ORAL PATHOLOGY AND MICROBIOLOGY

MARCH 2010

CERTIFICATE

This is to certify that this dissertation titled "**ESTIMATION OF ADVANCED GLYCATION END PRODUCTS IN SALIVA OF DIABETIC PATIENTS"** is a bonafide record of work done by **S.V. ARATHI SAGAR** under our guidance during her postgraduate study period between 2007-2010.

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Aims & Objective:

1. To estimate and compare salivary and blood glucose level in diabetic (controlled and uncontrolled) and non diabetic patients.

2. To estimate and compare salivary and blood AGE level in diabetic (controlled and uncontrolled) and non diabetic patients.

3. Assess the periodontal status of diabetic (controlled and uncontrolled) and non diabetic patients and its correlation to salivary glucose and salivary AGE levels.

Hypothesis

1. There is increase in salivary and serum AGE level in diabetic patients compared to non diabetic patients.

2. Salivary AGE level is directly proportional to blood AGE level.

3. Increase in salivary glucose and salivary AGE level is associated with poor periodontal status in diabetic patients

STUDY SETTING

Diabetic patients attending (n = 50) the Department of Diabetology, Voluntary Health service (VHS), Chennai and non-diabetic controls (n = 25) attending the department of Oral and Maxillofacial Pathology, Ragas Dental College & Hospital were included in the study. Verbal consent was obtained from every individual participating in the study .The study was approved by Institutional Review Board.

The patient were divided into three groups

GROUP 1: (n = 25)

Comprised of controlled diabetic patients of 40-60 years of age. Controlled diabetes was defined as those having random non-fasting plasma glucose values >120 mg/dl and < 200mg/dl (controlled diabetics)

GROUP 2: (n = 25)

Comprised uncontrolled diabetic patients of 40-60 years of age. Uncontrolled diabetes was defined as those having random non fasting plasma glucose values >200mg/dl (uncontrolled diabetics)

GROUP 3: (n = 25)

Comprised of non -diabetic patients whose age and sex were matched with Group I and Group II with random non fasting plasma glucose values <120mg/dl (non-diabetics)

EXCLUSION CRITERIA

- Patient with removable partial or complete dentures.
- Patients who had taken antibiotics or antifungal medication for any condition for a period of 15 days before the sample collection.
- Patients with immunocompromised condition other than Diabetes mellitus
- Pregnant women
- Patients who had the habit of tobacco/areca nut use

MATERIALS **(figure 1 and 2)**

- Test tubes
- Tourniquet
- Sterile disposable syringes
- Mouth mirror
- Explorer
- Light source
- Eppendof tubes
- Saliva spitting container
- Ice flask
- Cotton
- Spirit
- CPITN (Community Periodontal Index for Treatment Needs)
- EDTA (Ethylene diamine tetraacetic acid)
- Fluoride
- NMR tubes

- NMR tube holder
- Acetone
- Pipette

REAGENTS AND CHEMICALS **(Figure 3)**

1. Glucose estimation kit: Glucose oxidase method

Contents of the reagent:

1000 ml Enzyme reagent

Phosphate buffer (ph 7.5) 0.1mmol/l

4-Aminophenazone 0.25mmol/l

Phenol 0.75mmol/l

Glucose oxidase >15KU/l

Peroxidase >1.5KU/l

Mutarotase >2.0KU/l

Stabilizers

3ml standard

Glucose 100mg/dl or 5.5mmol/l

2. Glycosylated hemoglobin kit: Ion-exchange resin method (figure 4)

Contents of the reagent:

Ion exchange resin 25x3ml

(Predispensed Tubes)

Lysing reagent 2.5ml

Control (10%GHb) 1x1ml

Resin separators 25nos

3, Nuclear magnetic resonance spectroscopy

Deuterium oxide 99 atom %

EQUIPMENTS **(figure 5, 6, and 7)**

- Autoclave
- Semi-automated analyzer
- Refrigerator
- 500 MHz Nuclear magnetic resonance spectroscopy
- NMR sample mixer
- NMR software

PROCEDURE

1. **History**

   A detailed case history was taken for all patients which included their habits, medication and oral hygiene habits

2. **Collection of blood sample**

   2ml of venous blood was drawn from the brachial vein for estimation of blood glucose and glycosylated hemoglobin levels.

3. Estimation of blood and salivary glucose level done by **glucose oxidase method**

4. Estimation of glycoslated hemoglobin was done using **ion-exchange resin method**

5. Collection of unstimulated saliva sample - Spit technique

   Patient was asked to sit in the dental chair with head tilted in front and instructed not to speak/swallow or do any head movements during the procedure In the beginning the patient was asked to swallow any saliva if present in the

mouth .After this the patient was instructed to spit in a sterile graduated container

6. Glucose levels of unstimulated saliva were estimated using glucose oxidase method in a semi-automated analyzer. The glucose standard was diluted ten times for estimation of salivary glucose levels.

LABORATORY METHODS:

1. Estimation of blood and salivary glucose:

100µl of saliva and 0.2ml of blood samples were mixed with the reagent in the ratio of 1:3 and incubated for 5 minute at 37 C. The absorbance of standard and the samples against the reagent blank were measured. For salivary glucose estimation 100mg glucose standard which is usually used for blood glucose estimation was diluted at 1: 10 ml to a final concentration of 10mg/dl

Principles:

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). Hydrogen peroxide that is formed is catalysed by peroxidase (POD) and reacts with phenol and 4-aminophenazone to give a red – violet colour that is indicative of the quinoneimine dye. The amount of coloured complex formed is proportional to the glucose concentration and can be photometrically measured.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4- aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{Qunoneimine} + 4\,H_2O$$

2. Estimation of glycosylated hemoglobin in blood:

A. Hemolysated preparation:

1. Dispense 0.5ml lysing reagent into tubes labeled into tubes labeled as control (C) and test (T).
2. Add 0.1ml of the reconstituted control and well mixed blood sample into the appropriately labeled tubes. Mix until complete lysis is evident.
3. Allow to stand for 5 minutes.

B. Glycosylated hemogobulin (GHb) separation:

1. Remove cap from Ion –exchange resin tubes and label as control and sample.
2. Add 0.1ml of the hemolysate from step A into the appropriately labeled Ion exchange resin tubes
3. Insert a resin separator into each tube so that the rubber sleeve is approximately 1 cm above the level of the resin suspension.
4. Mix the tubes on a mixer continuously for 5 minutes.
5. Allow the resin to settle, push the resin separator into the tubes until the resin is firmly packed.
6. Pour or aspirate such supernatant directly into the cuvette and measure each absorbance against distilled water

Total Hemoglobin (THb) fraction:

1. Dispense 5ml of distilled water into the tubes labeled as control and sample
2. Add to it 0.02 ml of hemolysate from step A into appropriately labeled tube. Mix well
3. Read each absorbance against distilled water.

Calculations:

$$\text{Ratio of control (Rc)} = \frac{\text{Abs. Control GHb}}{\text{Abs. Control THb}}$$

$$\text{Ratio of sample (Rs)} = \frac{\text{Abs. Sample GHb}}{\text{Abs. Sample THb}}$$

Using the conversion chart in (**Annexure 2)** HbA1c % is obtained from total glycosylated hemoglobin ($GHbA_1$)

3. Estimation of Salivary AGE level using NMR [7]

To quantitate AGE concentration, proton-NMR (^{1}H-NMR) measurements were carried using a Jeol ECA 500 FT spectrometer (Jeol Japan) ® operating field strength of 500 MHz. Sample were made with 0.1ml of saliva mixed with 0.6ml of D_2O (99.95% purity,fisher scientific) and mixed thoroughly by vortex for 3 minutes at room temperature. The sample was poured into a Wilmad precision glass® NMR test tube with an inner diameter of 5mm.All spectra were recorded at a controlled probe temperature of 21/25˚C The spectra processing was carried out using Jeol Delta software®. The signal from deuterium oxide was used to optimize the homogeneity of

the magnetic field, and the fine adjustment was done by inspection of the free – induction decay obtained without water suppression. A field frequency was provided by detecting the deuterium signal of deuterium oxide. The proton spectra were obtained by using a flip angle of 90 degree. Pulse conditions were 128 accumulations. The relative intensity of the resonance of AGEs were determined by comparison with the intensity of the resonance of D_2O.Resonance intensity can be used to determine concentration, provided that the line widths are comparable. Peak height can be used to determine concentrations, provided that the line widths are comparable. Using the internal references, concentrations were calculated from the peak heights of identified substances which revealed a linear relationship between concentration and peak height. Therefore, the resonance intensity of the respective substance was used for quantification.

Assessment of periodontal status [8] (Figure 8)

Clinical attachment loss / attachement level was the criteria used to determine the periodontal status of individual teeth. Clinical attachment loss is the distance between the base of the pocket and a fixed point on the tooth such as cementoenamel junction. A manual CPITN probe was used to determine the pocket depth. The pocket depth was measured on six sites of the all the teeth present. The surfaces probed were mesio buccal/labial, mid-Buccal /labial, disto- buccal / labial, mesio-lingual / palatal, mid-lingual / palatal, disto-Lingual / palatal and the scores were recorded in the appropriate box in the chart (Annexure I).The measurements were taken when a slight resistance to the probe penetration was felt and care was taken to assure that all the measurements were made with the probe tip parallel to the tooth axis.

Determining the level of attachment:

When the gingival margin is located on the anatomic crown, the level of attachment is determined by subtracting the distance from the gingival margin to the CEJ from the depth of the pocket. If both are the same, the loss of attachment is zero.

When the gingival margin coincides with the CEJ, the loss of attachment equals the pocket depth.

When the gingival margin is located apical to the CEJ, the loss of attachment is greater than the pocket depth, and therefore the distance between the CEJ and gingival margin should be added to the pocket depth.

All the teeth with probing depth ≥ 4mm or clinical attachment loss of ≥ 1mm was considered as involved with periodontal disease. The total number of teeth with periodontal diseases was counted. The number of teeth in the oral cavity was counted and a percentage was derived to determine whether the disease was localized or generalized. if less than thirty percent of teeth were involved it was considered as localized periodontitis and more than thirty percent was considered as generalized periodontitis.

Codes:

Code – 0 – no periodontal disease (Healthy periodontium)

Code – 1 – Bleeding observed during or after probing

Code – 2 – Calculus or other plaque, retentive factors either seen or felt during probing

Code – 3 – Pathological pocket 4 to 5 mm in depth

Gingival margin situated on the black band of the probe

Code – 4 - Pathological pocket of 6mm or more in depth, black band of the probe not visible.

Data entry, database management and all statistical calculations were performed with the aid of the Statistical Package for the Social Sciences (SPSS, version 11.5) software. Descriptive statistics were calculated for all variables. Differences in means between more than two groups were assessed using the one way (ANOVA) and post hoc analysis using Tukey's test. Relationships between variables were evaluated by Pearson's correlation coefficient. Linear regression line was drawn to find the scatterness of the points between random blood glucose and HbA_1C, random blood glucose and salivary glucose, random blood glucose and salivary AGE, salivary AGE and HbA_1C, salivary glucose and salivary AGE levels, salivary glucose and periodontal status, salivary AGE and periodontal status. A p value of <0.05 was considered to be statistically significant. When the observed value of the correlation coefficient did not show statistical significance, the result was considered as a non – linear relationship.

INTRODUCTION REVIEW

HISTORY (2004) [4]

The word Diabetes was first used by Aretaeus of Cappadocia in the 2nd century AD. It comes from the Greek, meaning siphon (because the fluid does not remain in the body, but uses the man's body as a channel whereby to leave it. Aretaeus gave a clinical description of the disease, noting the increased flow, thirst and weight loss.The sweet, honey taste of urine in polyuric states, which attracted ants and insects, was reported by Hindu physicians such as (Susruta) during the 5th and 6thcentury AD. John Rollo (d.1809) was the first to apply the adjective Mellitus to the diasease. 19th century Claude Bernard (1813-1878) discovered that sugar appeared in urine was stored in the liver.Oskar Minkowski and Joseph Von Mering (1849-1908) showed pancreatic disorder causes diabetes.Paul Langerhans (1847-1888) was the first to describe the clusters of cells in the pancreas and named it as islets of langerhans and suggested that they were endocrine tissues of the pancreas that produced a glucose –lowering hormone.Insulin was discovered in 1921 at the university of Toronto, Canada by Frederick et al .By 1950 it was accepted that tissue complications, such as occur in the eye and kidney continued to develop in long standing diabetes in spite of insulin treatment.

DEFINITION

Diabetes mellitus by (WHO) is a condition in which the pancreas no longer produces enough insulin or when cells stop responding to the insulin that is produced, so that glucose in the blood cannot be absorbed into the cells of the body. Symptoms include frequent urination, lethargy, excessive thirst, and hunger. The treatment includes changes in diet, oral medications, and in some cases, daily injections of insulin[4]

CLASSIFICATION OF DIABETES (2004) [9]

1. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency) IDDM

a. Immune-mediated diabetes. - Previously encompassed by the terms insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Seen in Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia.

b. Idiopathic diabetes - These patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Individuals with this form of diabetes suffer from episodic ketoacidosis

2. Type 2 diabetes (NIDDM)

Ranges from predominantly insulin – resistant, with relative insulin deficiency, to predominantly insulin- secretory defect, with or without insulin resistance

3. Other specific types

a. Genetic defects of β-cell function

Chromosome 12,HNF-1α(formerly MODY-3)(maturity- onset diabetes of the young

Chromosome 7, glycokinase (formerly MODY-2)

Chromosome 20, HNF-4α (formerly MODY-1)

Mitochondrial DNA

Insulinopathies

B. Genetic defects of insulin action

1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipoatrophic diabetes

C. Diseases of the exocrine pancreas

1. Pancreatitis
2. Trauma/pancreatectomy
3. Neoplasia
4. Cystic fibrosis
5. Haemochromatosis
6. Fibrocalculous pancreatopathy

D. Endocrinopathies

1. Acromegaly
2. Cushing's syndrome
5. Hyperthyroidism
6. Somatostatinoma

3. Glucagonoma

4. Plaeochromocytoma

7. Aldostatinoma

E. Drug induced

1. Glucocorticoids

2. Thiazides

3. Vacor

4. Pentamidine

5. Nicotinic acid

6. Thyroid hormone

7. Diazoxide

8. Dilantin

9. Interfron – α

F. Infections

1. Congenital rubella

2. Cytomegalovirus

G. Other genetic syndromes sometimes associated with diabetes

1. Down's syndrome

2. Klinefelter's syndrome

3. Turner's syndrome

4. Wolfram's syndrome

5.Huntington's syndrome

4. Gestational diabetes

DIAGNOSTIC CRITERIA FOR DIABETES (2008) [9]

Diagnosis of diabetes and impaired glucose homeostasis, ADA guidelines (Standards of medical care in diabetes, 2007)

Fasting glucose

Normal	<100mg/dl
Prediabetes: impaired fasting glucose	100-125mg/dl
Diabetes	>126mg/dl

(oGTT) or random glucose

Normal	<140mg/dl
Prediabetes: impaired glucose tolerance	140-199mg/dl
Diabetes	>200mg/dl

Glycoslyated hemogloblin

Normal	<7 %
Diabetes	>7 %

TESTING OF GESTATIONAL DIABETES (GMD) [9]

Pregnant women who fulfill all of these criteria need not be screened for GDM.

This low-risk group comprises women who

- are <25 years of age
- are a normal body weight
- have no family history (i.e., first-degree relative) of diabetes
- have no history of abnormal glucose metabolism
- have no history of poor obstetric outcome

Role of glycosylated hemoglobin (A1C) level as a criterion for the diagnosis of diabetes

Soon after the introduction of the glycosylated hemoglobin assay as an index of glycemia, its use for the diagnosis of diabetes was considered. Measurement of glycohemoglobin (A1C) for this purpose has numerous advantages.

1) A1C measures average glycemic levels in a time scale of weeks, whereas plasma glucose varies greatly within any given day and from day to day. Thus, an elevated A1C indicates a chronic state of hyperglycemia.

2) The patient does not have to fast or otherwise prepare, and a blood sample can be drawn any time of day.

3) In reference laboratories, the precision of A1C measurement is similar to the measurement of plasma glucose

4) A test that can be used to diagnose diabetes and evaluate the results of treatment is an attractive measurement, as compared with our current situation, which calls for using different tests to diagnose the disease and then monitor treatment.

5) There is a threshold level of A1C associated with risk for retinopathy.

6) A1C assays had a mean sensitivity of 66% and a specificity of 98%.

Disadvantages.

1, Procedure not standardized all countries

2, A1C values may be affected by other conditions (e.g,hemoglobinopathy, pregnancy, uremia, blood transfusion, and hemolytic anemia)

3, A chemical preparation to create uniform calibration standards has only recently been established

DIABETES CONTROL AND ITS MEASUREMENT [4]

INDEX	MAIN CLINICAL USE
Urine glucose	Only crude index of BG (blood glucose), last resort in type 2 diabetes
Blood glucose A, Fasting B, diurnal/circadian profiles	 Correlated with mean daily BG and HbA_{1C} in type 2 diabetes Self monitoring of BG, hospital assessment
Glycated hemoglobin	Glycaemic control (mean) over preceding 1-3 months
Glycated serum protein eg fructosamine	Glycaemic control (mean) over preceding 2 weeks
Urine ketones	Insulin deficiency, warning of DKA (diabetic ketoacidosis)
Other blood metabolites/hormones A, Cholesterol B, Triglyceride	 Cardiovascular risk factor Cardiovascular risk factor

PATHOPHYSIOLOGY OF DIABETES (2003) [10]

I. Type 1 (previously called insulin dependent diabetes mellitus (IDDM) or juvenile-onset diabetes)

A. Causes

1. Genetic predisposition.
2. Environmental exposure: virus, toxin, stress.
3. Autoimmune reaction: beta-cells that produce insulin in the pancreas are destroyed. When 80-90% of the beta-cells are destroyed, overt symptoms occur.

B. Characteristics

1. Usually occurs before 30 years of age, but can occur at any age. Peak incidence occurs during puberty, around 10-12 years of age in girls and 12-14 years in boys.
2. Abrupt onset of signs and symptoms of hyperglycemia: increased thirst and hunger, frequent urination, weight loss, and fatigue.
3. Ketosis prone.

II. Type 2 (previously called non-insulin dependent diabetes mellitus, NIDDM, or adult-onset diabetes)

A. Causes

1. Insulin resistance: unable to utilize insulin that the body makes because of cell-receptor defect; glucose is unable to be absorbed into cells for fuel.
2. Decreased insulin secretion: pancreas does not secrete enough insulin in response to glucose levels.
3. Excess production of glucose from the liver: result of defective insulin secretory response.

B. Characteristics

1. Usually occurs after 30 years of age, but is now occurring in children and

adolescents.

2. Increased prevalence in some ethnic groups, e.g., African Americans, Hispanic/Latino, Native Americans, Asian Americans, and Pacific Islanders.
3. Strong genetic predisposition.
4. Frequently obese.
5. Not prone to ketoacidosis until late in course or with prolonged hyperglycemia.
6. May or may not have symptoms of hyperglycemia.
7. May also have extreme tiredness, blurred vision, delayed healing, numbness and tingling of hands and feet, recurring yeast infection.
8. Children between the ages of 10-19 that have one or more of the following are at an increased risk:

• Family history

• Member of certain ethnic populations

• Overweight

• Sedentary lifestyle

• Pre-puberty.

• Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans [dirty-neck syndrome] Acanthosis Nigricans can help identify persons who have high insulin levels and who may be at-risk for developing diabetes, hypertension [high blood pressure], dyslipidemia [lipoproteins inbalance], polycystic ovarian syndrome[PCOS]

MECHANISM OF HYPERGLYCEMIA INDUCED DAMAGE (2004)[4]

Four major hypotheses about how hyperglycemia causes diabetic complication

1. Increased influx of glucose and other sugars through the polyol pathway.
2. Increased intracellular formation of advanced glycation end products (AGE).
3. Activation of protein kinase C (PKC) isoforms.

4. Overactivity of hexasamine pathway.

POLYOL PATHWAY (2003)[11]

Diabetes causes increased oxidative stress in various tissues as evidenced by increased levels of oxidized DNA, proteins, and lipids. Besides damaging the functions of these molecules, oxidative stress also triggers a series of cellular responses, including the activation of protein kinase C (PKC), transcription factor NF-κB, and JNK stress-associated kinases , and so forth. Inappropriate activation of these important regulatory molecules would have deleterious effects on cellular functions, and it is thought to contribute to the pathogenesis of various diabetic complications. However, it is not clear how hyperglycemia leads to increased oxidative stress. It is most likely the combined effects of increased levels of reactive oxygen species (ROS) and decreased capacity of the cellular antioxidant defense system. Glucose auto-oxidation, nonenzymatic glycation, and the interaction between glycated products and their receptors , overproduction of ROS by mitochondria , and the polyol pathway , all are potential sources of hyperglycemia-induced oxidative stress.

ADVANCED GLYCATION END PRODUCT (AGE)(2003)[2]

Formation of AGE

1. Endogenously
2. Exogenous sources-

a, Tobacco smoke, for example, is a well-known exogenous source of AGEs. The combustion of various pre-AGEs in tobacco during smoking gives rise to reactive and toxic AGEs. Serum AGEs or LDL-linked AGEs are significantly elevated in cigarette smokers. Diabetic smokers, as a result, are reported to exhibit greater AGE deposition in their arteries and ocular lenses.

b Diet is a significant exogenous source of highly reactive AGEs. Food

processing, heating in particular, has a significant accelerating effect in the generation of glyco- and lipoxidation products.

AGEs form at a constant but slow rate in the normal body, starting in early embryonic development, and accumulate with time. However, their formation is markedly accelerated in diabetes because of the increased availability of glucose.

AGEs are a heterogeneous group of molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids. The initial product of this reaction is called a Schiff base, which spontaneously rearranges itself into an Amadori product, as is the case of the well-known hemoglobin A_{1c} (A1C). These initial reactions are reversible depending on the concentration of the reactants. A lowered glucose concentration will unhook the sugars from the amino groups to which they are attached;conversely, high glucose concentrations will have the opposite effect, if persistent.A series of subsequent reactions, including successions of dehydrations, oxidation- reduction reactions, and other arrangements lead to the formation of AGEs. Several compounds, e.g., N-carboxymethyl-lysine, pentosidine, or methylglyoxal derivatives, serve as examples of well-characterized and widely studied AGEs.

EFFECTS OF AGE

1. Retinopathy
2. Diabetic nephropathy- thickened glomerular basement membrane and mesangial expansion, are accompanied by accumulation of AGEs, leading to glomerulosclerosis and interstitial fibrosis
3. Increased AGE accumulation in the diabetic vascular tissues has been associated with changes in endothelial cell, macrophage, and smooth muscle

cell function. In addition, AGEs can modify LDL cholesterol in such a way that it tends to become easily oxidized and deposited within vessel walls, causing streak formation and, in time, atheroma. AGE-crosslink formation results in arterial stiffening with loss of elasticity of large vessels

PROTEIN KINASE C(1998)[12]

The activation of protein kinase C (PKC) and increased diacylglycerol (DAG) levels initiated by hyperglycemia are associated with many vascular abnormalities in retinal, renal, and cardiovascular tissues. Among the various PKC isoforms, the beta- and delta-isoforms appear to be activated preferentially in the vasculatures of diabetic animals, although other PKC isoforms are also increased in the renal glomeruli and retina. The glucose-induced activation of PKC has been shown to increase the production of extracellular matrix and cytokines; to enhance contractility, permeability, and vascular cell proliferation; to induce the activation of cytosolic phospholipase A2; and to inhibit Na+-K+-ATPase. Treatment with D-alpha-tocopherol was able to prevent many glucose-induced vascular dysfunctions and inhibit DAG-PKC activation.

HEXASAMINE PATHWAY [13]When glucose is high inside a cell, most of that glucose is metabolized through glycolysis, going first to glucose-6 phosphate, then fructose-6 phosphate, and then on through the rest of the glycolytic pathway.

COMPLICATION OF DIABETES

(The DCCT Research group, 1993)[14]

Chronic complication includes

Microvascular

1. Retinopathy-Impaired vision,blindliess
2. Nephropathy-Proteinuria,chronic kidney disease,dialysis
3. Neuropathy- a, Peripheral: sensory and motor neuropathy

   b, Autonomic: gastroparaesis, postural hypotension, impotence

Macrovascular

1. Coronary artery disease-myocardial infraction
2. Peripheral vascular disease-claudication,ulcers,amputation
3. Cerebrovascular disease-stroke

Oral disease(Petrou-Amerikanou et al,1998)

1. Gingivitis
2. Periodontitis
3. Xerostomia
4. Candidiasis
5. Oral lichen planus
6. Oral cancer
7. Leukoplakia

Acute complication

1. hyperosmolar hyperglycemia
2. Diabetic ketocidosis
3. acute infections

ANALYSIS OF SALIVA IN DIABETES (SALIVARY PROTEMICS)

- Salivary EGF levels reduced in diabetic patients
- Salivary matrix metalloproteinase (MMP-8) levels and gelatinase (MMP-9) Activities in patients with type 2 diabetes mellitus
- Salivary thromboplastic activity in diabetics and healthy controls
- Effects of diabetes mellitus on salivary secretion and its composition in the human

- Levels of immunoglobulin A1 and messenger RNA for interferon gamma and tumor necrosis factor alpha in total saliva from patients with diabetes mellitus type 2 with chronic periodontal disease
- Structural and functional salivary disorders in type 2 diabetic patients

Peck AB, Tanaka Y, Humphreys-Beher MG (2000) [15]

Oral problems such as periodontitis are recognized major complications associated with diabetes. salivary derived growth factors, including epidermal growth factor (EGF), are thought to play a role in helping maintain levels of oral health, promoting wound healing, and maintaining mucosal integrity. In the present study, salivary levels of EGF in diabetic vs. healthy control patients was evaluated. Twenty-one diabetic patients participated in this study. Age, race, sex and smoking histories were matched with 21 systematically healthy nondiabetic patients. Three milliliters of unstimulated resting whole saliva was collected from each patient at 6 h intervals up to 42 h and whole saliva protein concentrations were determined for each sample. EGF concentrations for each sample were quantitated spectrophometrically utilizing an immunoassay. Diabetic patients had greater salivary protein concentrations over 42 h of collection with a mean of 1.502+/-0.09 vs. 1.242+/-0.05 mg/ml for healthy control patients. The EGF concentration was significantly lower ($p<0.05$) for the diabetic patients compared to control patients, whether expressed relative to 1 ml volume of saliva (873.43+/-106.5 vs. 1101.09+/-116.8 pg/ml) or 1 mg whole saliva protein (629.18+/-92.6 vs. 931.20+/-124.6 pg/mg saliva protein). This study suggests that reduced levels of salivary EGF in diabetic patients may contribute to the development of oral and systemic complications of diabetes, which may have future clinical applications.

Salo T, Ronka H, Konttinen YT [2000] [16]they studied the salivary levels and activities of the matrix metalloproteinases (MMP) -8 and -9 in 45 type 2 diabetic patients and 77 control subjects. The patients' mean glycosylated haemoglobin (HbA1c) was 8.7%, indicating an unsatisfactory metabolic control of the disease. The MMP levels were further related to the clinical and microbiological periodontal findings as well as to salivary flow rate and other factors. The salivary flow rate, albumin and amylase concentrations were similar in type 2 diabetic patients to those in the control group. The mean gingival and periodontal pocket indexes were higher in the diabetes group. The number of potential periodontontal pathogenic bacteria was lower, however, in the diabetic than in the control group. Zymography and immunoblotting revealed that the major MMPs in the type 2 diabetic patients' saliva were MMP-8 and MMP-9. Salivary MMP levels and activities in type 2 diabetic patients were in general similar to those in the control group. However, the correlation coefficients using multiple regression analysis revealed that gingival bleeding, pocket depths and HbA1c were associated with increased MMP-8 levels which, in turn, were negatively predicted by elevated plasma lipid peroxide levels in the diabetic group, data on salivary MMP-8 and -9 do not support the concept of generalized neutrophil dysfunction in unbalanced diabetes. Moreover, plasma lipid peroxidation levels reflecting the increased oxidative burden, which is generated mainly by triggered neutrophils, do not indicate neutrophil dysfunction due to diabetes, but may rather be related to the increased tissue damage in an uncontrolled disease. However. advanced periodontitis in type 2 diabetes seems to be related to elevated salivary MMP-8 levels which might be useful in monitoring periodontal disease in diabetes.

Akyuz S, Ipbuker A, Emekli N [Mar 2004] [17]Coagulative function of saliva derives from the thromboplastin found in saliva. It may establish hemostasis in the mouth. salivary disfunction and changes in salivary composition and are frequent

complications of diabetes. This study investigated the influence of some local etiologic and systemic factors on salivary thromboplastic activity (STA) in diabetics. In this study, cytological smears and biochemical tests were used. STA was measured by Quick's one stage method, serum glucose by the glucose oxidase method, and salivary protein by the method of Lowry. STA was almost the same in the diabetic and control groups. The only statistically significant difference within the diabetic group was found to be due to antibiotic usage. STA, i.e. clotting time, was 30% longer (114 s) ($p<0.05$) and salivary protein (4.07 mg ml(-1)) ($p<0.1$) was lower in diabetics not taking antibiotics than in those taking them. No such differences were observed in the healthy controls. Significant linear correlations ($p<0.05$) with respect to STA were with salivary protein in the control group ($r=0.61$) and in the diabetic group ($r=0.51$) and with antibiotic usage ($r=0.29$), with leukocyte cell count ($r=0.27$) in the diabetic.

Francisco H, Santos C, Mesquita MF (2004)[18]This study investigated the effects of diabetes mellitus (types I and II) on human salivary gland function compared to healthy age-matched controls. The results have shown that both type I and type II diabetic patients secrete significantly ($p < 0.05$) less resting and stimulated saliva compared to healthy age-matched controls (AMC). It was also found that the diabetic patients have an increased resting and stimulated salivary protein concentration compared to healthy participants. However, the secretory capacity (stimulated minus resting values) was markedly reduced compared to controls. The level of calcium ($Ca2+$) in the saliva of diabetic patients was significantly ($p < 0.05$) elevated compared to the AMC. In contrast, the levels of magnesium ($Mg2+$), zinc ($Zn2+$) and potassium ($K+$) in the saliva of diabetic patients were significantly ($p < 0.05$) reduced compared to the values obtained in AMC. These results indicate that diabetes mellitus can lead to marked dysfunction of the secretory capacity of the salivary glands. In

these patients a modified fluid, organic and inorganic salivary secretion may be responsible for the increased susceptibility to oral infections and impaired wound.

Afonso-Cardoso SR, Buso AM [Jun 2006] [19]Diabetes mellitus and periodontal disease have high incidence in the general population and are associated with various degrees of dysfunction in the immune system. It has been shown that diabetic patients with severe periodontal disease have more complications of diabetes and less effective metabolic control compared with diabetic patients with healthy gingiva. Patients with diabetes and severe periodontal disease present higher levels of serous immunoglobulin A (IgA). Elevation of the IgA1 isotype is thought to contribute to this phenomenon. Another important event in the diabetes-periodontitis association is the disturbance in local and systemic production of inflammatory cytokines. In this study they tested the hypothesis that type 2 diabetic patients with chronic moderate periodontal disease have differences in salivary IgA1 titers and cytokine expression when compared with the chronic severe periodontal disease cases. We utilized a jacalin-IgA capture assay to determine the IgA1 titers in total saliva and reverse transcriptase-polymerase chain reaction to detect mRNA for interferon gamma (IFN-gamma) and tumor necrosis factor alpha (TNF-alpha) in total saliva samples of 13 patients with chronic moderate periodontal disease and 10 with chronic severe periodontal disease, they observed a predominance of IgA1 titers of 64 (45.5%) in saliva samples from chronic severe periodontal disease patients and titers averaging 512 (30.8%) in chronic moderate periodontal disease patients. Detected mRNA for IFN-gamma in six out of 10 chronic severe periodontal disease subjects and in two out of 13 chronic moderate periodontal disease patients. TNF-alpha expression was similar in both groups, data suggest that higher levels of IgA1 may exert partial protection of the periodontal tissue in chronic moderate periodontal disease diabetic patients when compared to severe periodontal disease. Despite the small number of

patients, IFN-gamma expression had a trend association with severity of periodontitis and TNF-alpha gene expression did not correlate with peridontitis.

Salom L, Gomez de Ferraris ME [Jul 2006] [20]Diabetes mellitus type 2 is the most common metabolic disorder and it causes an important morbimortality. The structural modifications in the parotid gland (sialosis) had already been described in these patients and could result in variations in the salivary composition, as well as an increase in periodontal and dental pathology.: To compare the biochemical findings in the saliva and to correlate these biochemical disturbances with the morphologic findings previously described. Clinical information were gathered about 33 patients, 17 had type 2 diabetes. Samples of whole saliva were obtained for biochemical analysis and serum samples to determine metabolic control. In the diabetics saliva they found urea and total proteins increased and reduced levels of microalbumina. salivary glucose was only augmented in patients with poor metabolic control. Clinical symptoms of xerostomia were present in 76,4% and dental and periodontal disease in 100%. The parotid gland was characterised by the presence of small acini, lipid intracytoplasmic droplets, as well as adipose stroma infiltration. The acinar cytoqueratins expression was heterogeneous and very positive in the hyperplasic ducts. These biochemical disorders in the saliva of the type 2 diabetic patients would be related with the structural changes previously observed in parotid gland

VARIOUS METHODS OF SALIVARY GLUCOSE ESTIAMTION

Gough H, Luke G. A, Beeley J. A. (1996) [21]Determination of glucose concentrations in small volumes of unstimulated fasting whole saliva. The technique involves high-performance ion-exchange chromatography at high pH and pulsed amperometric detection. It has a high level of reproducibility, a sensitivity as low as 0.1 μmol/l and requires only 50 μl samples. The procedure can be used for the analysis of other

salivary carbohydrates and for monitoring the clearance of dietary carbohydrates from the mouth.

Di Gioia ML, Leggio A (2004) [22] The methodology was developed for the determination of human salivary glucose concentration. The technique involves the glucose derivatization with acetic anhydride and subsequent analysis of glucose penta-acetylated by gas chromatography combined with mass spectrometry. Glucose concentration in the biological fluid depends on the physiological status of the donor.

Schlapfer P, Mindt W and Racine P.H. (1974) [23]Glucose concentrations of biological samples can be conveniently measured using glucose oxidase and the standard oxygen electrode. Blood samples are an exception because the variable oxygen concentration of the contained hemoglobin affects the results to avoid this error , Hexacyanoferrate(III), a threshold-type acceptor, proved to be satisfactory for glucose measurements.

George G. Guilbault, Palleschi G(1995)[24] Biosensors have been developed and applied for the non invasive determination of metabolites in body fluids. Advantages of saliva or sweat analysis are the ease of sample collection and that samples can be collected more frequently with much less stress on the patient. The biosensor was applied to the determination of sera and saliva glucose content.

CORRELATION BETWEEN SALIVA GLYCATED AND BLOOD GLYCATED PROTEINS

Ichiro N, Kanehisa M, TatsuyaT (2003) [25]Blood and saliva samples of 51 male workers were collected. The fructosamine and hydrazine methods were used to measure saliva glycated protein. HbA1c, fructosamine and blood glucose were measured as indices of blood glycated protein, and the correlation between blood glycated protein and saliva glycated protein was examined . Saliva fructosamine glycated protein showed a significant correlation with HbA1c and blood glucose.

Blood glycated protein and blood glucose could be estimated by measuring saliva glycated protein.

Forbat LN, Collins RE, Maskell GK, Sönksen PH. **(1981)**[26] Glucose concentration in venous blood and parotid saliva taken from 31 diabetics attending a diabetic clinic showed values ranging respectively from 3.9 to 19.1 mmol/l and 0.06 to 0.83 mmol/l (means 9.6 mmol/l and 0.32 mmol/l respectively). Linear regression of salivary glucose on blood glucose gave a simple correlation coefficient of 0.18 (NS). Since salivary glucose levels did not reflect blood glucose levels, the possibility of diabetics regulating their metabolic control by the noninvasive technique of monitoring salivary glucose concentrations is not possible

SCREENING OF DIABETES MELLITUS WITH GINGIVAL CREVICULAR BLOOD

Müller HP, **Behbehani E (2004)**[27] To test the feasibility of using gingival crevice blood (GCB) collected during routine periodontal examination to estimate blood glucose levels using a novel and very sensitive self-monitoring device Forty-six patients (20 male, 26 female; age range 12-56 years, mean age 36 +/- 11 years) seeking dental treatment took part in the study. 24 had gingivitis, of which 22 were moderate or advanced periodontitis. Periodontal probing depth (PD) and clinical attachment loss was measured at 6 sites of every tooth present, and bleeding on probing (BOP) was recorded. A site with profuse BOP was chosen for glucose determination. Measurements in GCB were compared with those of conventional capillary fingerstick blood (CFB) Agreement between the two measurements was low, the mean difference was -1.22.The present study failed to provide any evidence for the usefulness of GCB for testing blood glucose during routine periodontal examination.

Beikler T, **Kuczek A**, **Petersilka G**, **Flemmig TF(2002)** [28] Diabetes mellitus (DM) is undiagnosed in approximately 1/2 of the patients actually suffering from the disease. In addition, the prevalence of DM is more than 2x as high in patients with periodontitis when compared to periodontally healthy subjects, thus a high number of patients with periodontitis may have undiagnosed DM, blood oozing from gingival tissues during routine periodontal examination can be used for determining glucose levels. 32 non-diabetic and 13 diabetic patients with moderate to severe periodontitis were enrolled and subjected to routine clinical periodontal examination. Periodontal pocket probing was performed using a standard force. Blood oozing from gingival tissues of anterior teeth following periodontal pocket probing was collected with the stick of a glucose self-monitoring device (Elite(R) 2000, Bayer Diagnostics GmbH, Munich). As control, fingerstick capillary blood was taken. Statistical analysis was performed by Pearson's correlation coefficient. The results suggested that blood oozing during routine periodontal examination may be used for diabetes mellitus screening in a dental office setting.

Khader YS, **Al-Zu'bi BN**, **Judeh A**, **Rayyan M (2006)** [29]**.**This study was conducted to assess the usefulness of the gingival crevicular blood for estimating the glucose level during routine periodontal examination using Xitux Diagnostics Smart-X self-monitoring blood glucose device among Jordanian patients attending dental teaching clinics. A total of 34 type 2 diabetic patients (18 males and 16 females) and 26 non-diabetic patients (14 males and 12 females) participated in this study. Glucose level was measured in a sample of gingival crevicular blood and in another sample obtained by finger puncture using a self-monitoring device. Glucose measurements from gingival crevicular blood samples, ranged from 57 to 250 mg dl-1 with a mean of 125.4+/-60.7 mg dl-1 (+/-SD) and glucose measurements obtained by finger puncture, ranged from 62 to 263 mg dl-1 with a mean of 131.9+/-61.1 mg dl-1.

Pearson's correlation coefficient was performed to assess the correlation between the glucose measurements in these two samples. gingival crevicular blood can provide an acceptable source for measuring blood glucose level. However, the technique to obtain an acceptable blood sample from gingival crevices is not always feasible which would limit its application as a clinical practice. Additional studies that refine this technique and use larger sample size are recommended.

Yamaguchi M, **Kawabata Y**, **Kambe S**, **Wårdell K**, **Nystrom FH**, **Naitoh K**, **Yoshida H (2004 May)**[30] To realize a non-invasive blood glucose monitor, the gingival crevicular fluid (GCF) was measured. A GCF-collecting device was developed that was designed to be disposable, biocompatible and small enough to be inserted in the gingival crevice for collection of a sub-microlitre sample of GCF. Also, a high-sensitivity glucose testing tape incorporated in the device was developed. Red laser light in a portable optical device measured the colour density of the testing tape. It was suggested that GCF could be used as a method of non-invasive blood glucose measurement as where was positive correlation with the blood glucose level

ORAL MANIFESTATION OF DIABETES

DIABETES AND PERIODONTITIS

Beena VT, Nair RG, Vijayakumar [1995] [3]Immunoglobulin in the saliva of diabetic patients with periodontitis The study was conducted to estimate the concentration of immunoglobulins in the saliva of diabetic and nondiabetic patients with periodontitis. The salivary immunoglobulins G, A and M (IgG, IgA, IgM) were determined in 50 patients with type II or noninsulin dependent diabetes mellitus (NIDDM) and 50 non diabetic patients with periodontitis. The values were compared with that of 50 age and sex matched controls. IgG, IgA were found to be significantly increased in diabetic patients with periodontitis, compared to nondiabetic patients and controls. Though an increase in IgM was found in diabetic patients it was not

significant. The altered immune response observed may be due to the response to a greater antigenic challenge which in turn may be responsible for the increased incidence of periodontitis.

Cucinotta M, Cicciu D, Passi P, Teti D [Sep 2006] [31]Salivary histamine level as a predictor of periodontal disease in type 2 diabetic and non-diabetic subjects Some previous investigations underscored the role of histamine in periodontal disease, especially in diabetic patients, but the behavior of this inflammatory mediator in the early phases of periodontal involvement remains unclear. The aim of the present study was to correlate the presence of histamine in saliva with clinical parameters in healthy, periodontitis-affected, and diabetic subjects to ascertain whether this amine may serve as a predictive index of periodontal risk.: For this purpose, subjects were selected as follows: 1) with newly diagnosed type 2 diabetes mellitus; 2) with neither diabetes nor periodontitis; 3) with no diabetes but with chronic, untreated periodontal disease. Histamine salivary levels were measured at the initial time (T0) and after 6, 12, and 24 months using high-performance liquid chromatography. The main periodontal indexes were recorded at the same time intervals. At T0, a very typical shape of the histamine chromatogram was found for all patients of the three groups; at this time, the salivary histamine levels of diabetic patients were increased and comparable to those of healthy patients with periodontal disease, whereas healthy subjects with no periodontitis showed reduced histamine levels. Further controls at 6, 12, and 24 months showed a statistically significant correlation between the increase of salivary histamine and the worsening of the periodontal indexes in diabetic and non-diabetic subjects

Yamalik N, Ozer N, Ersoy F, Yeniay I [1996] [32]

The alterations of whole saliva constituents in patients with diabetes mellitus The present study was undertaken in order to determine the possible alterations in

whole saliva and the periodontal status in patients with diabetes mellitus (DM), and was conducted on 17 patients with DM and 17 systemically and periodontally healthy subjects. When the subjects were evaluated clinically, significantly increased probing depths were noticed in the DM group when compared with the healthy subjects. In whole saliva samples, sodium, potassium, total protein, amylase, thiocyanate, and secretory IgA levels were determined in both groups. Difference between the two groups regarding the mean salivary potassium levels were found to be statistically significant since the mean salivary potassium levels in the DM and the control groups were 2.470 +/- 9.04 mmol/L and 14.30 +/- 8.88 mmol/L, respectively. The mean salivary total protein, amylase and secretory IgA levels in the DM group were 2.41 +/- 1.0 mg/mL, 124.2 +/- 79.7 U/mL and 6.86 +/- 3.50 mg/L, all being significantly higher than the control group. However, no significant differences could be shown for the salivary sodium and thiocyanate levels. Nor was there any difference between non-insulin dependent diabetes mellitus (NIDDM) and insulin-dependent diabetes mellitus (IDDM). The findings of the present study suggest that, besides the clinical examinations, the determination of the possible alterations in the composition of whole saliva might also be helpful in understanding the increased severity of periodontitis.

Diabetes mellitus and oral lichen planus

Van Dis M, Parks E. (2009)[33]-The purpose of this study was to determine the prevalence of oral lichen planus in a population of patients with diabetes mellitus compared with a control population and to determine if patient medications had any influence on the presence of such lesions. Two hundred seventy-three patients with diabetes and an identical number of age-, gender- and race-matched controls were examined for clinical evidence of oral lichen planus. Patient medication histories were

also obtained from each group. Eleven diabetic patients (4%) and eight control patients (3%) had clinical evidence of oral lichen planus. Ingestion of nonsteroidal anti-inflammatory drugs or angiotensin-converting enzyme inhibitors was associated with the presence of oral lichen planus lesions in six patients. There was no apparent association of diabetes and oral lichen planus in this population, and the ingestion of medications known to cause lichenoid mucosal reactions had no influence per se on the presence of oral lichen planus lesions ($p>0.05$). However, the type of medication ingested by those patients who had oral lichen planus lesions was either nonsteroidal anti-inflammatory drug or angiotensin-converting enzyme inhibitor, which was a significant association ($p<0.00$).

Petrou-Amerikanou C; Markopoulos A K; Belazi M; Karamitsos D; Papanayotou P (1998)[34]The purpose of the present study was to determine the prevalence of oral lichen planus (OLP) in a population of patients with diabetes mellitus (DM) as compared with a control population. One hundred and thirty-nine patients with type I DM, 353 patients with type II DM and 274 controls were examined for clinical evidence of OLP. The clinical evidence of OLP in the diabetic and control patients was confirmed by histopathological examination The prevalence of OLP in type I diabetic patients was 5.76%, in type II 2.83%, and 1.82% in the controls. The prevalence of OLP was significantly higher in patients with type I DM and slightly higher in patients with type II DM in comparison to the prevalence in the control sample. The fact that type I diabetes and OLP are characterized by autoimmune phenomena and T cell immune responses respectively, suggest that the immune system may play a critical role in the appearance of OLP in patients with type I DM.

Bagan JV, **Donat JS**, **Penarrocha M**, **Milian MA**, **Sanchis JM (1993)** [35] -A study was made of 72 patients with oral lichen planus associated (n = 28) or not with

diabetes mellitus (n = 44). No significant differences were observed between both groups in terms of the location of the lichen planus lesions on the buccal mucosa, palate, gums or floor of the mouth. On the other hand, the diabetics exhibited a greater frequency of oral lichen planus on the tongue. Atrophic-erosive lesions were more common in patients with lichen planus associated with diabetes

Oral leukoplakia and diabetes mellitus

Albrecht M, **Bánóczy J**, **Dinya E**, **Tamás G Jr**. (**1992)** [36]-The occurrence of oral leukoplakia in 1600 patients with diabetes mellitus (815 type 1: insulin-dependent, 761 type 2: non-insulin-dependent)-under care at the International Medicine Department-was studied. Precancerous lesions and conditions were diagnosed and grouped according to internationally accepted criteria. The prevalence of oral leukoplakia in diabetic patients was 6.2%, as compared to 2.2% in the healthy controls. Leukoplakia showed the highest occurrence in the second year of established diabetes, and their prevalence was higher among insulin-treated diabetics. Smokers were more often affected

Diabetes mellitus and tumors of the oral cavity

Ujpál M, **Matos O**, **Bíbok G**, **Somogyi A**, **Szigeti K**, (**2004 Apr**) [37]Diabetes mellitus (DM) is a risk factor for the development of periodontal diseases and various inflammatory lesions in the oral mucosa. A possible correlation between DM and oral premalignancies and tumors was examined in this study. Stomato-oncological screening was carried out on 200 DM patients in the medical departments: The lesions found were classified in three groups: inflammatory lesions, benign tumors and precancerous lesions. Benign tumors were found in 14.5%, and precancerous lesions in 8% of diabetics.A retrospective DM screening of 610 inpatients with histologically

confirmed oral malignancies was also performed. The control group comprised 574 complaint- and tumor-free adults. Fasting blood glucose levels were determined in both groups, and the tumor location was registered in the cancer patients. The combination of DM and smoking means a higher risk for oral precancerous lesions and malignancies. DM may be a risk factor for oral premalignancies and tumors.

Diabetes mellitus in odontogenic infections and oral candidiasis

Ueta E, **Osaki T**, **Yoneda K**, **Yamamoto T**. (**1993 Apr)** [38] -The prevalence of diabetes mellitus (DM) in odontogenic infections and oral candidiasis was examined, and influences of DM on the clinical manifestations of the infections and neutrophil functions were investigated. Among 21 severe and 221 mild odontogenic infections, DM was detected in 5 cases in each group. Of 64 cases of symptomatic oral candidiasis, 8 cases were complicated with DM which was detected by blood examination during treatment. During the period of infection, the mean fasting blood sugar level was 16.0 +/- 4.4 and 9.8 +/- 1.2 mmol/l in the DM-complicated odontogenic infections and candidiasis, respectively. All white blood counts,

C-reactive protein levels and erythrocyte sedimentation rates were more elevated in DM(+) odontogenic infection cases than in DM(-) ones. In DM(+) candidiasis. The polymorphonuclear leukocytes from diabetic patients, especially those with candidiasis, produced less free oxygen radicals and exhibited reduced phagocytosis and intracellular killing of Candida cells associated with this reduced O2- generation during the infection. These suppressed neutrophil functions increased after treatment but did not reach control levels. These results indicate that DM is a predisposing condition for odontogenic infections and oral candidiasis. DM-complicated infections become severe because of neutrophil suppression, and that examination of blood sugar level should be essential for patients with oral infections

Geerlings SE, **Hoepelman AI (1999 Dec)** [39]-Patients with diabetes mellitus (DM) have infections more often than those without DM. The course of the infections is also more complicated in this patient group. One of the possible causes of this increased prevalence of infections is defects in immunity, no disturbances in adaptive immunity in diabetic patients have been described. Different disturbances (low complement factor 4, decreased cytokine response after stimulation) in humoral innate immunity have been described in diabetic patientscellular innate immunity most studies show decreased functions (chemotaxis, phagocytosis, killing) of diabetic polymorphonuclear cells and diabetic monocytes/macrophages compared to cells of controls. In general, a better regulation of the DM leads to an improvement of these cellular functions. Furthermore, some microorganisms become more virulent in a high glucose environment. Another mechanism which can lead to the increased prevalence of infections in diabetic patients is an increased adherence of microorganisms to diabetic compared to nondiabetic cells. This has been described for Candida albicans. Possibly the carbohydrate composition of the receptor plays a role in this phenomenon.

Soysa NS, **Samaranayake LP**, **Ellepola AN (2006 May)** [40] -It has been reported that poor glycaemic control predisposes to oral candidal infection in diabetic patients. For instance, the carriage of Candida species and the density of candidal growth in the oral cavity is frequently claimed to be increased in patients with diabetes mellitus.

Darwazeh AM, **MacFarlane TW**, **McCuish A**, **Lamey PJ (1991 Jul)** [41]-The glucose concentration in unstimulated mixed saliva and serum was assayed and correlated with oral candidal colonization in 41 diabetics and 34 healthy control subjects. In diabetic patients, salivary glucose concentration was significantly higher than in the controls and was directly related to blood glucose concentration ,diabetic

patients who carried Candida intraorally had significantly higher salivary glucose concentrations than those in whom Candida could not be isolated

Xerostomia in diabetes mellitus

Sreebny LM, **Yu A**, **Green A**, **Valdini A** (**1992 Jul**) [42]-The prevalence of xerostomia in a group of ambulatory diabetic patients and to compare the following in patients with and without xerostomia: 1) flow rates of saliva and lacrimal fluid, 2) the presence of other symptoms suggestive of oral and extraoral dryness, 3) indexes of glycemic control, and 4) noninvasive measures of cardiovagal autonomic nervous system function .Forty adult diabetic patients and an equal number of age- and sex-matched healthy nondiabetic control subjects were studied .A questionnaire was administered to all patients, and the following tests were performed: resting and stimulated flow rates on whole saliva; Schirmer's test (lacrimal fluid), serum glucose and HbA1, expiration-inspiration ratio, 30:15 ratio, Valsalva ratio, and the systolic blood pressure response to standing .The salivary flow rates of the diabetic subjects was consistently lower than those of healthy, nondiabetic control subjects. The mean, resting, and whole-saliva flow rate was abnormally low in the diabetic patients who complained of xerostomia; Dry mouth is a common complaint among ambulatory diabetic patients. It is strongly associated with objective measurements of poor salivary flow and with other oral and extraoral symptoms

Moore PA, **Guggenheimer J**, **Etzel KR**, **Weyant RJ**, **Orchard T (2001 Sep)** [43] - The Oral Health Science Institute at the University of Pittsburgh has completed a cross-sectional epidemiologic study of 406 subjects with type 1 diabetes and 268 control subjects without diabetes that assessed the associations between oral health and diabetes. This report describes the prevalence of dry-mouth symptoms (xerostomia), the prevalence of hyposalivation in this population, and the possible

interrelationships between salivary dysfunction and diabetic complications .The subjects with diabetes were participants in the Pittsburgh Epidemiology of Diabetes Complications study who were enrolled in an oral health substudy. Control subjects were spouses or best friends .Of the medical diabetic complications studied (ie, retinopathy, peripheral and autonomic neuropathy, nephropathy, and peripheral vascular disease), only neuropathy was found to be associated with xerostomia and decreased salivary flow measures .Subjects with type 1 diabetes who had developed neuropathy more often reported symptoms of dry mouth as well as symptoms of decreased salivary flow rates.

ESTIMATION OF SALIVARY AGE LEVEL

Grootveld M, Christopher J.L, Silwood [44] -The applications of high resolution ^{1}H NMR analysis as a diagnostic probe for human saliva are reviewed with special reference to diabetes mellitus, and a recently published report regarding the ability of this technique to detect advanced glycation endproducts (AGEs) in this biofluid

Zidek W, Henning L, Schluter H [Oct 2004] [6] Patients with diabetes mellitus are prone to develop increased advanced glycation endproducts causing local complications and increased overall morbidity and mortality. Nuclear magnetic resonance spectra were determined in saliva of 52 consecutive patients with diabetes mellitus and 47 age-matched healthy control subjects. Resonance spectra showed specific peaks at 2.3, 7.3, and 8.4 ppm in saliva from patients with diabetes mellitus. These peaks could be generated by incubation of saliva from healthy control subjects with hypochloric acid in vitro, indicating the presence of advanced glycation endproducts. The presence of advanced glycation endproducts in patients with diabetes mellitus was associated with approximal plaque index, indicating increased

periodontal damage. The study indicates that increased advanced glycation endproducts are involved in the pathogenesis of diabeticcomplication

USE OF NMR IN PREDICTION OF DIABETES RISK

Chatellier G, Menard J, Panhard X, (2007) [45] **-**It has been proposed that nuclear magnetic resonance (NMR) plasma analysis can improve lipoprotein subclass discrimination and predict coronary artery disease (CAD) the mortality and morbidity for CAD in type 2 diabetes patients is two to four times higher than in nondiabetic subjects, making cardiovascular disease the first cause of death and disability amongst this population. Moreover, CAD is often undetected in diabetic patients. To assess whether NMR spectroscopy can predict cardiovascular disease a randomized clinical trial, involved high-risk type 2 diabetic patients who were followed for 4 years and who, during that period, had fatal or nonfatal acute myocardial infarction Patients of the 'case' group were defined as those who presented either a fatal or nonfatal acute myocardial infarction or who underwent sudden death during the 4-year follow-up. Control patients were defined as patients not presenting any cardiovascular events. Control patients were matched to the case patients for age ± 2 years, sex and geographical origin obtained blood samples after an overnight fast, using tubes without anticoagulant, stored sera at −80 °C before NMR analysis., acquired spectra at 500 MHz (Varian Inova) with a 1.5-s presaturation of the water signal the discriminatory capacity of multivariate analyses of NMR spectroscopic data is not high enough to render the technique feasible for clinical use. With respect to cardiovascular risk prediction, global statistical analyses, including classical biochemical and clinical risk factors at baseline, should be considered in addition to NMR data.

Hodge AM, Jenkins AJ, English DR, O'Dea K, 2009 Jan [46] To determine whether nuclear magnetic resonance (NMR)-determined lipoprotein profiles predict type 2 diabetes. 813 male and female participants in the Melbourne Collaborative Cohort Study, aged 40-69 years at baseline (1990-1994), and with a baseline fasting plasma glucose <7.0mmol/L. Incident type 2 diabetes was identified in 1994-1998. Concentration of very low density lipoprotein (VLDL) particles (positive) and high density lipoprotein (HDL) particle size (negative) were selected by stepwise regression as predictors of type 2 diabetes. Factor analysis identified a factor from NMR variables, explaining 47% of their variation, and characterized by a positive correlation with VLDL, particularly large and medium sized; more low density lipoprotein (LDL) that were smaller; and relatively smaller, but not more HDL particles. This factor was positively associated with diabetes incidence, but not independently of triglycerides. identified an atherogenic NMR lipoprotein profile in people who developed diabetes, but this did not improve diabetes prediction beyond conventional triglyceride levels.

Cotch MF, Jenkins AJ, Shea S (2009) [47]

To examine the associations between nuclear magnetic resonance (NMR) defined lipoproteins and diabetic retinopathy (DR) in a population-based sample of adults with type 2 diabetes. 921 persons with diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA) were included. DR was assessed from retinal photographs. Lipoproteins were measured by NMR spectroscopy. The lack of association between NMR-defined lipoproteins and DR does not support clinical use of NMR spectroscopy for management of patients with DR.

VARIOUS USES OF NMR TECHNIQUE

Silwood C.J.L, Lynch A.W.D, Claxson (2002)[48]- NMR technique was used to detect 60 metabolites from oral health care products

Takeda I , Stretch C, Bhatnager K(2009)[49]

NMR was used to measure metabolites from saliva of smokers and nonsmokers and found that citrate, lactate, pyruvate, and sucrose to be higher and formate to be lower in concentration in smokers compared with non-smokers

Harada H, Shimizu H, Maeiwa M(1987)[50] NMR was applied to the study of metabolites in human saliva specimens. Acetate, lactate, ethanol, glucose and some other substances were simultaneously identified and quantitated from the $^{(1)}$H-NMR spectra of saliva specimens treated with D_2O. The experiments demonstrated the value of $^{(1)}$H-NMR as an analytical method in the field of forensic science and clinical pathology and toxicology.

Michael Y. Tsai, Poulos AG (2004)[51] NMR is used as an alternative method for the measurement of postprandial TG-rich lipoproteins ,Chylomicron and chylomicron remnant/VLDL fraction in individuals consuming a high-fat meal.

Age and gender of subjects in the study groups (Table 1, Graph I)

The study population comprised of 75 patients, 25 in group I, 25 in group II, and 25 in group III. There were 35 males (Group I = 8, Group II = 14, group III = 13) and 40 females (Group I = 17, Group II = 11, group III = 12). The mean age in group I was (males = 57.57 ± 12.69, females = 50.72 ± 7.47). The mean age in group II was (males = 58.57 ± 6.80 , females = 51.27 ± 10.00). The mean age in group III was (males = 54.42 ± 8.15, females = 51.67 ± 8.06).

Mean random blood glucose in each group (Table II, Graph II)

The mean blood glucose values in mg/dl were,

Group I 87.08 (± 18.07), Group II 257.44 (± 54.47) and Group III 79.86 (± 17.05)

There was statistically significant difference between group I and II and between group II and group III (p = 0.000**)

There was no statistically significant difference between group I and III (p = 0.742)

Mean HbA_1C in the study population (Table III, Graph III)

The mean HbA_1C values in % were,

Group I 6.7 (± 1.4), Group II 8.6 (± 1.8) and Group III 4.9 (± 0.80)

There was statistically significant difference between group I and II, group I and III and group II and III (p = 0.000**)

Mean salivary glucose in the study population (Table IV, Graph IV)

The mean salivary glucose values in mg/dl were,

Group I 9.7(±7.6), Group II 12.1(± 9.6) and group III 8.6(± 7.5)

The difference was not statistically significant between any of the two groups

Group I and II (p = 0.357), Group I and III (p = 0.927), Group II and III (p = 0.356)

Mean salivary AGE in the study population (Table V, Graph V)

The mean salivary AGE values in ppm were,

Group I 3.5 (± 1.2), Group II 6.3 (± 2.11), group III 1.99 (± 0.80)

There was statistically significant difference between group I and II and between Group II and III (p = 0.000**). There was no statistically significant difference between group I and III (p = 0.180)

Pearson's correlation analysis of random blood glucose with HbA_1C in the study groups: (Table VI, Graph VIa, VIb ,VIc and VId)

Pearson's correlation analysis of random blood glucose with HbA_1C showed statistically significant negative correlation in group I (r = -0.546, p = 0.005**). There was positive correlation which was not statistically significant in group II (r = 0.242, p = 0.243) and group III (r =0.113, p = 0.591)

There was highly significant positive correlation when all the groups (I, II, III) were assessed together (r = 0.620, p = 0.000**)

Pearson's correlation analysis of random blood glucose with salivary glucose between all the groups: (Table VII, Graph VIIa, VIIb, VIIc and VIId)

Pearson's correlation analysis of random blood glucose with salivary glucose showed Non significant negative correlation in group I (r = -0.119, p = 0.572), positive correlation which was statistically insignificant in group II (r = 0.351, p = 0.086) and Significant negative correlation in group III (r = -0.414, p = 0.040*)

There was statistically non significant positive correlation when all groups (I, II, III) were assessed together (r = 0.199, p = 0.086).

Pearson's correlation analysis of salivary glucose and salivary AGE between all groups: (Table VIII, Graph VIIIa, VIIIb, VIIIc and VIIId)

Pearson's correlation analysis of salivary glucose and salivary AGE showed statistically non significant negative correlation in group I (r = -0.083, p = 0.799), statistically non significant positive correlation in group II (r = 0.134, p = 0.573) and group III (r = 0.291, p = 0.576).

Statistically significant negative correlation was observed when all groups (I, II, III) were assessed together (r = -0.380, p = 0.019*).

Pearson's correlation analysis of salivary AGE and HbA_1C between all the groups (Table IX, Graph IXa, IXb, IXc and IXd)

Pearson's correlation analysis of salivary AGE and HbA_1C showed statistically non significant negative correlation in group I (r = -0.100, p = 0.758), statistically non significant positive correlation in group II (r = 0.095, p = 0.692) and group III (r = 0.433, p = 0.391)

Statistically significant negative correlation was observed when all groups (I, II, III) were assessed together (r = -0.419, p = 0 .009**)

Pearson's correlation analysis of blood glucose and salivary AGE between all the groups (Table X, Graph Xa, Xb, Xc and Xd)

Pearson's correlation analysis of blood glucose and salivary AGE showed statistically non significant positive correlation in group I (r = 0.199, p = 0.556) and group II (r = 0.126, p = 0.598). There was statistically significant negative correlation in group III (r = -0.852, p = 0.031*)

Statistically significant negative correlation was observed when all groups (I, II, III) were assessed together ($r = -0.369$, $p = 0.022$*)

Pearson's correlation analysis of salivary glucose and periodontal status between all the groups (Table XI, Graph XI)

Pearson's correlation analysis of salivary glucose and periodontal status showed statistically significant positive correlation between salivary glucose and periodontal status when all groups were assessed together ($r = 0.245$, $p = 0.034$*).

Pearson's correlation analysis of salivary AGE and periodontal status between all the groups (Table XII, Graph XII)

Pearson's correlation analysis of salivary AGE and periodontal status showed statistically significant positive correlation between salivary AGE and periodontal status when all groups were assessed together ($r = 0.538$, $p = 0.003$**)

Table I: Gender distribution in the study population (n = 75)

Group	n = 75	Male			Female		
		n	%	Age Mean ± S.D	n	%	Age Mean ± S.D
Group I	25	8	32	57.57 ± 12.69	17	68	50.72 ± 7.47
Group II	25	14	56	58.57 ± 6.80	11	44	51.27 ± 10.00
Group III	25	13	52	54.42 ± 8.15	12	48	51.67 ± 8.06

Graph I: Gender distribution in the study population (n = 75)

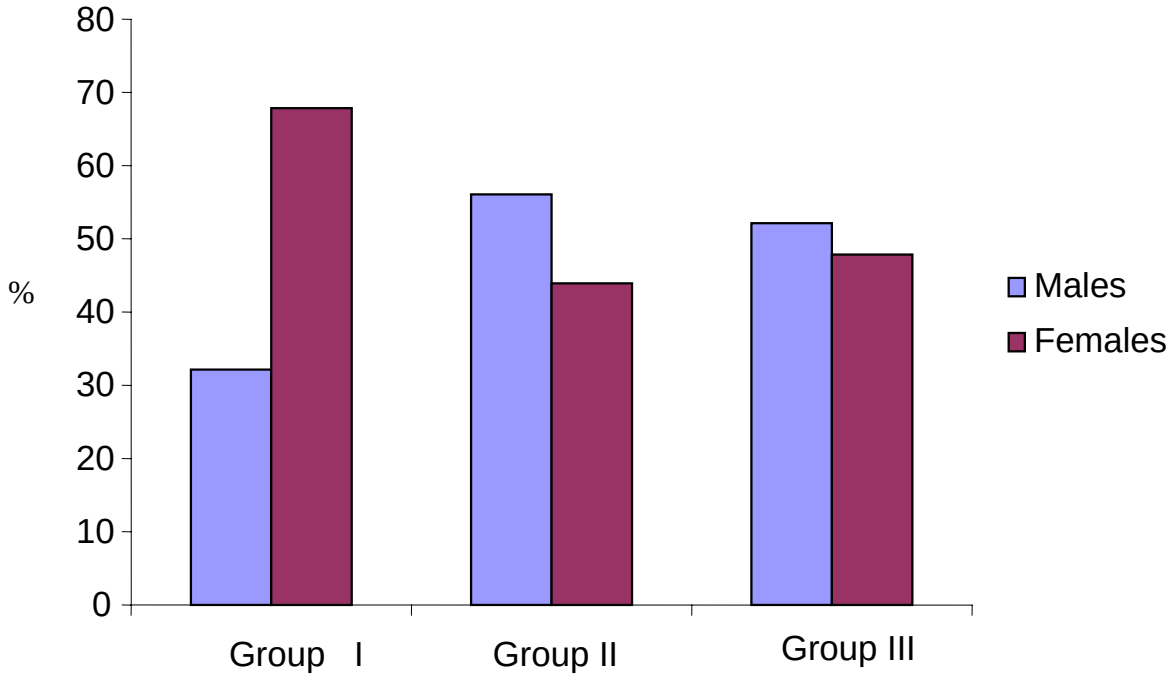


Table II: Mean blood glucose in each group

Variable	Study groups (Mean ± SD)			p-value		
	I (n=25)	II (n=25)	III (n=25)	I & II	I & III	II & III
Random blood glucose in mg/dl	87.08 ± 18.07	257.44 ± 54.47	79.86 ± 17.05	0.000**	0.742	0.000**

Controlled blood glucose : 120-200mg/dl
Uncontrolled blood glucose: >200mg/dl
Normal blood glucose : 80 -120mg/dl

Graph II: Mean blood glucose in each group (n=75)

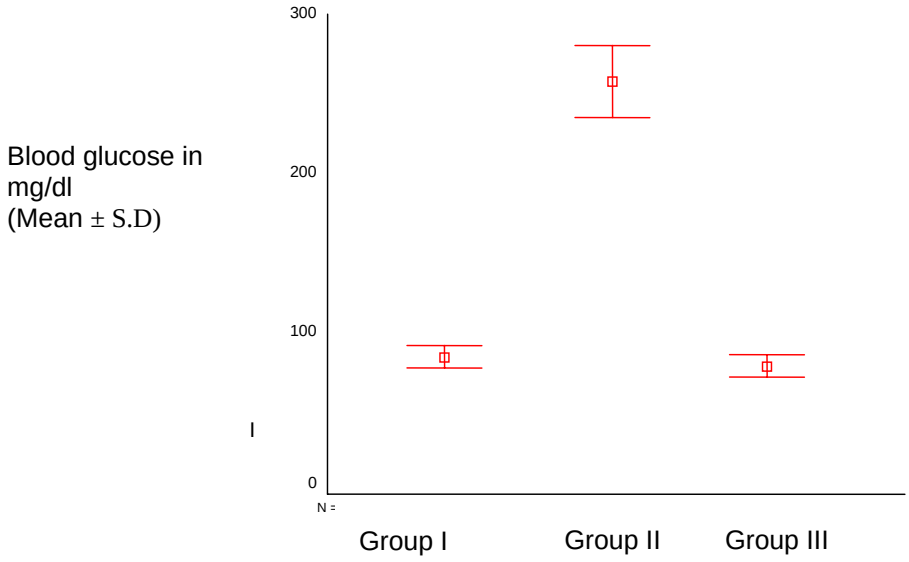


Table III: Mean HbA_1C values in each group

Variable	Study groups (Mean ± SD)			p-value		
	I (n=25)	II (n=25)	III (n=25)	I & II	I & III	II & III
HbA_1C in %	6.7 ± 1.4	8.6 ± 1.8	4.9 ± 0.80	0.000**	0.000**	0.000**

Normal HbA_1C: 5 - 8%

Graph III: Mean HbA_1C values in the study groups (n=75)

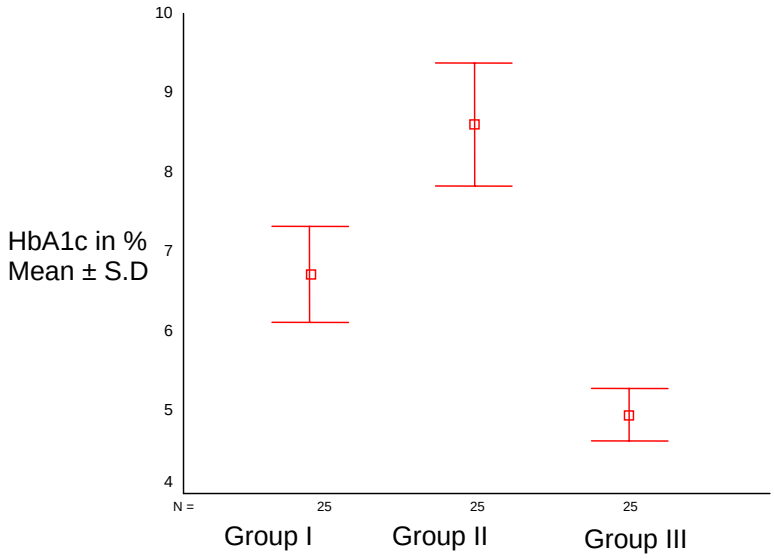


Table IV: Mean salivary glucose values in each group

Variable	Study groups (Mean ± SD)			p-value		
	I (n=25)	II (n=25)	III (n=25)	I & II	I & III	II & III
Salivary glucose in mg/dl	9.7 ± 7.6	12.1 ± 9.6	8.6 ± 7.5	0.357	0.927	0.356

Graph IV: Mean salivary glucose values in each group (n=75)

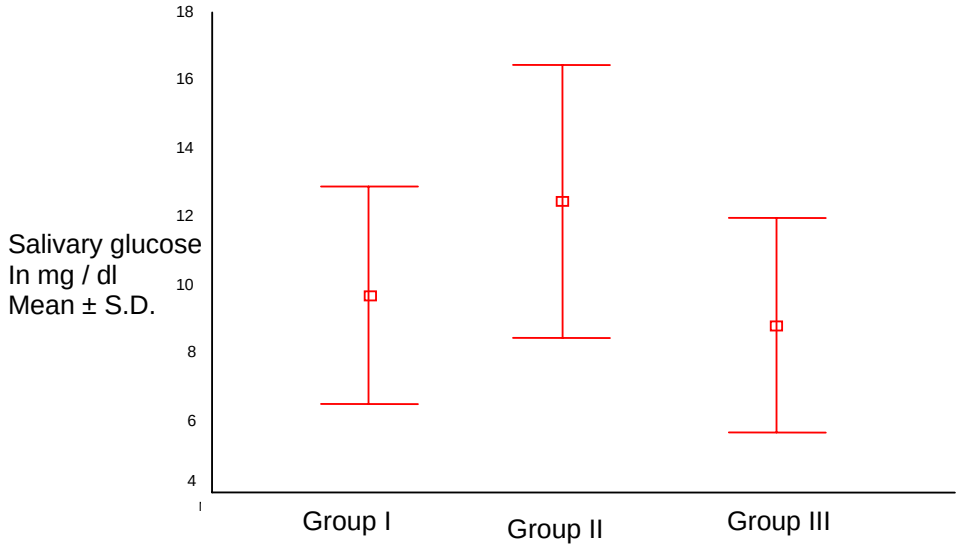


Table V: Mean salivary AGE values in each groups

Variable	Study groups (Mean ± SD)			p-value		
	I (n=25)	II (n=25)	III (n=25)	I & II	I & III	II & III
Salivary AGE in ppm	3.5 ± 1.2	6.3 ± 2.11	1.99 ± 0.80	0.000**	0.180	0.000**

Graph V: Mean salivary AGE values in each groups (n =38)

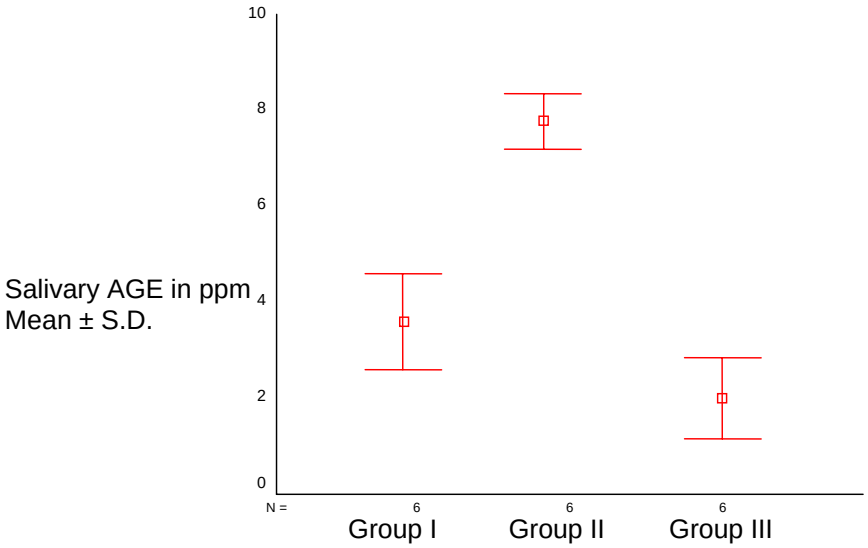
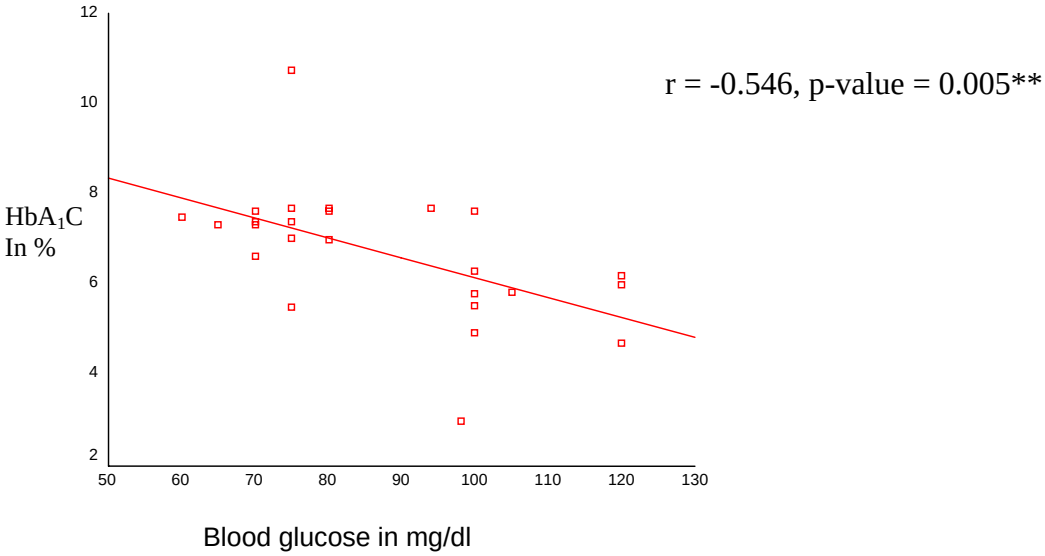


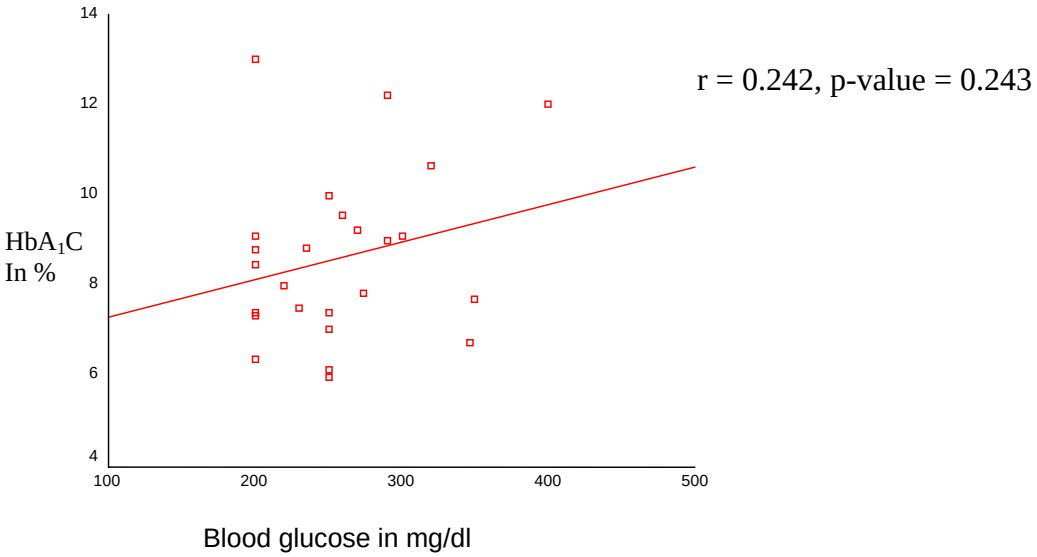
Table VI: Pearson's correlation analysis of blood glucose and HbA_1C in the study groups

Groups	Correlation coefficient (r)	p- value
Group I (controlled)	-0.546	0 .005**
Group II (uncontrolled)	0.242	0 .243
Group III(non diabetic)	0 .113	0 .591
Groups I, II, III	0 .620	0 .000**

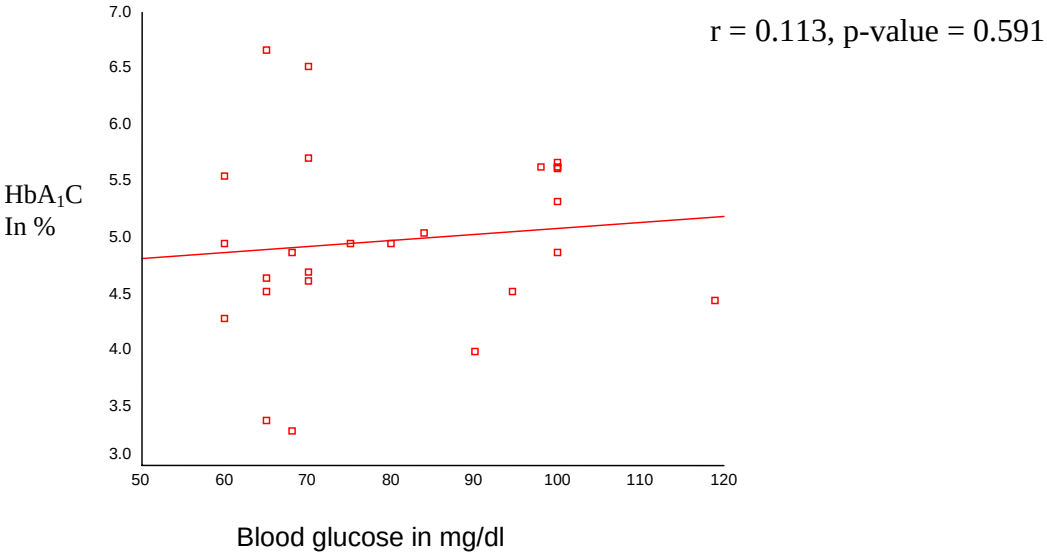
Graph VIa: Pearson's correlation analysis of blood glucose and HbA_1C in group I(controlled) n=25



Graph VIb: Pearson's correlation analysis of blood glucose and HbA_1C in Group II (uncontrolled) n=25



Graph VIc: Pearson's correlation analysis of blood glucose and HbA_1C in Group III (non diabetic) n=25



Graph VId: Pearson's correlation analysis of blood glucose and HbA_1C in all the groups (n=75)

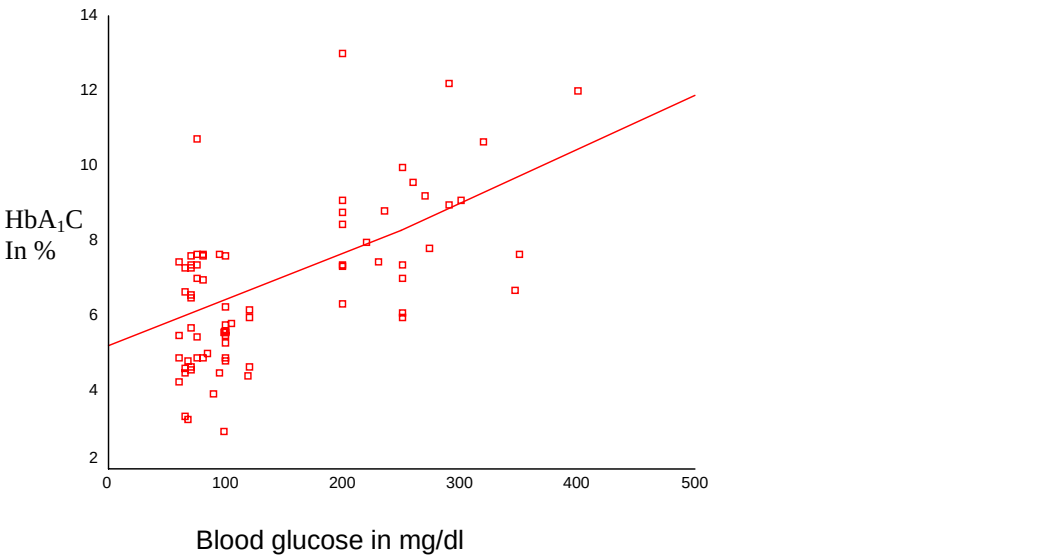
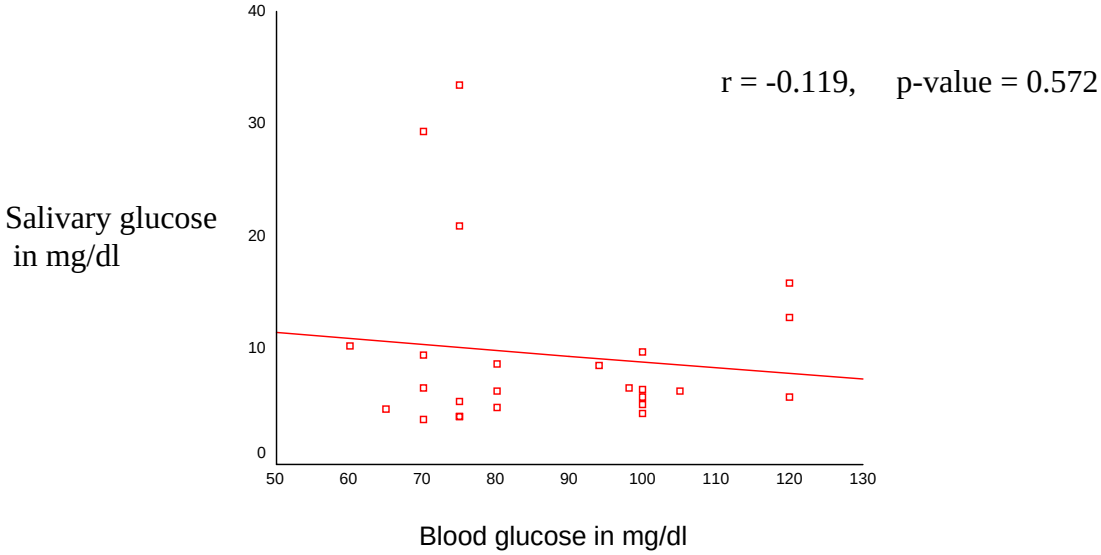


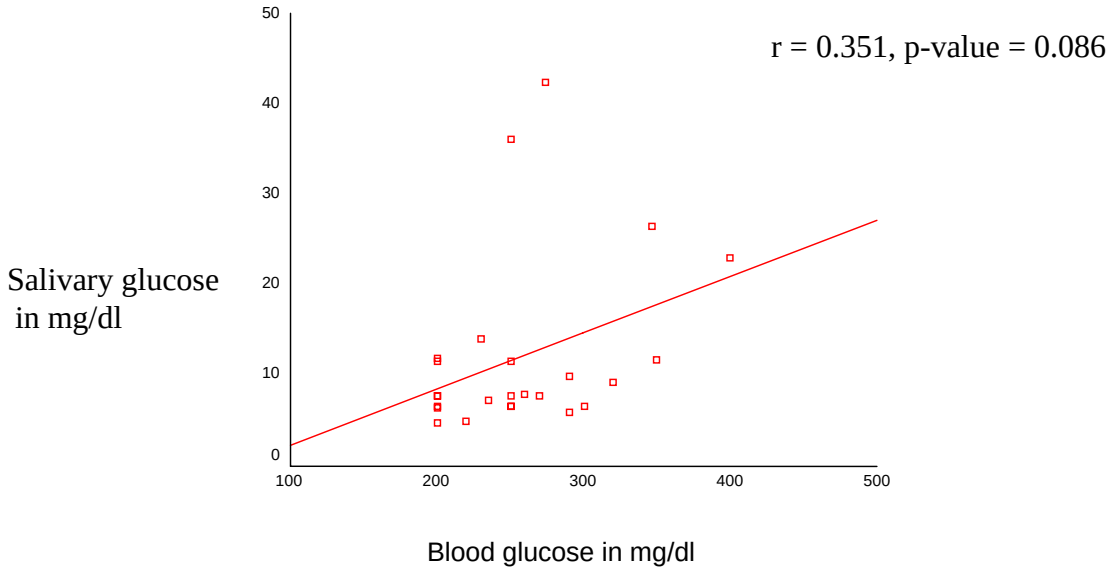
Table VII: Pearson's correlation analysis of blood glucose and salivary glucose in the study groups

Groups	Correlation coefficient (r)	p- value
Group I (controlled)	-0.119	0.572
Group II (uncontrolled)	0.351	0.086
Group III(non diabetic)	-0.414	0.040*
Group I,II,III	0.199	0.086

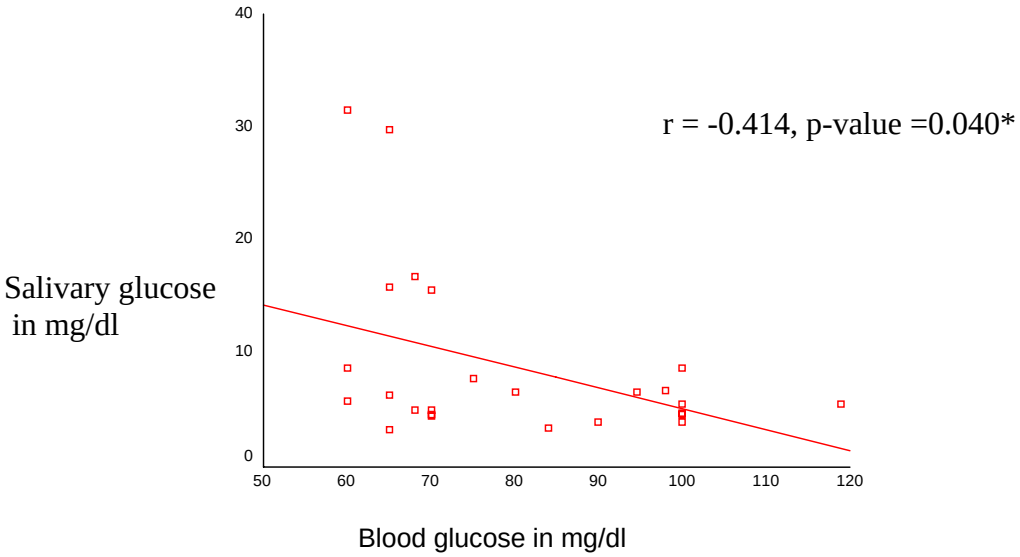
Graph VIIa: Pearson's correlation analysis of blood glucose and salivary glucose in group I(controlled) n=25



Graph VIIb: Pearson's correlation analysis of blood glucose and salivary glucose in group II(uncontrolled) n=25



Graph VIIc: Pearson's correlation analysis of blood glucose and salivary glucose in group III (non diabetic) n=25



Graph VIId: Pearson's correlation analysis of blood glucose and salivary glucose in all the groups (n=75)

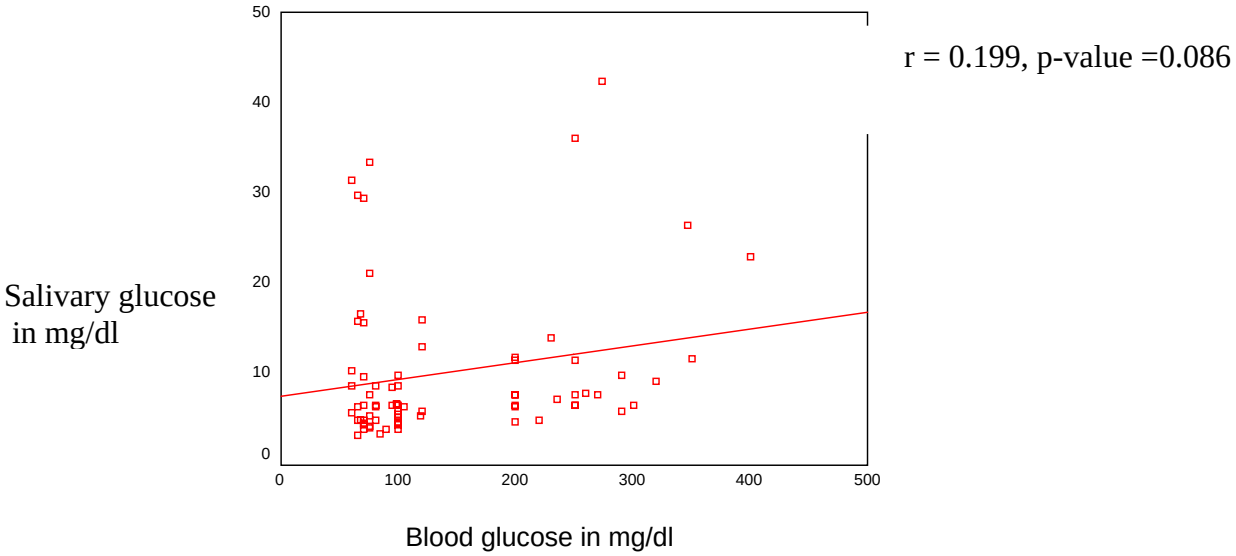
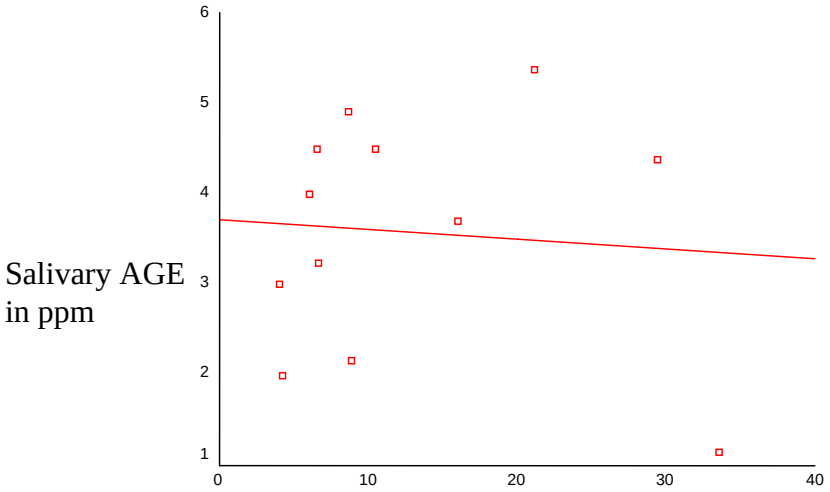


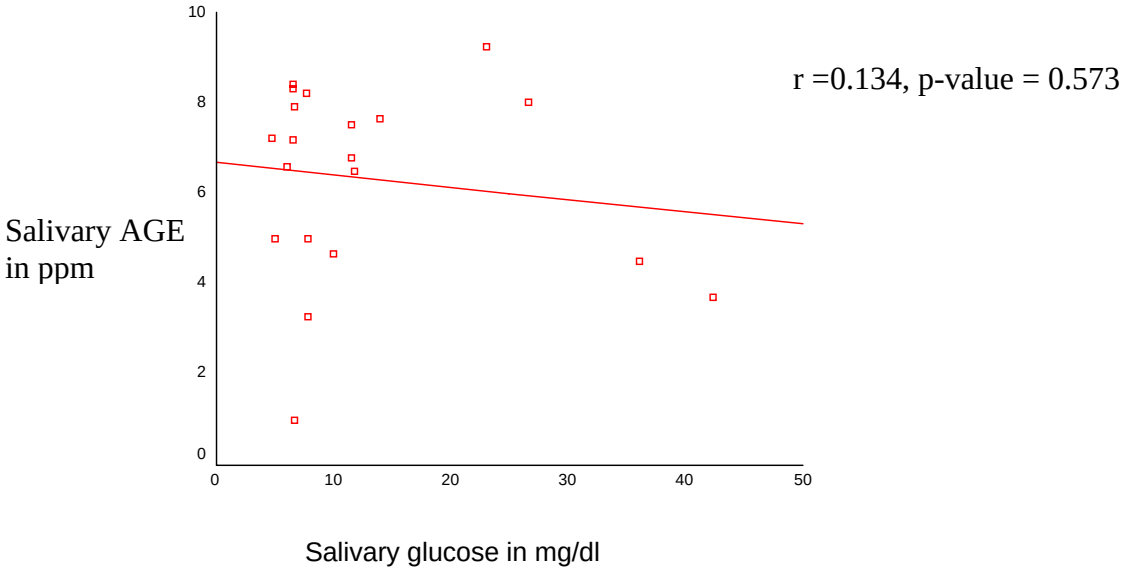
Table VIII: Pearson's correlation analysis of salivary glucose and salivary AGE in the study groups

Groups	Correlation coefficient (r)	p- value
Group I (controlled)	-0.083	0 .799
Group II (uncontrolled)	0.134	0 .573
Group III(non diabetic)	0.291	0 .576
Group I,II,III	-0.380	0 .019

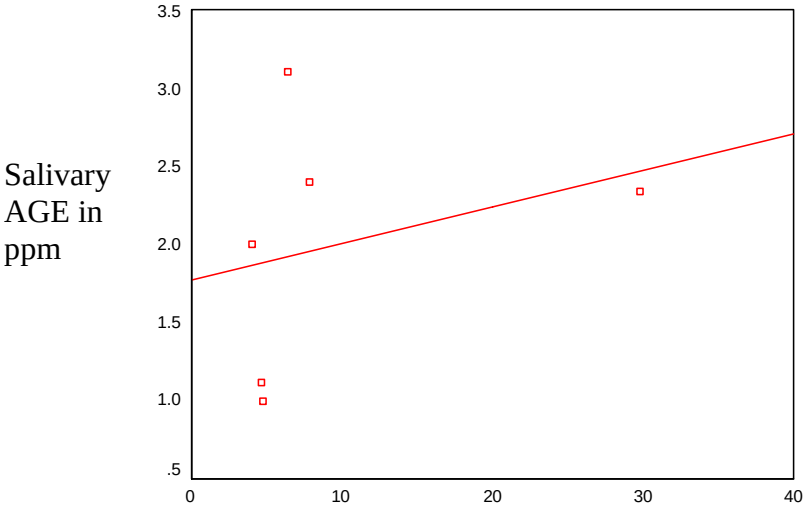
Graph VIIIa: Pearson's correlation analysis of salivary glucose and salivary AGE in group I (controlled) n=20



Graph VIIIb: Pearson's correlation analysis of salivary glucose and salivary AGE in group II (uncontrolled) n=12



Graph VIIIc: Pearson's correlation analysis of salivary glucose and salivary AGE in group III (non diabetic) n= 6



r = 0.291, p-value = 0 .576

Graph VIIId: Pearson's correlation analysis of salivary glucose and salivary AGE in all the groups n = 38

r = -0.380, p-value = 0.019*

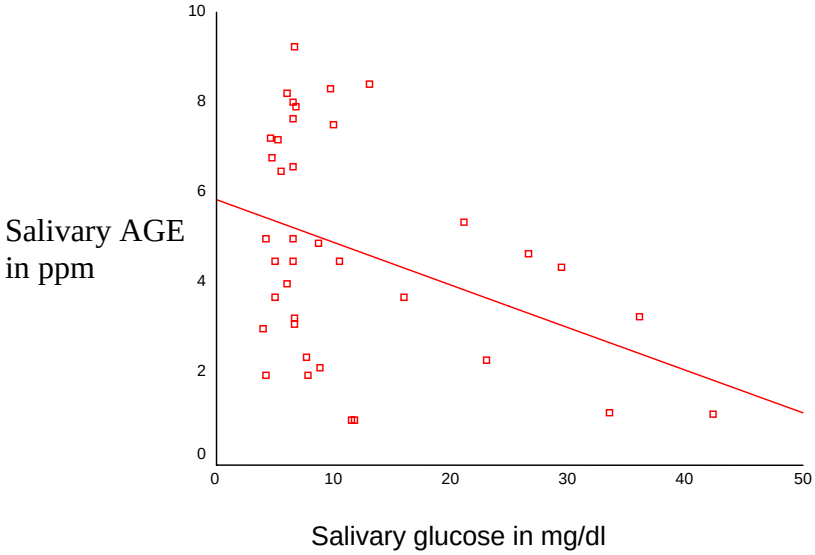
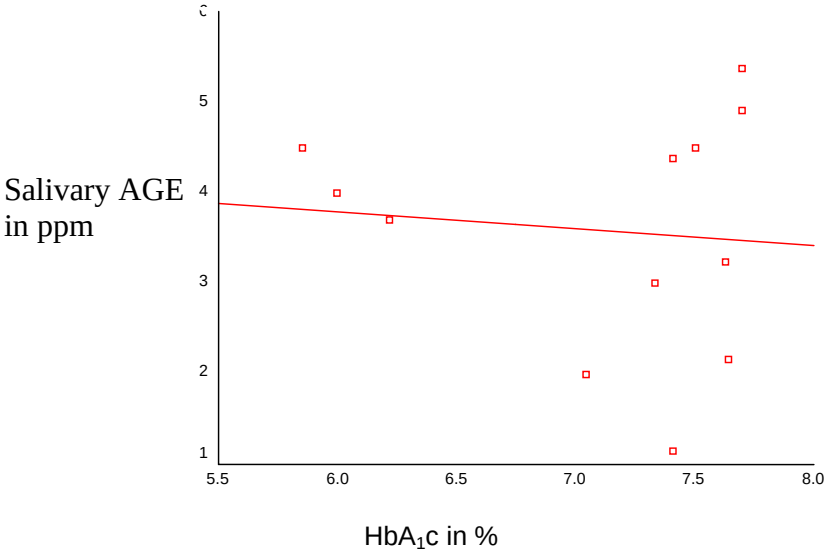


Table IX: Pearson's correlation analysis of salivary AGE and HbA_1C in the study groups

Groups	Correlation coefficient (r)	p- value
Group I (controlled)	-0.100	0.758
Group II (uncontrolled)	0 .095	0.692
Group III(non diabetic)	0 .433	0.391
Group I,II,III	-0.419	0.009**

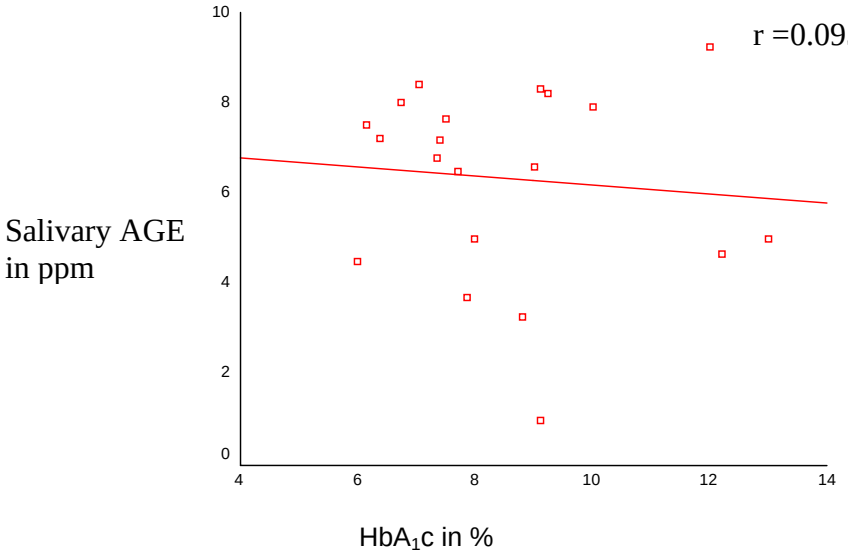
Graph IXa: Pearson's correlation analysis of salivary AGE and HbA_1C in group I (controlled) n=20

r = -0.100, p-value =0 .758

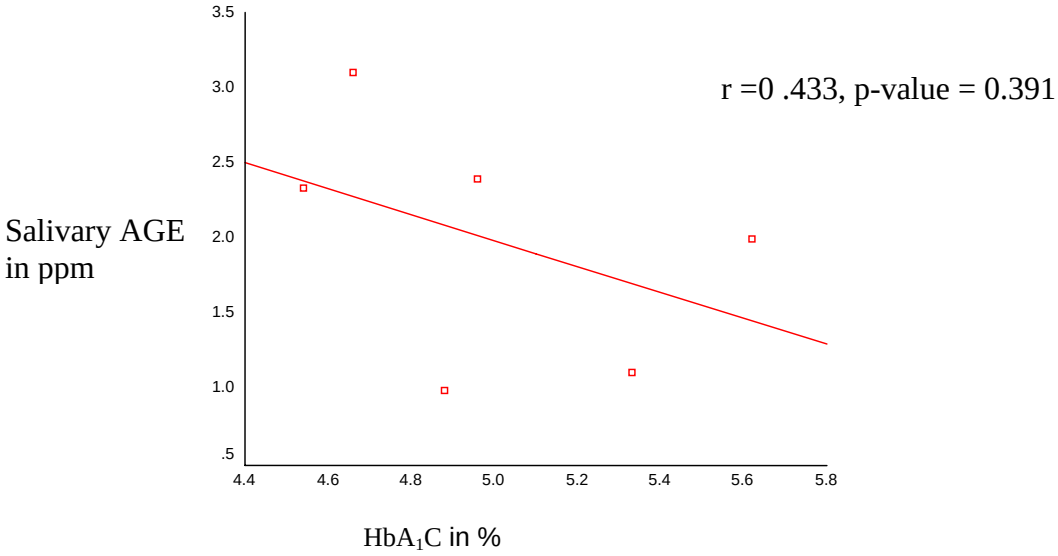


Graph IXb: Pearson's correlation analysis of salivary AGE and HbA_1C in group II (uncontrolled) n=12

r =0.095, p-value = 0.692



Graph IXc: Pearson's correlation analysis of salivary AGE and HbA_1C in Group III (non diabetic) n=6



Graph IXd: Pearson's correlation analysis of salivary AGE and HbA_1C between all the groups n = 38

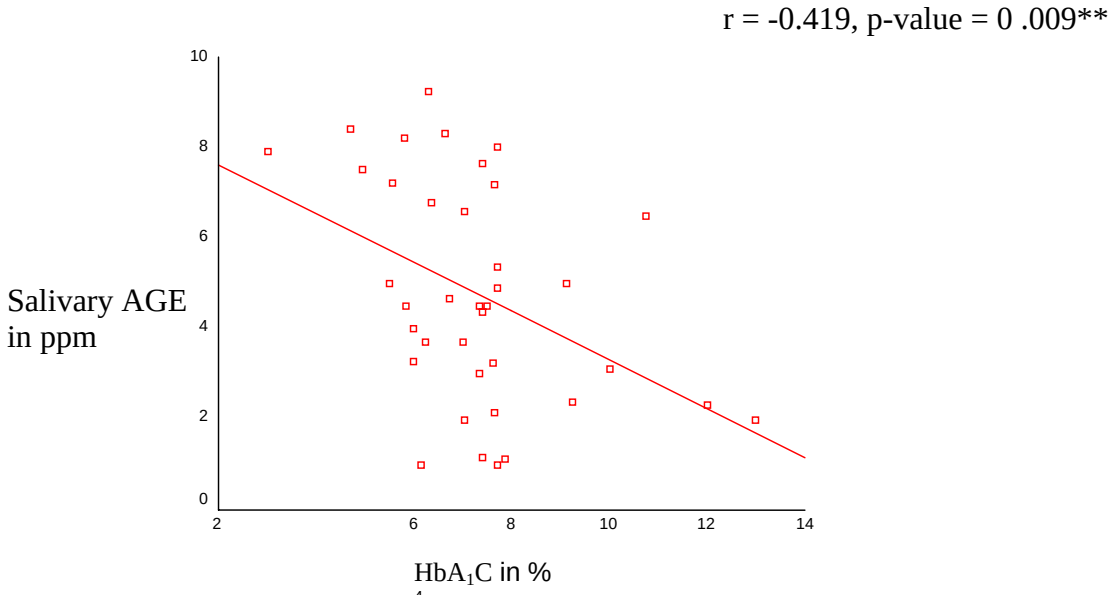
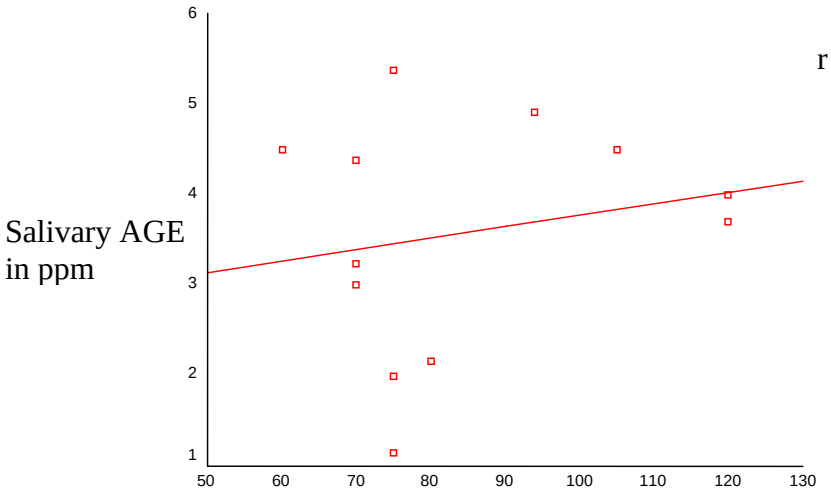


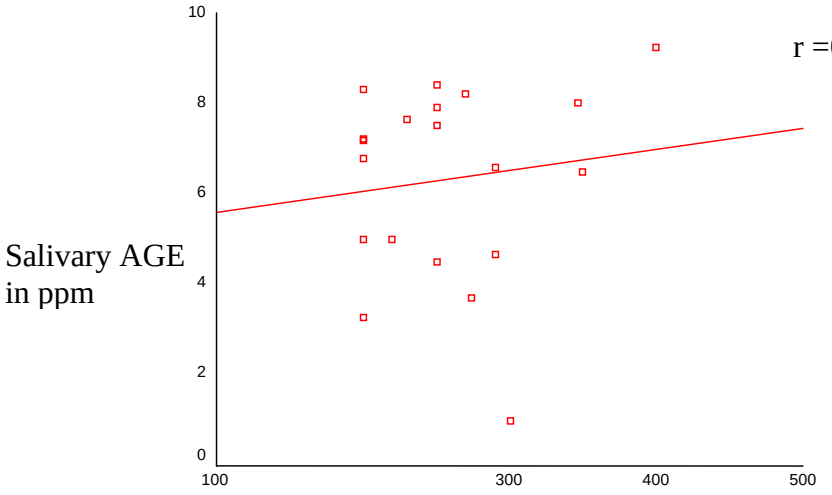
Table X: Pearson's correlation analysis of random blood glucose and salivary AGE levels in the study groups

Groups	Correlation coefficient (r)	p- value
Group I (controlled)	0.199	0.556
Group II (uncontrolled)	0.126	0.598
Group III(non diabetic)	-0.852	0 .031*
Group I,II,III	-0.369	0.022*

Graph Xa: Pearson's correlation analysis of random blood glucose and salivary AGE in Group I(controlled diabetes) n = 20

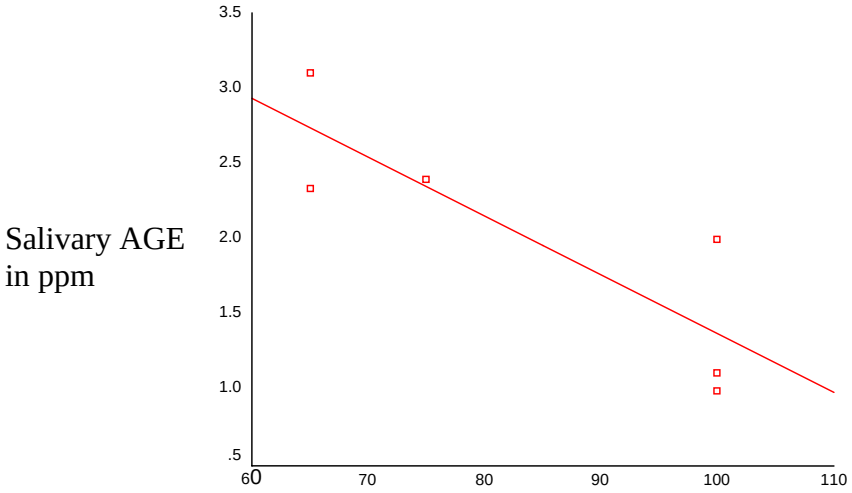


Graph Xb: Pearson's correlation analysis of random blood glucose and salivary AGE in Group II(uncontrolled diabetes) n = 12



Graph Xc: Pearson's correlation analysis of random blood glucose and salivary AGE in Group III (Non diabetic) n = 6

r = -0.852, p-value = 0.031*



Graph Xd: Pearson's correlation analysis of random blood glucose and salivary AGE between all the groups (n = 38)

r = -0.369, p-value = 0.022*

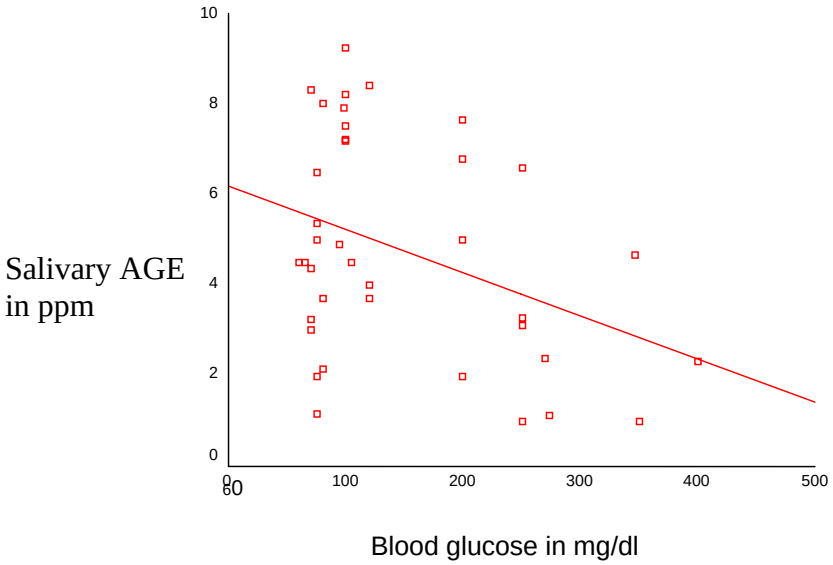


Table XI: Pearson's correlation analysis of salivary glucose and periodontal status in the study groups(n = 75)

Groups	Correlation coefficient (r)	p- value
Group I,II,III	0 .245	0 .034*

Graph XI: Pearson's correlation analysis of salivary glucose and periodontal status in the study groups (n = 75)

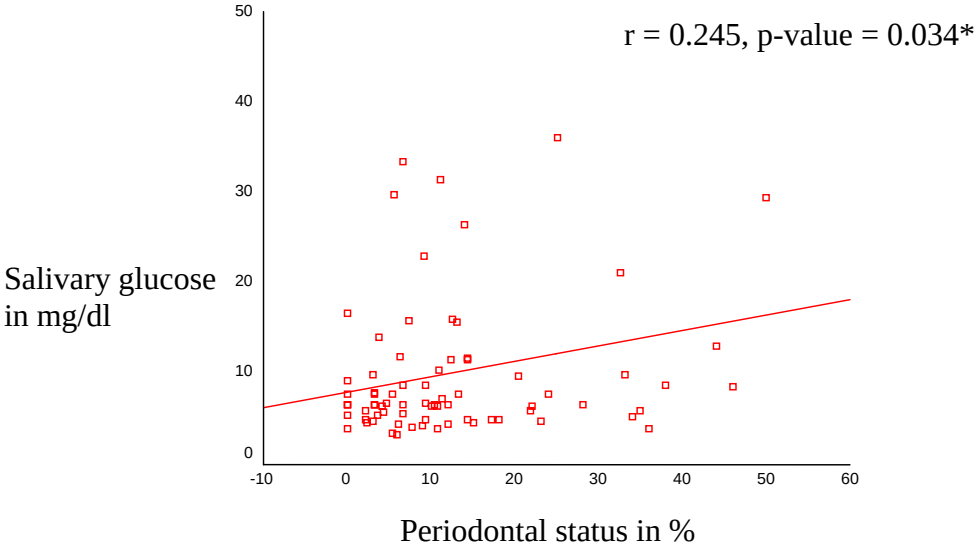


Table XII: Pearson's correlation analysis of salivary AGE and periodontal status in the study groups(n = 38)

Groups	Correlation coefficient (r)	p- value
Group I,II,III	0 .538	0.003**

Graph XII: Pearson's correlation analysis of salivary AGE and periodontal status In the study groups (n = 38)

r = 0.538, p-value = 0.003**

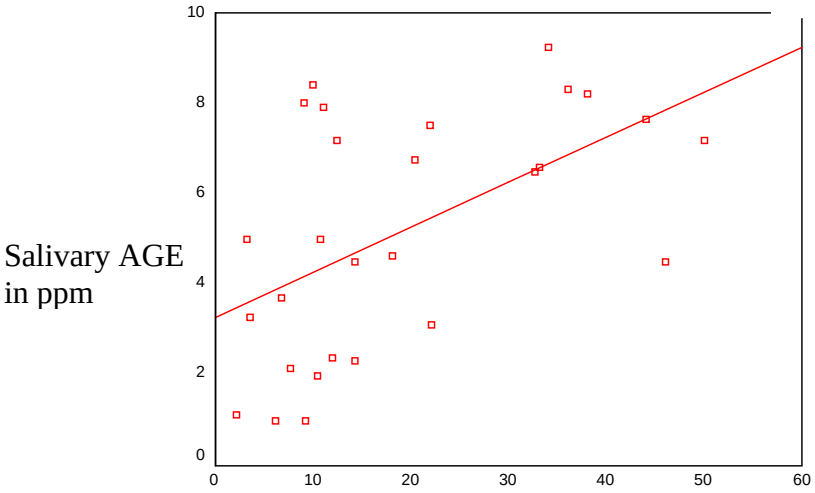


Figure 1: ARMAMENTARIUM FOR ORAL EXAMINATION

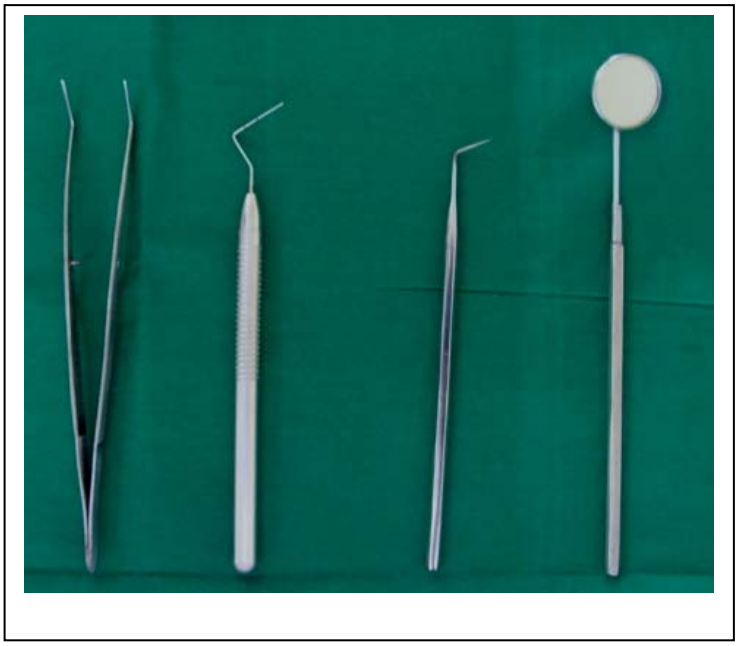


Figure 2: ARMAMENTARIUM FOR SAMPLE COLLECTION

Figure 4: ARMAMENTARIUM FOR ESTIMATION OF BLOOD GLUCOSE AND SALIVARY GLUCOSE

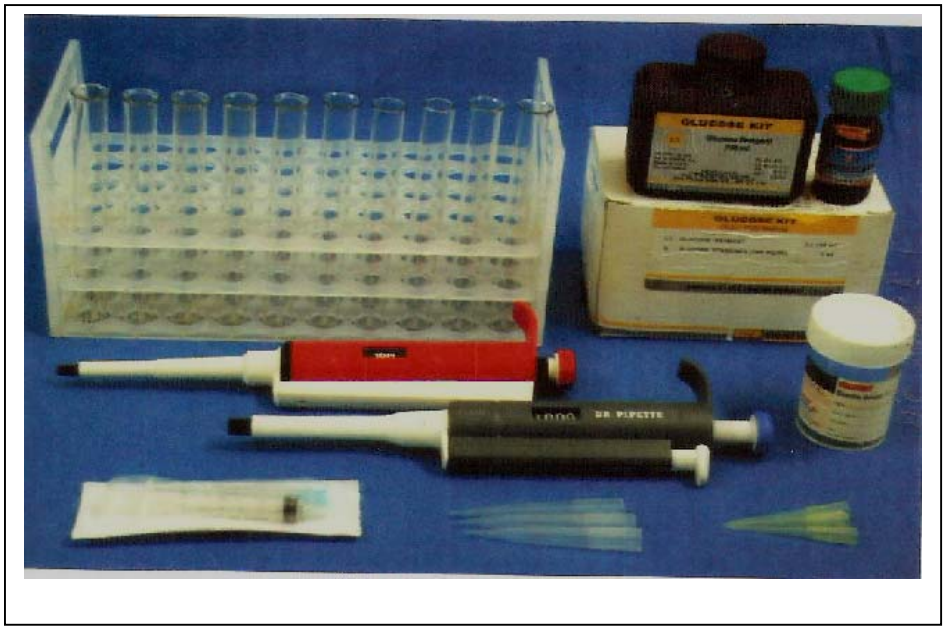


Figure 3: ARMAMENTARIUM FOR ESTIMATION OF GLYCOSLYATED HEMOGLOBIN

Figure 5: Nuclear Magnetic Resonance Spectroscopy

Figure 6: Sample mixer

Figure 7: Wilmad NMR tubes

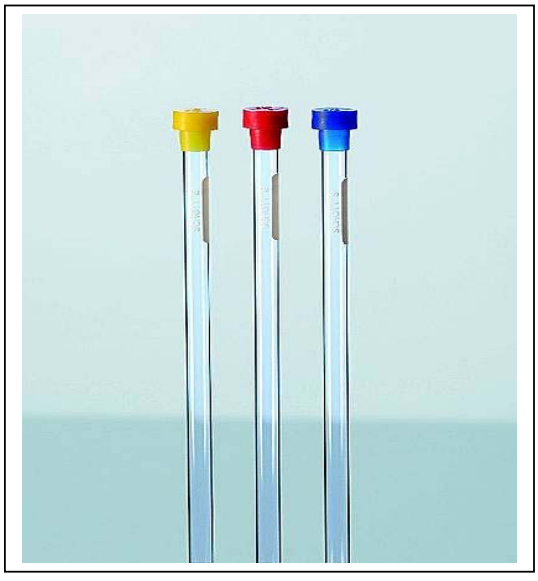


Figure 8: **ARMAMENTARIUM FOR PERIODONTAL STATUS ASSESSTMENT**

Diabetic patients are prone to develop increased Advanced Glycation End Products (AGE) which are irreversible products of non-enzymatic glycation and oxidation of proteins causing local complications, overall morbidity and mortality. The presence of advanced glycation endproducts in patients with diabetes mellitus was associated with increased periodontal damage as proposed by **Zidek *et al* (2003)** [6].

The present study population comprised of 75 patients, 25 each in group. Group I - controlled, Group II - uncontrolled and Group III - non-diabetic. Of the 75 patients, 35 were males and 40 were females. The number of females were higher compared to males in our study. This could be due to the fact that females tend to seek treatment early and also one of the exclusion criteria in our study was smoking. Although the mean age of patients with uncontrolled diabetes (group II) was higher than that in controlled diabetes (group I) the difference was statistically insignificant.

Although the mean random blood glucose levels showed difference between the three groups (Group II > Group I > Group III), the difference was statistically significant only between group I and II (p = 0.000**) and between group II and III (p = 0.000**). This was consistent with the findings reported by **Ichiro *et al* (2003)** [25]

In our study mean HbA_1C levels were higher in diabetics than in non-diabetics (Group II > Group I > Group III). This difference was statistically significant when compared between any of the two groups (p = 0.000**). This was consistent with the findings of **Dodds *et al* (2000)**[52] who showed a statistically significant difference in HbA_1C levels between diabetic and non diabetic patients.

Although salivary glucose levels were higher in diabetics than in non-diabetics (Group II > Group I > Group III) the difference was not statistically significant when

compared between any of the two groups. [Group I & II (p = 0.357), group I & III (p = 0.927), group II & III (p = 0.356)]. This is similar to findings reported by **Aryeh *et al* (1998)**[53] where in salivary composition like salivary glucose, potassium and total protein were elevated in diabetic patients compared to non diabetic. Also, **Reuterving *et al* (1987)**[54] have shown that except for salivary glucose the other composition in saliva did not show any difference between diabetic ad non diabetic patients.

The salivary AGE levels were higher in diabetics than in non – diabetics (Group II > Group I > Group III). This difference was statistically significant when compared between group I and II, group II and III (p = 0.000**).Group I and II and between group II and III (p = 0.000**).This is consistent with the findings reported by **Zidek *et al* (2004)**[6] who worked on 52 diabetic and 47 non diabetic patients showing significant difference in salivary AGE level between diabetic and non diabetic patients

HbA_1C concentration increased significantly with increase in random blood glucose levels (r = 0.620, p = 0.000**) in the study groups. The concentration of HbA_1C is an indicator of average blood glucose concentration for the preceding 2-3 months. Our results are consistent with **Nathan *et al* (2008)**[55] who worked on 268 diabetic and 80 nondiabetic patients. Also in our study there was significant negative correlation in controlled diabetes (r = -0.546, p = 0.005). This is due to the fact that HbA_1C value is unaffected by insulin, diet and exercise, the value remains the same till the life of an red blood cells and unaffected by short term fluctuation in blood glucose levels.

In group II (r = 0.351, p = 0.086) there was an increase in salivary glucose levels with increase in random blood glucose levels. This positive correlation was also observed when all the study groups were assessed together(r = 0.199, p = 0.086). While in group I

as the random blood glucose levels increases the salivary glucose level decreased. There was negative correlation in group I (r = -0.119, p = 0.572) and III (r = -0.414, p = 0.040*) Several factors may account for the varying observed correlation between blood and salivary glucose concentrations. They include oral retention of alimentary carbohydrates, glucose utilization by oral bacteria, release of carbohydrates from salivary glycoproteins, and contamination of saliva by a large outflow of crevicular fluid in patients with a poor gingival status. The negative correlation in group I and III is consistent with the findings reported by **Reuterving *et al* (1987)**[54] who observed that salivary glucose levels was low during the period of better metabolic control, therefore in well controlled individuals with altered glucose metabolism there was lower salivary glucose levels. Also**,** the observed correlation is consistent with the findings of **Amer *et al* (2001)**[56] who reported that salivary glucose increases as serum glucose increases, while the salivary samples from age matched non-diabetic did not show any correlation

We observed that the salivary AGE concentration significantly decreased as the levels of salivary glucose increased and this negative correlation was statistically significant (r = -0.380, p = 0.019*).The salivary glucose is affected by bacteria and enzymes in the mouth. The microorganism utilizes the glucose rapidly so it is difficult to measure it without ultra- filtration. Salivary AGE is not formed from salivary glucose. Reports have suggested that the increased glucose formed in the serum can be elicited in the gingival crevicular fluid and this glucose is utilized in the formation of salivary AGE. **Beikler *et al* (2002)**[28] who worked on 32 non-diabetic and 13 diabetic patients with moderate to severe periodontitis found that blood oozing during routine periodontal examination may be used for diabetes mellitus screening in a dental office setting.

Khader_ *et al* (2006) [29] in his study compared gingival crevicular blood sample and r sample obtained by finger puncture using a self-monitoring device and found that there was correlation, there by stating that Gingival crevicular blood can provide an acceptable source for measuring blood glucose level.

When all the groups were assessed together, as the values of HbA_1C increases the levels of AGE in saliva decreased significantly (r = - 0.419, p = 0.009). Our results are consistent with the results of **Ichiro *et al* (2003)[25].** HbA1c in erythrocytes cannot be measured with saliva.

The salivary AGE levels increased when there was an increase in blood glucose levels in both group I and II. However this positive correlation was not statistically significant in Group I (r = 0.199, p = 0.556) and in group II (r =0.126, p = 0.598). Our results are consistent with the results of **Zidek *et al* (2004)** [6] who reported that NMR signal was more in diabetics compared to that in non – diabetic patients.

There was a significant association between periodontal status and salivary glucose levels. As the levels of salivary glucose increased the periodontal status deteriorated (r = 0.245, p = 0.034*). **Tenovvuo *et al* (1986)[56]** reported that increased exposure of bacteria as a consequence of elevated salivary glucose was seen in controlled and uncontrolled diabetic. The salivary glucose increased the substrate for bacteria and altered plaque micro-flora favoring periodontal diseases. **Oates *et al* (2002)[57]** stated that high circulating levels of glucose inhibit peripheral leukocyte chemotaxis and provide a nutrient-rich environment for bacterial or fungal replication. **Kaisa *et al* (2000)** [58] reported that in diabetics, there is a decreased salivary flow rate and an increase in salivary glucose levels. Higher salivary microbial counts, especially yeast counts, are

related to the low salivary flow rates and high salivary glucose levels which contribute to periodontitis.

There was a significant association between periodontal status and salivary AGE levels ($r = 0.538$, $p = 0.003$**). However, given the limited sample size in this subgroup (n=38) and other confounding factors (age, gender, regular oral hygiene maintenance, early diagnosis, use of other substances, maintenance of diabetic status, food habits and genetic status). We state the above results confirm an association between diabetes mellitus and periodontitis but a cause and effect relationship has to be established taking into account the confounding factors. AGE is responsible for various changes leading to collagen crosslinking, and basement membrane disintegration. AGE can also bind to macrophage receptors and induce cytokin (IL- 1 & TNF-α) up-regulation. This in turn induces alteration in the matrix metalloproteinase 8 (MMP 8) periodontal tissue of diabetics [60]. **Killi M *et al* (2002)**[61] showed that MMP type 8 originates from polymorphonuclear (PMN) leukocyte, epithelial cells and plasma cells and is detected in the gingival cervicular fluid of inflamed periodontium. **Villela B *et al* (1987)**[62] reported that there was correlation between GCF collagenase and pocket depth.

Brown *et al* (1998)[63] reported that one of the major complications of hyperglycemia is alteration of circulating proteins. These proteins when exposed to aldose sugar undergo non-enzymatic glycation and oxidation resulting in AGE formation. **Valssara *et al* (1992)**[64] reported that AGE act on target cells via their binding to cell surface polypeptide receptor, the binding being the immunoglobulin superfamily called the Receptor for AGE (RAGE). AGE can interact with RAGE on cells such as macrophages, endothelial cells and fibroblast which stimulate the production of MMP.

Neutrophils release diacyl glycerol, protein kinase C and superoxide which also stimulate MMP. **Schmidt *et al* (2009)** [65] reported that AGE in the gingival of the diabetics was associated with oxidative stress a potential mechanism of accelerated tissue injury. **Wolowczuk *et al* (2008)**[66]showed that binding of AGE to their receptor RAGE initiates cellular signals that activate NF-kappaB, which results in transcription of proinflammatory factors causing chronic inflammatory diseases.

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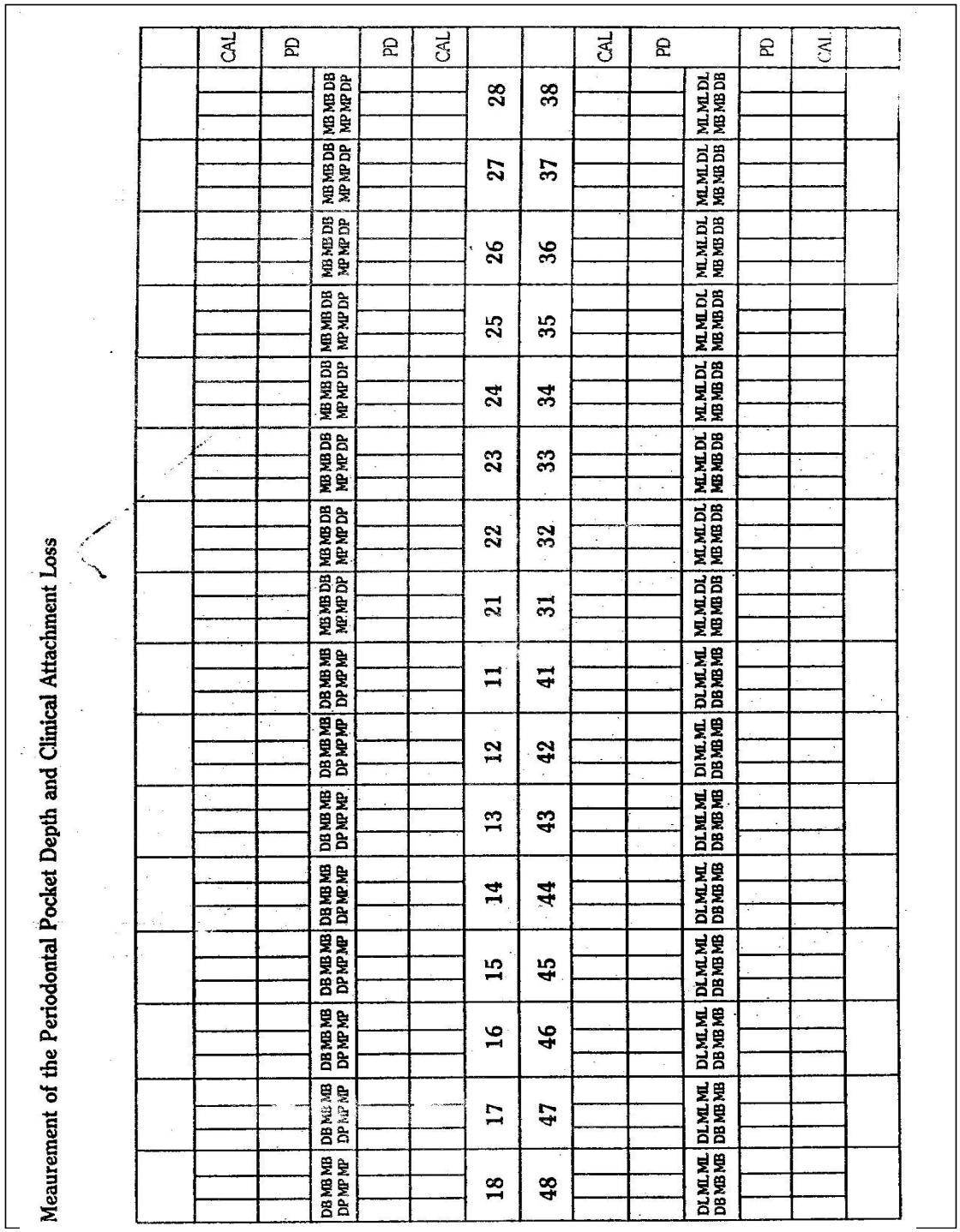
Annexure 1


Meaurement of the Periodontal Pocket Depth and Clinical Attachment Loss
CAL
PD
DB MB MB DP MP MP
DL ML ML DB MB MB
MB MB DB MP MP DP
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18 17 16 15 14 13 12 11 21 22 23 24 25 26 27 28
48 47 46 45 44 43 42 41 31 32 33 34 35 36 37 38

Annexure 2

CONVERSION CHART OF GLYCOSYLATED HEMOGLOBIN A1% TO MEAN BLOOD GLUCOSE AND GLYCOSYLATED HEMOGLOBIN A1C %

A1	A1c	MBH	A1	A1c	MBH
6.0	4.30	35	7.9	5.88	105
6.1	4.38	39	8.0	5.97	109
6.2	4.46	43	8.1	6.05	112
6.3	4.54	46	8.2	6.14	116
6.4	4.63	50	8.3	6.22	120
6.5	4.71	54	8.4	6.30	123
6.6	4.79	58	8.5	6.39	127
6.7	4.88	61	8.6	6.47	131
6.8	4.96	65	8.7	6.55	134
6.9	5.05	68	8.8	6.64	138
7.0	5.13	72	8.9	6.72	142
7.1	5.21	76	9.0	6.81	145
7.2	5.30	79	9.1	6.89	149
7.3	5.38	83	9.2	6.97	153
7.4	5.46	87	9.3	7.06	156
7.5	5.55	90	9.4	7.14	160
7.6	5.63	94	9.5	7.22	164
7.7	5.72	98	9.6	7.31	167
7.8	5.80	101	9.7	7.39	171

A1	A1c	MBH	A1	A1c	MBH
9.8	7.48	175	12.1	9.40	259
9.9	7.56	178	12.2	9.49	263
10.1	7.64	186	12.3	9.57	266
10.2	7.73	189	12.4	9.65	270
10.3	7.81	193	12.5	9.74	274
10.4	7.89	197	12.6	9.82	277
10.5	7.98	200	12.7	9.99	285
10.6	8.06	204	12.8	10.07	288
10.7	8.15	207	12.9	10.16	292
10.8	8.23	211	13.0	10.24	295
10.9	8.31	215	13.1	10.33	299
11.0	8.40	219	13.2	10.41	304
11.1	8.48	222	13.3	10.49	309
11.2	8.56	226	13.4	10.58	314
11.3	8.65	230	13.5	10.66	320
11.4	8.73	233	13.6	10.74	326
11.5	8.82	237			
11.6	8.90	241			
11.7	8.98	244			
11.8	9.07	248			
11.9	9.15	252			
12.0	9.24	255			

Annexure 3

LIST OF ABBREVATIONS

DM	-	Diabetes mellitus
AGEs	-	Advanced glycation end products
FPG	-	Fasting blood glucose
PG	-	Postprandial glucose
OGTT	-	Oral glucose tolerance test
HbA_1C	-	Glycosylated hemoglobin
THb	-	Total hemoglobin fraction
GOD	-	Glucose oxidase
POD	-	Perioxidase
NMR	-	Nuclear magnetic resonance spectroscopy
CPITN	-	Community periodontal index for treatment needs
MMP	-	Matrix metalloproteinase
RAGE	-	Receptor for advanced glycation end product