ASSOCIATION BETWEEN SALIVARY FRUCTOSAMINE, PLASMA GLYCATED HEMOGLOBIN AND PLASMA GLUCOSE LEVELS AMONG TYPE II DIABETES MELLITUS AND NON DIABETIC INDIVIDUALS —A CROSS SECTIONAL STUDY

Dissertation Submitted to
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In Partial Fulfillment for the Degree of
MASTER OF DENTAL SURGERY

BRANCH VII
PUBLIC HEALTH DENTISTRY
APRIL 2017
THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY
CHENNAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled “Association between Salivary Fructosamine, Plasma Glycated Hemoglobin and Plasma Glucose levels among Type II Diabetes mellitus and Non Diabetic individuals – A cross sectional study” is a bonafide and genuine research work carried out by me under the guidance of Dr. P. D. Madan Kumar, MDS, Professor and Head, Department of Public Health Dentistry, Ragas Dental College and Hospital, Chennai.

Place: Chennai

Dr. SADHANA. K,
Post Graduate Student
Department of Public Health Dentistry,
Ragas Dental College and Hospital,
Chennai.
THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY
CHENNAI

DECLARATION BY THE GUIDE

I hereby declare that this dissertation titled “Association between Salivary Fructosamine, Plasma Glycated Hemoglobin and Plasma Glucose levels among Type II Diabetes mellitus and Non Diabetic individuals – A cross sectional study” is a bonafide and genuine research work carried out by Dr. Sadhana K, Post Graduate Student in the Department of Public Health Dentistry, Ragas Dental College and Hospital, Chennai under my guidance in partial fulfillment for the requirement of the degree of Master of Dental Surgery (Public Health Dentistry).

Date: 8.1.2016
Place: Chennai

Dr. P. D. Madan Kumar MDS,
Professor and Head,
Department of Public Health Dentistry,
Ragas Dental College and Hospital,
Chennai,

Dr. P. D. MADAN KUMAR, M.D.S
PROFESSOR
DEPT. OF PUBLIC HEALTH DENTISTRY
RAGAS DENTAL COLLEGE & HOSPITAL
THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY
CHENNAI

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT
AND HEAD OF THE INSTITUTION

This is to certify that this dissertation titled “Association
between Salivary Fructosamine, Plasma Glycated Hemoglobin and
Plasma Glucose levels among Type II Diabetes mellitus and Non
Diabetic individuals – A cross sectional study” is a bonafide and
genuine research work done by Dr. Sadhana. K, under the guidance of
Dr. P.D. Madan Kumar, MDS., Professor and Head, Department of
Public Health Dentistry, Ragas Dental College and Hospital, Chennai.

Dr. P.D. Madan Kumar, MDS.,
Professor and Head,
Department of Public Health
Dentistry,
Ragas Dental College and Hospital,
Chennai.

Date: 28.11.2016
Place: Chennai

Dr. N.S Azhagarasan, MDS.,
Principal,
Ragas Dental College and Hospital,
Chennai.

Date: 28.11.2016
Place: Chennai
THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY
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Signature of the Candidate
Dr.Sadhana.K
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This agreement herein after the “Agreement” is entered into on this day 28th December 2016, between the Ragas dental college and hospital represented by its Principal having address at Ragas dental college and hospital, Chennai-119, (herein after referred to as, ‘the College’)  

And

Dr. P. D. Madan Kumar, MDS, aged 39 years, working as Professor and Head of the Department of Public Health Dentistry at the college, having address at Department of Public health dentistry, Ragas Dental college and hospital, (herein after referred to as the ‘Researcher and Principal investigator’)  

And

Dr. Sadhana. K, aged 26 years, currently studying as Post Graduate student in the Department of Public Health Dentistry (herein after referred to as the ‘PG/Research student and Co-investigator’).

Whereas the ‘PG/Research student as part of her curriculum undertakes to research on the study titled “Association between Salivary Fructosamine, Plasma Glycated Hemoglobin and Plasma Glucose levels among Type II Diabetes mellitus and Non Diabetic individuals – A cross sectional study” for which purpose the Researcher and Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator.

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In witness whereof of the parties herein above mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its Principal
PRINCIPAL
RAGAS DENTAL COLLEGE AND HOSPITAL
UTHANDI, CHENNAI - 600 119.

Witnesses
1. (Dr. K. Ibrahim)
2. (Dr. B. B. Bande)

Student Researcher

Student Guide

Dr. P.D. MADAN KUMAR, M.D
PROFESSOR
DEPT. OF PUBLIC HEALTH DENTISTRY
RAGAS DENTAL COLLEGE & HOSPITAL
CHENNAI - 600 119
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“Nothing grows in the shadow of want without the sunlight of acknowledging your fullness.” - Bryant McGill

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Truly,

Dr. Sadhana K
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Introduction
INTRODUCTION

“Health is wealth” goes the old maxim. Health is a direct source of human welfare and also an instrument for raising economic standards.\(^1\) Health is a result of common cultural endeavor. During the past few decades there has been an awakening that health is a fundamental human right and a worldwide social goal has been established that health is essential to the satisfaction of basic human needs to improve the quality of life.\(^2\) An understanding of health is the foundation of all health care. Over the centuries health has evolved as a concept from individual care to a worldwide social goal and encompasses the whole quality of life.\(^3\)

The term disease is often used more broadly to refer to any condition that causes pain, dysfunction, distress, social problems, or death to the person afflicted or similar problems for those in contact with the person.\(^4\) In recent times due to the advances in infection control, antibiotic therapy and preventive measures, the burden of communicable disease has considerably reduced when compared to non communicable diseases. It has been estimated that by the year 2020 non communicable disease would cause seven out of every ten deaths in developing countries.\(^5\)

Industrial revolution, rapid urbanization added to economic growth in the last 200 years has led to detrimental changes in the diet and lifestyle of humans. Recent innovations in food processing technology coupled with
marketing techniques has led to the increased consumption of saturated fat and sugar rich diet.\textsuperscript{5} Tobacco use, physical inactivity and harmful use of alcohol are the other factors that have contributed to the increased prevalence of non communicable diseases.\textsuperscript{7}

Non communicable diseases which include cardiovascular diseases, cancer, respiratory disease, and Diabetes mellitus kill about 38 million people per year out of which Diabetes mellitus has led to the death of around 4.9 million people in the year 2014.\textsuperscript{7} Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both.\textsuperscript{8} The American Diabetes Association (ADA) etiopathogenetic classification of Diabetes mellitus includes Type I, Type II and Gestational Diabetes. The chronic hyperglycemic state due to insulin deficiency causes progressive tissue and vascular damage which in due course leads to micro and macro vascular complications.\textsuperscript{8}

Type I Diabetes is classified into Type Ia which is due to immunological destruction of pancreatic $\beta$ cells resulting in insulin deficiency; and Type Ib is idiopathic in nature. Type Ia is characterized by the presence of auto antibodies against islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD). There is no known etiology for Type Ib Diabetes mellitus. It is marked by permanent insulinopaenia, but has no evidence of autoimmunity.\textsuperscript{9}
Type II Diabetes is the most common form of Diabetes and is characterized by disorders of insulin secretion and insulin resistance. It has a strong genetic predisposition and strongly associated with sedentary lifestyle, reduced physical activity and obesity. In recent times the role of autoimmunity as a pathological component of Type II Diabetes has been widely studied. Circulating antibodies against beta cells, glucose lowering receptors of the body and self-reactive T cells have been identified amongst Type II Diabetes patients.

Advanced Glycated End Products (AGE) formed by the non-enzymatic glycation of proteins, lipids and nucleic acids are responsible for the severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. The receptors for Advanced Glycated End Products (RAGE) are present on macrophages, adipocytes, endothelial cells, and vascular smooth muscle cells. Thus stimulation of these receptors by the AGE results in the generation of pro-inflammatory cytokines, release of vasoconstrictors, expression of adhesion molecules and increases thrombomodulin activity, which in turn leads to a pro-coagulant state. AGE products are also responsible for the generation of hydroxyl ions resulting in oxidative stress in Diabetes patients.

Diabetes is the most common endocrine disorder. According to the International Diabetes Federation (IDF); in the year 2014 an estimated 387 million people suffered from Diabetes and it has led to the death of
Introduction

4.9 million people in the year 2014. There has been an alarming rise in the prevalence of all types of Diabetes, and it has been projected that the number will increase by 55% by the year 2035.\textsuperscript{13}

India has the world’s largest population living with Diabetes after China.\textsuperscript{14} The IDF reported that in the year 2014 about 66.8 million Indians suffer from Diabetes and it may rise to 109 million by the year 2025. The National prevalence rate of Diabetes is 8.63 % and nearly 1 million Indians die due to Diabetes every year.\textsuperscript{13}

Short term and long term glycemic control in diabetic patients is monitored conventionally by evaluating the Plasma Fasting Glucose levels and Glycated Hemoglobin levels respectively.\textsuperscript{15} The ADA 2014 (American Diabetes Association) list of investigations for the diagnosis of Diabetes includes the Fasting Plasma Glucose (FPG) level or the two hour Plasma Glucose (2-h PG) value after 75-g Oral Glucose Tolerance Test (OGTT). In recent times Glycated Hemoglobin (HbA1C) was added to the list.\textsuperscript{16}

Screening for Diabetes has a remarkable impact on individual’s health, health systems and society as a whole.\textsuperscript{17} Diabetes is associated with significant mortality and morbidity; nevertheless the disease can be controlled when diagnosed before the onset of Diabetes associated complications, further the diagnostic tests are acceptable and accessible to patients. The disease has an asymptomatic stage that may be present for up to seven years before it is diagnosed.\textsuperscript{18}
Type II Diabetes mellitus frequently remains undetected for a long time, about one-half of diabetic patients present with one or more irreversible complications at the time of diagnosis.\textsuperscript{19,20} Studies reports that one in two people with Diabetes do not know that they have Diabetes, thus 175 million people are currently undiagnosed and a huge amount of people with Diabetes are progressing towards complications unaware of their health condition. In India there are around 35.4 million people undiagnosed with Diabetes. Hence early detection and monitoring of blood glucose improves micro vascular outcomes, helps both patients and their health care professionals assess the responses to therapy.\textsuperscript{13}

Early detection and periodic monitoring of Diabetes enhances the longevity and quality of life, by prevention or delay of long term diabetic complications. This also reduces hospital admissions and length of stay; consequently it minimizes the health care spending and helps in the redistribution of health care resources. The Diabetes Prevention Programme (DPP) Research group reports that periodic screening helps in the identification of individuals with Impaired Glucose Tolerance (IGT), and thus helps to plan timely life style modification programs to prevent or delay the transition from IGT to Diabetes.\textsuperscript{17}

In recent times the performance of diagnostic test for Diabetes has been reviewed extensively. Although the tests recommended by the ADA are
widely used each of them have their pros and cons, hence screening programs include a combination of these tests. \(^{17}\)

Diabetes can be diagnosed if the patient has Fasting blood glucose level of 126 mg per dL or greater on two separate occasions. The limitations of Fasting Plasma Glucose (FPG) testing are overnight fast, venous puncture, a 12 to 15 percent day-to-day variance in FPG values and a slightly lower sensitivity for predicting micro vascular complications.\(^{18,20}\) The sensitivity of FPG lies between 40% and 65% and specificity is > 90%.\(^{17}\)

The Oral Glucose Tolerance Test is considered as a first-line diagnostic test; however it has the following limitations such as venous puncture, poor reproducibility and patient compliance. The high osmolarity of the glucose solution causes delayed gastric emptying which results in nausea vomiting, and abdominal bloating frequently.\(^{21}\) The sensitivity and specificity of Oral Glucose Tolerance Test is reported to be 78% and 62% respectively.\(^{22}\)

Maillard reaction is the process by which carbonyl group of reducing sugars such as glucose react non enzymatically with amino groups in proteins, lipids and nucleic acid to form aldimines or early glycation products (Schiff’s Base). These early glycation products consequently undergo the Amadori rearrangement and form intermediate glycation products which includes Plasma Glycated Hemoglobin (HbA1C) and Plasma Fructosamine. The advanced glycated end products (AGE) are formed by classic rearrangement of the intermediate glycation products. Pentosidine, Pyralline, Carboxyethyl
lysine (CEL), Carboxymethyl lysine (CML), Methyl glyoxal lysine dimer (MOLD), Deoxyglucosone–lysine dimer (DOLD) are the AGE products.\textsuperscript{12}

Plasma Glycated Hemoglobin (HbA1C) is formed by the glycation of N–terminus of the valine and lysine residues in the alpha and beta chains of hemoglobin. Similarly Plasma Fructosamine is formed by the glycation of plasma protein albumin at multiple sites, predominantly at the epsilon amino groups of lysine residues. The half-life of hemoglobin is 60 days and that of albumin is 14-20 days. Chronic hyperglycemia in Diabetic patients results in increased glycation of hemoglobin and albumin. Hence Plasma HbA1C and Plasma Fructosamine levels are elevated in diabetic patients. Thus Plasma HbA1C levels indicate the glycemic control for six to eight weeks, and Plasma Fructosamine levels indicate the mean blood glucose concentration over two to three weeks.\textsuperscript{15}

Unlike Amadori products such as Plasma HbA1C and Plasma Fructosamine, AGE products affect long living proteins, such as the structural component of the connective tissue matrix, or basement membrane, which includes collagen, myelin, complement C\textsubscript{3}, tubulin, plasminogen activator, fibrinogen, ocular lens protein, peripheral nerve protein, and elastin. Thus advanced glycation reflects glycemic control over a long period. At present there is no universally accepted method to detect AGE, and no internationally recognized standard unit of measurement, making comparison of results between different laboratories difficult. Hence Plasma HbA1C and Plasma
Fructosamine values are widely used to estimate the glycemic control over a period of one to eight weeks.\textsuperscript{12,15} 

Plasma HbA1C measurement has been recently included by the American Diabetes Association (ADA) as a diagnostic and screening tool for Diabetes. The chief advantages of using HbA1C measurement is the ease of testing because it does not require fasting and acute perturbations such as stress, diet, exercise and smoking does not affect HbA1C levels, further microangiopathic complications are strongly associated with HbA1C levels as it reflects chronic hyperglycemia.\textsuperscript{23} 

The limitations of HbA1C testing include, venous puncture, variation among different races, and added to this, conditions such as anemia and some medications may influence the results.\textsuperscript{16} The risks that diabetic patients face on a daily basis are not accurately represented, as it does not readily reflect the degree of glycemic variability that a patient may experience during a given day.\textsuperscript{24} The sensitivity and specificity of HbA1C are 36\% and 100\% respectively.\textsuperscript{17} 

Currently Plasma Fructosamine levels are used to monitor the glycemic control over a period of one to three weeks. Research has proved that the Plasma Fructosamine levels correlate with Plasma HbA1C level and it is a more responsive marker than Plasma HbA1C levels. Kennedy et al have reported that in a group of uncontrolled diabetic patients after a week of improved care, Plasma Fructosamine values dropped by 37\%, however
HbA1C values decreased only by 8%. Plasma Fructosamine is unaffected by disorders of red blood cells and medications. Sensitivity, specificity values are 67.3% and 97.3%. The limitation of Plasma Fructosamine test is that the test results must be interpreted with caution in case of protein loosing disorders such as nephropathy and liver disorder and it is requires venous puncture to draw blood.

Extensive research about use of saliva as an adjunct test medium to aid in conventional medical assessment of systemic diseases has been carried out in recent times. Saliva contains constituents that are frequently altered in the presence of systemic diseases. Thus, due to its simplicity in collection, saliva may be collected repeatedly with minimal discomfort to the patient, thereby rendering saliva as a very desirable diagnostic medium. These significant characteristics have led to finding bio markers in saliva for the detection of systemic illness on the national health care agenda of developed countries and are of great interest for most salivary researchers.

Proteins secreted from the salivary glands and plasma contributes to the abundant proteins present in saliva. Studies have reported that the glycation of saliva proteins correlates with Plasma Glucose levels and Plasma Glycated proteins. Thus Salivary Fructosamine levels have been reported to correlate with Plasma Glucose and Plasma HbA1C levels.

Salivary Fructosamine can be used in a manner similar to Plasma HbA1C levels to monitor the glycemic control over a period of one to three
weeks. Since collection of saliva is simple and painless it can be used as a non-invasive tool for monitoring the glycemic control in diabetic patients, thus reducing the necessity of repeated venous punctures to draw blood.\textsuperscript{28}

A substantial body of evidence suggests that Asian Indians are genetically more susceptible to Type II Diabetes mellitus, mainly due to the Pro 1 2 Ala polymorphism which is not protective against Diabetes or insulin resistance among Asian Indians unlike in Caucasoids. The Thr 394 Thr polymorphism is associated with increased total, visceral and subcutaneous body fat, thus contributing to central obesity and increased waist circumference among Asian Indians. Coupled with the above factors, lack of physical activity due to urbanization and substitution of unrefined cereals by polished cereals, and high fat sugar rich diet has escalated the prevalence of Type II Diabetes to alarming levels in India.\textsuperscript{29,30}

To the best of our knowledge there are only few studies conducted in India on the levels of Salivary Fructosamine amongst Diabetes mellitus patients. Since the etiopathology of the two types of Diabetes mellitus are different and Type II Diabetes mellitus being the most common form among the two types accounting to 90\% of all the affected individuals, the present study was contemplated to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Post Prandial Glucose levels amongst Type II Diabetes mellitus patients and Non Diabetic controls in Chennai.
Hypothesis
HYPOTHESIS

RESEARCH QUESTION:

Is there an association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Post Prandial Glucose levels amongst Diabetes mellitus patients?

RESEARCH HYPOTHESIS:

There is an association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Post Prandial Glucose levels amongst Diabetes mellitus patients.

NULL HYPOTHESIS:

There is no association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Post Prandial Glucose levels amongst Diabetes mellitus patients.
Aim and Objectives
AIM AND OBJECTIVES

AIM:

To determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Post Prandial Glucose levels among Type II Diabetes mellitus patients and Non Diabetic healthy controls.

OBJECTIVES:

1. To obtain saliva and blood samples from Type II Diabetes mellitus patients and Non Diabetic healthy controls.
2. To estimate the level of Salivary Fructosamine in saliva samples.
3. To estimate the levels of Plasma glycated hemoglobin, Plasma Fasting and Post Prandial Glucose levels in blood samples.
4. To determine the association between Salivary Fructosamine, Plasma Glycated Hemoglobin and Plasma Fasting and Post Prandial Glucose levels.
Review of Literature
NOMENCLATURE

GLYCATION:

The term glycation according to the IUPAC-IUB (International Union of Pure and Applied Chemistry –International Union of Biochemistry), is any reaction linking a sugar and a protein.\textsuperscript{15}

PLASMA FRUCTOSAMINE:

The non enzymatic reaction between a sugar (generally glucose) and a protein (generally albumin) results in the formation of ketoamine referred to as Fructosamine.\textsuperscript{15}

Johnson et al (1982) introduced the term Fructosamine in clinical chemistry literature.\textsuperscript{34} Earlier it was also referred to as GSA (Glycated Serum Albumin), Glycated Plasma Albumin (GPP).\textsuperscript{15} The term Glycated Albumin or protein has also been widely used in literature since quantitatively Glycated Albumin is the most important glycated plasma protein.\textsuperscript{35}

GLYCATED HEMOGLOBIN (HbA1C):

Earlier the term Glycosylated Hemoglobin was used to refer to the product of non enzymatic reaction between glucose and the free amino groups of hemoglobin. Later the term Glycated Hemoglobin was suggested by Roth, on behalf of the IUPAC-IUB Joint Commission on Biochemical Nomenclature
Review of Literature

(JCBN), in 1983. The JCBN finally recommended the term Glycohemoglobin in 1986.\textsuperscript{36}

**GLYCOPROTEIN:**

The term glycoprotein usually refers to a protein (mostly globulin), containing a carbohydrate component, that was added enzymatically during the synthesis of the protein.\textsuperscript{15}

**CHEMISTRY OF GLYCATION**

**FRUCTOSAMINE:**

The reaction between amino acid and reducing sugar to form ketoamine adducts was first described by Maillard. Carbonyl groups of reducing sugars such as glucose reacts, non enzymatically with amino group of proteins and results in the formation of Schiff base (aldimines). The Schiff base (labile aldimine) undergoes Amadori rearrangement to form stable Fructosamine. Further the straight chain Fructosamine, if formed from glucose undergoes cyclization to a hemiketal furanose or pyranose ring structure for added stability.\textsuperscript{15}

Iberg N, Fluckiger R, reported that glycation of albumin occurs at multiple sites and the principle site was Lys -525 (epsilon amino group of lysine residues).\textsuperscript{37}
GLYCATED HEMOGLOBIN (HbA1C):

Glycation of Hemoglobin occurs when, valine residue of beta chain, epsilon amino groups of lysine (in the alpha and beta chain) and the N terminus (in the alpha beta chain) reacts with glucose.\textsuperscript{15,38}
FORMATION OF GLYCATED HEMOGLOBIN

\[
\text{Glucose} \xrightarrow{\text{\(\beta\)-N-valyl-}1-deoxyglucose} \xrightarrow{\text{\(\beta\)-N-valyl-1-deoxyfructose}}
\]

REFLECTION OF GLYCEMIC CONTROL

PLASMA FRUCTOSAMINE:

The half life of albumin is about 14-20 days; hence it reflects the glycemic control over a period of two to three weeks.\(^{15}\)

GLYCATED HEMOGLOBIN (HbA1C):

The half-life of hemoglobin is 60 days; hence it reflects the glycemic control over a period of six to eight weeks.\(^{15}\)
REVIEW OF LITERATURE

Campbell MJA (1965) compared the levels of Plasma and Salivary Glucose levels among diabetic patients and healthy controls. Blood samples and unstimulated saliva samples were collected two hours after breakfast. The study was carried out using two methods to estimate the glucose levels. In the first experiment Somogyi blood glucose estimation technique was used, and in the second experiment, the paper chromatographic technique (to isolate the sugars), spot test (to identify the sugars), and an ultra-micro-technique (to quantitatively estimate the glucose levels) was used. In the first experiment the Somogyi blood glucose estimation technique identified the presence of glucose in the saliva of only diabetic patients. The Salivary Glucose levels ranged from 0.00 and above 10.00 mg/100 ml among diabetic patients. No significant correlation was observed between Plasma Glucose and Salivary Glucose levels (r=0.14). The second experiment identified the presence of glucose in saliva of both the non-diabetic and diabetic participants. The salivary glucose levels in non-diabetic and diabetic participants were between 0.24 and 3.33 mg/100 ml and 0.44 and 6.33 mg/100 ml respectively. There was a significant difference in the level of Salivary Glucose between diabetic and non-diabetic individuals. However no significant correlation was observed between Plasma and Salivary Glucose levels. The study concluded that saliva of diabetic patients differed from that of non diabetic individuals.
Guthrow CE, Morris M et al (1979) conducted a cross sectional study among 22 diabetic patients (including Type I and Type II diabetic patients) and 25 healthy controls. Venous blood samples were collected from the participants to estimate Plasma Glycated Hemoglobin (HbA1C) and Serum Glycated Albumin levels. The study reported that Glycated Albumin was formed by non enzymatic glycosylation of Albumin, resulting in the formation of Schiff base, between the aldehyde form of the sugar and free amino group in protein, followed by Amadori rearrangement to a ketoamine derivative. The study compared the use of an ion exchange chromatographic method and a calorimetric method (with thiobarbituric acid) to determine Plasma Glycated Albumin levels. The study results showed significant correlation between the two methods ($r = 0.99$). The mean level of Glycated Albumin among normal individuals, determined by chromatographic and thiobarbituric acid assays were $7.0 \pm 1.9\%$ and $8.3 \pm 2.2\%$, respectively; levels as high as $30\%$ was observed in poorly controlled diabetics. However no significant correlation was observed between HbA1C and Serum Glycated Albumin. This was attributed to the difference in half-life of erythrocytes (120 days) and Albumin (17 days). The study concluded that since the half-life of Albumin is less than that of erythrocytes, the plasma concentration of Glycated Albumin should be expected to reflect short-term control of hyperglycemia in Diabetes.

Yue DK, Morris S et al (1980) conducted a cross sectional study among 45 individuals. Plasma Glucose, Glycated Hemoglobin (HbA1C) and
Glycated Plasma Proteins were estimated from the blood samples (collected during routine clinical visit at 10 am or 2 pm). The study results showed highly significant correlation between Plasma Glycated Proteins and Plasma Glycated Hemoglobin ($r=0.74$, p value $<0.005$). The mechanism of formation of Glycated Plasma Proteins were found to be similar to that of HbA1C. The study reported that Glycated Albumin was quantitatively the most important Glycated Plasma Protein, although the C labeled glucose was shown to bind to all protein components, the major peak corresponded to the position of Albumin. The study concluded that the measurement of Glycated Plasma Proteins is a suitable alternative to determine the levels of Plasma Glycated Hemoglobin since it reflects the glycemic control over a short period of time. It was also reported to be more accurate than Glycated Hemoglobin in conditions such as hemolytic anemia, hemoglobinopathy and recent blood transfusions.

Dolohofer R, Weiland OH (1980), conducted a prospective study among 65 diabetic patients and 10 healthy individuals over a period of 20 days. Venous blood samples were collected to determine Plasma Fasting Glucose, Glycated Hemoglobin and Serum Glycated Albumin levels. The mean Glycated Albumin levels among healthy controls and diabetic subjects, were 64 (56-71) and 124 (61-255) picomoles of HMF per nanomole of Albumin respectively. Glycated Albumin showed a positive correlation with mean blood glucose concentration ($r=0.715$) and Glycated Hemoglobin
(r=0.88). No correlation was observed between Glycated Albumin and Fasting Glucose levels (r=0.3). During the study period of 20 days it was observed that among insulin treated diabetic patients, there was only 15% drop in HbA1C levels (from 20.7 to 17.5%), however there was more than 50% drop in Glycated Albumin levels (from 208 to 91 pmol HMF per nanomole of Albumin). Thus the study concluded that Glycated Albumin is a valuable tool to monitor short term glycemic control, since the turnover of Albumin is comparatively faster than that of erythrocytes.

Forbat LN, Collins RE et al (1981)\textsuperscript{42} conducted a cross sectional study among 31 diabetic patients. Venous blood samples were obtained to estimate blood glucose levels. Pure samples of parotid saliva was obtained by cannulation to estimate Salivary Glucose levels. Salivation was stimulated by external parotid massage or by a drop of lemon juice on the tongue. The mean glucose concentrations in blood and saliva samples were 9.6 mmol/l and 0.32 mmol/l respectively. No significant correlation was observed between blood and Salivary Glucose levels (r=0.18). The study concluded that due to the weak correlation between blood and Salivary Glucose levels, the possibility of a noninvasive technique of monitoring Diabetes using Salivary Glucose concentrations is not possible.

Kennedy AL, Thomas J (1981)\textsuperscript{43} conducted a prospective study over a period of eight weeks among 14 diabetic patients (8 with insulin dependent and 6 with non-insulin dependent) and 80 healthy controls. Blood samples
were collected at 1, 2 and 8 weeks to determine Plasma Glucose, HbA1C and Glycated Serum Albumin levels. The mean glucose levels during the above days was estimated by collecting venous blood at seven intervals (fasting, 2 hours after breakfast, before lunch, 2 hours after lunch, before dinner, 2 hours after dinner and at bed time). At the end of one week there was a 30% fall in mean glucose levels which was accompanied by 34% fall in Glycated Albumin levels (p value <0.01), however only 7.1% fall in HbA1C levels was observed. At the end of 2 weeks 37% fall in glucose levels, 40% fall in Glycated Albumin levels and 18% fall in HbA1C levels was observed. At the end of 8 weeks both HbA1C and Glycated Albumin showed the same level of glycemic control. The study concludes that Glycated Albumin can be used to access the preceding one to two weeks glycemic control.

Johnson RN, Metcalf PA, Baker JR (1982)\textsuperscript{34} introduced the term Fructosamine into clinical chemistry literature. The study reported that Fructosamine is a ketamine derivative of the non enzymatic reaction between a sugar (chiefly glucose) and a protein (chiefly albumin). A simple colorimetric method designed to measure Serum Fructosamine as an index of diabetic control was tested in the study. The method relied on the ability of ketoamine (Fructosamine) to act as reducing agents in alkaline solution. The results showed significant difference in the levels of Plasma Fructosamine between diabetic and non diabetic individuals (p value<0.001). A significant correlation was observed between Plasma Fructosamine and Plasma Fasting
Glucose levels. The study concluded that estimation of Plasma Fructosamine levels allows clear discrimination between diabetic and normal individuals.

Jones IR et al (1983)\(^{44}\) conducted a prospective study among 12 newly diagnosed diabetic patients (2 Type I diabetic and 10 Type II diabetic patients) aged 14 to 66 years. The participants were followed weekly over a period of 8 weeks from the time of diagnosis. Venous blood was collected weekly, to determine Plasma Fasting Glucose, Glycated Hemoglobin and Glycated Albumin levels. At 4 weeks significantly (p value<0.01), greater fall was observed in Glycated Albumin levels (58%) when compared to HbA1C levels (39%). The fall in fasting glucose levels (72%) was similar to that of Glycated Albumin, and significantly differed from that of HbA1C levels. Glycated Albumin showed a significant positive correlation with Fasting Plasma Glucose levels (r = 0.70) and with HbA1C, levels (r = 0.91) over the 8 week period. At the end of 8 weeks there was no significant difference in the fall of HbA1C (76%), Glycated Albumin (83%) and Fasting Plasma Glucose (88%). The study concluded that Glycated Albumin provides earlier objective evidence of the metabolic response to therapeutic intervention, and can be regarded as an intermediate index of diabetic control.

Roberts AB, Baker JR et al (1983)\(^{45}\) conducted a study among 79 non diabetic pregnant women, 20 women with gestational Diabetes and 19 pregnant women with established Diabetes. Fasting Plasma Glucose, Post Prandial Glucose levels after 3 hours Oral Glucose Tolerance Test (OGTT),
and Plasma Fructosamine levels were determined. Women with gestational Diabetes had significantly higher Plasma Fructosamine levels ($1.90 \pm 0.18$ m mol/lit) when compared to non diabetic women ($1.54 \pm 0.18$ m mol/lit). Women with established Diabetes had even higher levels of Plasma Fructosamine ($2.34 \pm 0.43$ m mol/lit). The Fructosamine test was able to detect 85% of women in gestational Diabetes group. In non-diabetic group significant correlation was observed between Plasma Fructosamine and Plasma Fasting Blood Glucose ($r=0.29$, p value <0.001). Plasma Fructosamine also showed significant correlation with area under OGTT ($r=0.26$, p value <0.001). Infants of women with gestational or established Diabetes had significantly higher birth weight ratio and skin fold thickness when compared to non diabetic women (p value <0.001). Cord blood of infants with diabetic mothers had significantly higher levels of C-peptide, insulin and Fructosamine. In the non diabetic group significant correlation was observed between maternal Plasma Fructosamine and fetal birth weight ratio ($r=0.23$, p value, 0.05), however in the diabetic group no significant correlation was observed ($r=0.29$). It was also observed that 4 diabetic women who had high Plasma Fructosamine levels (2.23, 2.60, 2.69, and 2.82 mmol/lit) in their first trimester had miscarriage. Thus the study concluded that Plasma Fructosamine estimation may provide a simple, in expensive means to screen for Diabetes among pregnant women.
Roberts AB, Baker JR (1986) conducted a cross sectional study among 3664 female volunteers (non pregnant) and 1200 pregnant women. Plasma Fructosamine levels were determined from blood samples obtained from these patients. No significant difference was observed in Fructosamine levels between non-pregnant and pregnant women during the first trimester (mean Fructosamine value = 2.46 ± 0.16 (SD) mmol/L). A significant difference was observed in Fructosamine levels between first- and second-trimester (mean second trimester Fructosamine = 2.41 ± 0.14 (SD) mmol/L) and between second- and third-trimester (mean third trimester Fructosamine = 2.34 ± 0.14 (SD) mmol/L). Glucose tolerance test was carried out in 167 pregnant women, out of which nine women were identified with gestational Diabetes. Fructosamine exceeded the ninety-fifth percentile for gestational age in eight of the nine individuals. A highly significant association (p < 0.001) was found between Fructosamine levels, Fasting Plasma Glucose and area under the Glucose tolerance curve. Sensitivity, specificity, positive and negative predictive value calculated for 63 patients were 86%, 95%, 66%, and 98%, respectively. The study concluded that Serum Fructosamine may be a useful screening test for gestational Diabetes.

Roberts AB, Baker JR (1987) conducted a study among 30 pregnant women. Serum Fructosamine concentrations at different stages of gestation with fetal growth (as determined by ultrasonography) and birth weight were determined. During the first trimester, Serum Fructosamine levels in mothers
of macrosomic infants were significantly higher (P <0.05) when compared to mothers of normal birth weight infants. A significant correlation was observed between first-trimester Fructosamine concentrations and birth weight ratio (r = 0.68, P < 0.0001), also with ultrasound measurements of fetal abdominal circumference and femur length. It was observed that, the fetus destined to be macrosomic had an enlarged abdomen in the second trimester, often before 20 weeks of gestation. The study concluded that maternal diabetic control during early gestation influences the fetal growth and contributes to the development of fetal macrosomia.

Zhang – Ru Gen et al (1990) conducted a cross sectional study among 81 participants. They were divided into two groups, 55 diabetic patients were included in Group I and 26 healthy individuals were included in Group II. Saliva and blood samples were collected from the participants. The study results showed that diabetic patients had significantly higher Salivary Fructosamine levels (0.61±0.19 mmol DMF/L) when compared to healthy individuals (0.38±0.07 mmol DMF/L). Salivary Fructosamine showed positive correlation with plasma fasting glucose (r=0.449), Plasma Fructosamine (r=0.526) and Plasma HbA1C (r=0.411).The study concluded that Salivary Fructosamine reflects the glycemic control of two to three weeks; hence it can be used to monitor glycemic control in diabetic patients.

Nakamoto I, Morimoto K, Takeshita T, Toda M (2003) conducted a cross sectional study among 51 male participants. They were divided into
three groups based on the glucose levels as Normal (109 mg/dl or lower), Impaired Glucose Tolerance (110 to 125 mg/dl) and Diabetic group (126 mg/dl or higher). Blood and saliva samples were collected from the individuals after overnight fasting. Plasma HbA1c, Fructosamine and Fasting Glucose were estimated from the blood samples. Salivary Glucose, Fructosamine and Hydrazine Glycated protein levels were estimated from the saliva samples. Correlation between Blood Glycated protein (HbA1C) and Saliva Glycated protein (Salivary Fructosamine and Hydrazine Glycated protein) was determined. The mean Salivary Fructosamine of 51 participants was 25.2±11.6 umol/g protein. The study results showed positive correlation between Salivary Fructosamine, Plasma HbA1c and Blood Glucose (r=0.449; p=0.001 and r=0.445; p=0.001, respectively). No correlation was observed between Salivary Fructosamine and Plasma Fructosamine (r=0.260; p=0.065). Salivary Hydrazine Glycated protein showed no correlation with HbA1C, Plasma Fructosamine and Plasma Fasting Glucose levels (r=0.204; 0.090 and 0147 respectively). Salivary Glucose had no correlation between Blood Glucose and other blood parameters. The study concluded that Salivary Fructosamine can be utilized as a non-invasive tool for the early diagnosis and also for monitoring glycemic control in diabetic patients.

Morenkova SA (2004)\textsuperscript{49} compared the levels of Salivary Fructosamine among 50 healthy controls, 55 Type I and Type II Diabetes patients. Participants from both the genders aged 25 to 65 years were included.
in the study. The study reported that there was an increase in Salivary Fructosamine concentration with an increase in Plasma Glucose levels among diabetic patients. The levels of Salivary Fructosamine in healthy controls, Type I diabetic patient and, Type II diabetic patients were 3.7 ±0.7 mmol/l, 6.2±1.7 mmol/l and 7.3±2.0 mmol/l respectively. The study concluded that Salivary Fructosamine can be used for the diagnosis of Diabetes and also as a benchmark reflecting effectiveness of treatment for Diabetes.

**Mittman N, Desiraju B et al (2010)** conducted a prospective study for three years among 100 diabetic patients. HbA1C and Fructosamine levels were estimated during enrolment and 6 months after enrolment. Fructosamine values were corrected for variations in the concentration of Serum Albumin according a formula by Lamb et al \[ \text{AlbF (Albumin Fructosamine)} = \text{Fructosamine (\(\mu\text{mol /l}\))}^* 100/\text{Albumin (g/l)} \]. Serum Albumin, total protein, serum creatinine and serum glucose were recorded every month. Morbidity data including number of hospitalizations, duration of hospitalization, and episodes of infections were recorded throughout the study period. A significant correlation was observed between HbA1C and Fructosamine \(r=0.67, p \text{ value}<0.001\). Also a significant correlation was observed between glucose measurements, HbA1C and Fructosamine. Patients who were hospitalized during the study period had higher AlbF levels compared with those who were not hospitalized (974 versus 863 mmol/g, p value =0.012). Also patients who underwent dialysis using AV (Arterio Venous) access
catheters and having an infection during the study period had significantly higher AlbF (998 versus 903 mmol/g, p value =0.03). AlbF was a significant positive predictor of hospitalization (odds ratio=1.005, p value = 0.016) and of infection (odds ratio=1.003, P value =0.039) in diabetic patients, however HbA1C did not predict hospitalization (odds ratio=1.01, P=0.96) or infection (odds ratio=1.34, p value =0.17). AlbF was a significant predictor of the number of hospitalizations per patient year (relative risk=1.12, p value =0.007), the duration of hospitalization per patient year (relative risk=1.9, p value <0.0001), and the number of episodes of infection per patient year (relative risk=1.29, p value =0.001), however HbA1C did not predict frequency, or duration of hospitalization, or of infection in patients receiving dialysis by AV access. The study concluded that Fructosamine levels corrected for AlbF was a better indicator for diabetic patients on hemodialysis when compared to HbA1C.

Freedman BI et al (2011) conducted a prospective study among 444 patients with Diabetes and End Stage Renal Disease (ESRD). Blood samples were obtained from diabetic patients who enrolled for dialysis. Quarterly levels of Glycated Albumin (GA), HbA1C were measured upto 2.3 years, and monthly glucose levels were estimated from the blood samples. The Mean ± SD of Glycated Albumin and HbA1c were 21.5% ± 6.0% (median 20.4%), and 6.9% ± 1.6% (median 6.6%), respectively. 156 deaths were observed during the period. Using the best fit model it was observed that
the risk of mortality increased by 14% for each 5% rise in GA. GA was associated with risk of hospitalizations within 17 days of measurement (p value =0.003). The results showed that the rate of hospitalizations per quintile of GA differed significantly between the lowest and highest quintiles (5.90% versus 9.67%, respectively; p value =0.02) and was also significantly different comparing the second lowest with the highest quintiles (6.23% versus 9.67%, respectively; p value = 0.03). However no such graded relationships were observed for hospitalization rates with either HbA1C or blood glucose levels. The study concludes that clinicians should consider measuring GA instead of HbA1C in patients with Diabetes on dialysis, as it is a better predictor of glycemic control.

Kosaryan M, Mahdavi MR et al (2012) conducted a cross sectional study among 33 Diabetes mellitus patients with beta thalassemia. 21 female and 12 male participants were included in the study. Blood samples were obtained to determine Fasting blood sugar, HbA1C, HbF (Fetal Hemoglobin), Ferretin and Fructosamine levels. The mean HbA1C and Fructosamine among the 33 patients were 8.9%±1.8% and 442±124 (mmol/L) respectively. A positive correlation was observed between HbA1C and Fasting blood glucose (r=0.75, p<0.01).Similarly Fructosamine showed positive correlation with Fasting blood glucose (r=0.54, p<0.01).The study concluded that since the levels of HbA1C in thalassemia may be affected by high levels of fetal
hemoglobin, Fructosamine levels serve as a suitable alternative to determine the glycemic control in thalassemia patients.

Manjrekar PA, Hedge A et al (2012)\textsuperscript{53} conducted a cross sectional study among 66 participants. They were divided into three groups as follows, 20 healthy controls were included in Group I, 23 non-diabetic first degree relatives of Type II diabetic patients were included in Group II and 23 Type II diabetic patients were included in Group III. Blood and saliva samples were obtained after an overnight fast of about 10 hours. Plasma Fasting Glucose, HbA1C, and Serum Fructosamine levels were estimated from the blood samples. Salivary Glucose and Fructosamine and total protein levels were estimated from the saliva samples. The study results showed that individuals in Group III had significantly higher levels of Salivary Fructosamine (202.1 ± 103.4 mg/dl) when compared to individuals in Group II (130 ± 71.6 mg/dl) and Group I (99.8 ± 50.1 mg/dl) (p value less that 0.05). However no significant correlation was observed between Salivary Fructosamine and Salivary Glucose with Plasma Fasting Glucose levels in all the three groups (Group I r value=0.061, Group II r value=0.171, Group II r value =-0.078) . Also no significant difference was observed in the Salivary Glucose levels and total protein levels between the three groups. The study concluded that although saliva collection is non-invasive, salivary parameters do not correlate with blood parameters hence; further research is required to validate the use of
Salivary Fructosamine and Glucose as a non-invasive tool in monitoring Diabetes.

**Shafi T et al (2013)** conducted a prospective study among 503 hemodialysis participants over a period of 3.5 years. Non-fasting pre-dialysis blood samples were collected from the patients to determine, Glucose, HbA1C, Fructosamine and Glycated Albumin levels. Glycated albumin (%) was computed as follows: \[\text{Glycated albumin in (g/dL)/Serum Albumin in (g/dL) \times 100/1.14} + 2.9\]. Mortality data, the first Cardio Vascular Disease (CVD) event, hospitalizations with sepsis were obtained from hospital records. Fructosamine and Glycated Albumin were moderately correlated with random Serum Glucose levels (Spearman correlations of 0.562 and 0.688, respectively). The study results showed that Fructosamine and Glycated Albumin were associated with all-cause mortality; adjusted Hazard Ratio (HR) per doubling of the biomarker was 1.96 (95% CI 1.38–2.79) for Fructosamine and 1.40 (1.09–1.80) for Glycated Albumin. CVD mortality was associated with both the markers [Fructosamine 2.13 (1.28–3.54); Glycated Albumin 1.55 (1.09–2.21)]. The markers were also associated with trends toward a higher risk of hospitalization with sepsis [Fructosamine 1.75 (1.01–3.02); Glycated Albumin 1.39 (0.94–2.06)]. The study concluded that Serum Fructosamine and Glycated Albumin are risk factors for mortality and morbidity in hemodialysis patients and, monitoring the levels of these markers may be valuable for the management of Diabetes in dialysis patients.
Willer AK, Kosi L et al (2015) conducted a patient level pooled data analysis of six randomized controlled trials. The aim of the study was to determine the impact of gender on glycemic control and the incidence of hypoglycemia in patients with Type II Diabetes Mellitus treated with insulin. The study included patients with Type II Diabetes Mellitus from multinational, multi centre, randomized open level trials with similar designs. The patients included in the analysis were between 20-80 years of age, with BMI of 20-40 kg/m², their HbA1C levels were between 7.5-12%. About 2600 patients were included in the pooled data analysis, out of which there were about 1251 women and 1349 men. When the baseline data were compared, the results of the study showed no significant effect of gender on HbA1C and Fasting Blood Glucose Levels (FBG). However the baseline measures of HbA1C and FBG were significantly higher in women within the BMI ≤ 28 kg/m². It was also observed that, although women weighed significantly less than men, they had higher BMI. The study states that glucose control depends on many variables such as insulin sensitivity, insulin secretion, hepatic glucose production and release of glucagon and incretins. As these factors are influenced by gender, glucose control may vary in men and women. It was also observed that due to the difference in glucose absorption, prolonged gut absorption, women had higher Post Prandial Glucose levels. The difference in HbA1C levels could not be explained by differences in body composition; hence this was attributed to the differences in ethnicity, genetic factors and age. The study concluded that in women, there was smaller improvement in
HbA1C and they experienced severe hypoglycemia when compared to men, hence physicians should be aware of the need for periodic monitoring of glycemic control.
Materials and Methods
MATERIALS AND METHODS

**Study Design:** Cross sectional study

**Study Setting:** Hospital based setting

**Study Duration:** 5 months. (From August 2015 to December 2015)

**Study Population:** Type II Diabetes mellitus patients.

**ETHICAL CLEARANCE:**

A detailed protocol of the study was prepared and submitted to the Institution Review Board of Ragas Dental College and Hospital, Chennai. The cross sectional study was started after obtaining ethical clearance from the review board (Annexure - I).

**PERMISSION FROM AUTHORITIES:**

Permission to conduct the study was obtained from the

1. Principal, Ragas Dental College and Hospital, Chennai
2. The Hospital Superintendent, Aringar Anna Government Hospital of Indian Medicine, Arumbakkam, Chennai.(Annexure – II)
3. The Head Clinical Lab, Anderson Laboratories, P.H Road, Chennai.
   (Annexure –III)
Informed consent (bilingual) was obtained from the study participants in the local language (Tamil) and English. (Annexure –IV and V)

**STUDY DESIGN:**

This cross-sectional study was designed to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Postprandial glucose levels amongst Type II Diabetes mellitus patients. The study participants were divided into two groups: Group 1 (Type II Diabetes mellitus patients), and Group 2 (Non Diabetic healthy individuals). Blood and saliva sample were collected from both the groups and the levels of Salivary Fructosamine, Plasma Glycated Hemoglobin, Plasma Fasting and Postprandial glucose levels were estimated. The study was conducted from August, 2015 to December, 2015.

**ELIGIBILITY CRITERIA:**

**INCLUSION CRITERIA:**

1. Patients above 18 years of age with Type II Diabetes mellitus having Plasma Fasting Glucose levels greater than or equal to 126 mg/dl were included in Group 1 (American Diabetes Association-Standards of medical care)13
2. Non Diabetic healthy individuals above 18 years of age (without any other systemic disease such as protein losing nephropathy, liver failure and anemia) having Plasma Fasting Glucose levels less than 126 mg/dl were included in Group 2 (American Diabetes Association-Standards of medical care)\textsuperscript{13}

3. Patients who gave consent to participate in the study.

**EXCLUSION CRITERIA:**

1. Patients who were not willing to participate in the study
2. Type II Diabetes mellitus patients with diabetes associated complications such as protein loosing nephropathy and liver failure (confirmed from their hospital reports)
3. Patients with hemolytic anemia or hemoglobinopathy
4. Patients who underwent recent blood transfusion

**SAMPLE SIZE ESTIMATION:**

The sample size for each group was calculated using the G power statistical software. The Pearson’s correlation coefficient from a previous study conducted by Nakamoto I et al was considered for estimating the sample size\textsuperscript{28}.

The Pearson’s correlation coefficient (r value) between Salivary Fructosamine and Plasma Glycated Hemoglobin was reported to be 0.645\textsuperscript{28}. 

\textsuperscript{13} American Diabetes Association-Standards of medical care

\textsuperscript{28} Nakamoto I et al.
On considering r value as 0.675, alpha error as 0.05, and power as 90%, we obtained a sample size of 42 for each group.

Hence for the present study we included 50 individuals, in each group. Thus a total of 100 participants were included in the study.

**RECRUITMENT OF THE STUDY SUBJECTS:**

Seven hospitals in Chennai city were approached to obtain permission to carry out the cross sectional study. The nature and purpose of the study, was explained in detail to the hospital authorities. Among the seven hospitals only one hospital granted permission (Aringar Anna Government Hospital of Indian Medicine, Arumbakkam, Chennai). The Permission was granted by The Hospital Superintendent, Aringar Anna Government Hospital of Indian Medicine, Arumbakkam, Chennai in July 2015, and the study was conducted from August 2015 to December 2015.

A total of 100 participants were included in the study. The participants were divided into two groups, 50 in each group: 50 patients with Type II Diabetes mellitus were included in Group 1 and 50 non Diabetic healthy individuals were included in Group 2.
Recruitment of Type II Diabetes mellitus patients (Group 1):

A total of 157 Type II Diabetes patients visiting The Department of Diabetology, Aringar Anna Government Hospital of Indian Medicine were approached out of which only 96 patients met the inclusion criteria. The nature and purpose of the study were explained in detail to the 96 patients and their queries were cleared. All the selected subjects were given an informed consent form, which was to be filled, and signed by them. Only those individuals who were willing to participate and returned the consent form were selected. Among the 96 patients 46 did not give consent; finally around 50 Type II Diabetes mellitus patients were included in the study.

Recruitment of non Diabetic healthy individuals (Group 2):

A total of 300 individuals who enrolled for the routine master health checkup conducted once a month by the General Out Patient Department (OPD), Aringar Anna Government Hospital of Indian Medicine were approached. These individuals were requested to fast overnight for the master health checkup. The Fasting Plasma Glucose levels and the Plasma HbA1C levels were analyzed immediately during the check up and those individuals who were identified to be Non Diabetic were approached to participate in the study. A total of 170 individuals were identified as non Diabetic healthy individuals and also met the inclusion criteria. The nature and purpose of the study were explained in detail to these patients and their queries were cleared. Informed consent form was given to the selected individuals. Finally 50 subjects accepted to participate in the study and gave consent.
FLOWCHART ILLUSTRATING THE METHODOLOGY OF THE STUDY:

Assessed for eligibility
(Group 1=157)
(Group 2= 300)
(Total=457)

Individuals who met inclusion criteria
Group 1=96
Group 2= 170
(Total =266)

Excluded (did not meet criteria)
(Group 1=61)
(Group 2=130)
(Total=191)

Excluded (did not give consent)
(Group 1=46)
(Group 2=120)

Group 1 (n=50)
Patients with Type II Diabetes mellitus

Group 2(n=50)
Non Diabetic individuals

Blood and saliva samples were collected from participants

- Blood samples were analyzed at Aringar Anna Hospital
- Plasma Fasting and Post Prandial Glucose and Plasma HbA1C levels was estimated
- Saliva samples were transported to Anderson Laboratory
- Salivary Fructosamine levels was estimated
DEMOGRAPHIC DATA:

A pre validated questionnaire (validated by Pan American Health Organization and World Health Organization) was administered to collect data regarding the socio demographic factors, family history of non communicable disease, tobacco usage and alcohol consumption.

SALIVA AND BLOOD COLLECTION:

Blood and saliva samples were obtained only as a part of their routine periodic checkup (Group 1) and master health checkup (Group 2).

BLOOD COLLECTION:

Blood samples were collected after an overnight fast. Blood was drawn from the ante-cubital vein and it was collected in vacutainers which contained fluoride and EDTA. The following procedure was carried out to draw blood by a trained phlebotomist.

1. A tourniquet was tied about 3 inches from the selected site
2. Site was cleaned with sterile alcohol prep
3. The selected vein was anchored by placing the thumb about two centimeters below the vein and pulling gently to make the skin a little taut.
4. After this, the needle, beveled upward, was pushed smoothly and quickly into the vein, to minimize the possibility of hemolysis as a result of vascular damage.
5. Immediately after the insertion, the tourniquet was released to minimize the effect of hemoconcentration.

6. 3 ml of blood was collected in a 5 ml syringe.

7. The samples were transferred to a 3ml K$_3$ EDTA vacationer tube.

8. Specimens were centrifuged and separated within 30-45 minutes following collection.

9. The samples were stored in the laboratory at a temperature of 4 degree Celsius.

The blood samples were analyzed at the Aringar Anna Government Hospital of Indian Medicine.

**COLLECTION OF SALIVA:**

After the participants rinsed their mouth with water for six times 3 ml of unstimulated saliva samples were collected in a sterile container. The saliva thus obtained were transferred to a sample collection box containing gel packs and transported to Anderson Laboratory, PH road, Chennai to estimate the Salivary Fructosamine levels. The samples were stored under 2 to 8 degree Celsius at the laboratory before carrying out the laboratory procedures.

The armamentarium required for blood and saliva collections were as follows.
Materials and Methods

Blood Collection: (Photograph 1 and 2)

1. Sterile gloves and Cotton rolls
2. Tourniquet
3. Sterile 5 ml syringe
4. Sterile K₃ EDTA vacationer

Saliva Collection: (Photograph 3 and 4)

1. Sterile collection tubs
2. Thermocol Ice Box (1 number)
3. Gel packs (2 numbers)
LABORATORY PROCEDURE:

LABORATORY EQUIPMENT FOR DETERMINATION OF PLASMA GLUCOSE AND GLYCATED HEMOGLOBIN:

1. Hexokinase-mediated reaction Roche/Hitachi Modular P Chemistry Analyzer:

   Auto analyzer to determine the Fasting and Postprandial Plasma Glucose levels (Photograph 5)

2. Tosoh A1C 2.2 plus Glycohemoglobin Analyzer:

   HPLC (High Perfusion Liquid Chromatography) analyzer - To determine the Plasma Glycated Hemoglobin level (Photograph 6)

AUTOMATED LABORATORY PROCEDURE TO DETERMINE PLASMA FASTING AND POST PARANDIAL GLUCOSE LEVELS AND PLASMA GLYCATED HEMOGLOBIN:

1. Specimens were centrifuged and separated within 30-45 minutes following collection.

2. The specimens were stored at 4 degree Celsius

3. Frozen specimens were allowed to thaw completely before analysis

4. The bar coded specimens were placed in the auto analyzers
5. Mod P computer terminal was clicked to <Start>

6. The calibration and control data was obtained as an automatic printout.

LABORATORY EQUIPMENTS AND REAGENTS REQUIRED FOR QUANTITATIVE DETERMINATION OF SALIVARY FRUCTOSAMINE:

1. Buffer –Carbonate detergents (200mmol/L)
2. Enzymes –Nitrotetrazolium chloride (NBT)(0.48mmol/L)  
   - Uricase (3000U/L)
3. Fructosamine cal-Calibrated lyophilized serum
4. NBT Kinetic –Spectrophotometer, measuring at 520 nm to determine the  
   Salivary Fructosamine levels. (Photograph 7)
5. Matched cuvettes 1.0 cm light path

LABORATORY PROCEDURE FOR QUANTITATIVE DETERMINATION OF FRUCTOSAMINE\(^3\): (Photograph 8 and 9)

The assay conditions were set as follows

- Wavelength -520(490-550) nm
- Cuvette-1 cm light path
- Temperature -37 degree Celsius
1. The instrument was adjusted to zero with distilled water

2. The reagents were pipette into a cuvette, mixed and incubated at 37 degree Celsius.

3. The absorbance of the calibrator (A1) was read after 10 minutes and, the absorbance of the sample (A2) was read after 15 minutes, against distilled water.

**CALCULATION**

\[ \Delta A = A_2 - A_1 \]

\[ (\Delta A)_{\text{Sample}} \quad * \text{Calibrator concentration = } \mu\text{mol/L of Fructosamine in the sample} \]

\[ (\Delta A)_{\text{Calibrator}} \]

**STATISTICAL ANALYSIS:**

The following procedures were carried out:-

1. Data compilation and presentation

2. Statistical analysis

I. Data compilation and presentation:

Data obtained were compiled systematically in Microsoft Excel spreadsheet. The dataset was subdivided and distributed meaningfully and presented as graphs and tables.
II. Statistical analysis:

Statistical analysis were performed using Statistical Package for Social Sciences software (SPSS version 20, USA). Normality test was done and it was found that among the quantitative variable only age was normally distributed and the other variables were not normally distributed. Depending upon the nature of the data, appropriate parametric and non parametric statistical tests were chosen, p value of 0.05 was considered to be significant.

Pearson- Chi square Test and Fisher’s Exact Test were used to analyze all the qualitative data (Demographic details). T- Test was used to compare the age among the two groups and Mann-Whitney U Test was used to compare the other quantitative variables (BMI, Salivary Fructosamine, Plasma Fasting and Postprandial Glucose and Plasma Glycated Hemoglobin levels). To test for any positive association between Salivary Fructosamine and, Plasma Glycated Hemoglobin, Plasma Fasting and Postprandial Glucose levels, simple linear regression was carried out and Spearman’s correlation coefficient was determined.
Photographs
ARMAMENTARIUM TO COLLECT BLOOD AND SALIVA

Photograph 1: STERILE GLOVE, SYRINGE AND COTTON ROLL

Photograph 2: EDTA VACUTAINER
Photograph 3: STERILE SALIVA COLLECTION TUB

Photograph 4: GEL PACKS FOR COLLECTION OF SALIVA
LABORATORY EQUIPMENT

Photograph 5: ROCHE/HITACHI MODULAR P CHEMISTRY ANALYZER TO ANALYZE PLASMA GLUCOSE

Photograph 6: TOSOH A1C 2.2 PLUS GLYCOHEMOGLOBIN ANALYZER TO ANALYZE PLASMA HbA1C
Photograph 7: NBT KINETIC –SPECTROPHOTOMETER TO
ANALYZE SALIVARY FRUCTOSAMINE
PHOTOGRAPH 8 AND 9: AUTOMATED PROCEDURE TO ANALYZE SALIVARY FRUCTOSAMINE
Results
RESULTS

The present study was conducted to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin, Plasma Fasting and Postprandial Glucose levels amongst Type II Diabetes mellitus patients and non diabetic healthy controls. This study was done among 100 subjects divided into two groups with 50 participants in each group. Patients with Type II Diabetes mellitus were included in Group 1 and normal healthy individuals were included in Group 2. The study was conducted over a period of 5 months.

Figure 1: Mean age distribution of the study population
Table 1: Mean age distribution of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>N=50 Mean (±SD) 54.38(±9.924)</td>
<td>N=50 Mean (±SD) 50.90(±9.990)</td>
<td>0.084*</td>
</tr>
</tbody>
</table>

*T-Test

Table 1 shows the mean age distribution of the study population. The mean age of the participants in Group 1 was 54.38(±9.924) and in Group 2 was 50.90(±9.990). There was no statistically significant difference in mean age between the two groups.

Figure 2: Gender distribution of the study population
Table 2: Gender distribution of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>N</td>
<td>N</td>
<td>0.072*</td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

*-Pearson Chi-Square Test

Table 2 shows the gender distribution of the study population. A total of 30 male and 20 female participants were included in Group 1. In Group 2, a total of 21 male and 29 female participants were included. There was no statistically significant difference in gender distribution between the two groups.

Figure 3: Distribution based on the race of the study population
Table 3: Distribution based on the race of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>P Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>P value</td>
<td>Male</td>
<td>Female</td>
<td>P value</td>
</tr>
<tr>
<td>Aryo–Dravidian</td>
<td>N</td>
<td>N</td>
<td>0.823*</td>
<td>N</td>
<td>N</td>
<td>0.418*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6</td>
<td></td>
<td>4</td>
<td>9</td>
<td>0.417*</td>
</tr>
<tr>
<td>Dravidian</td>
<td>20</td>
<td>12</td>
<td></td>
<td>17</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Indo-Aryan</td>
<td>2</td>
<td>2</td>
<td></td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*- Fischer’s Exact Test

Table 3 shows the distribution based on the race of the study population. Among 30 male participants in Group 1, 8 participants belonged to the Aryo–Dravidian race, 20 participants belonged to the Dravidian race and 2 participants belonged to the Indo–Aryan Race. Out of the 20 female participants in Group 1, 6 participants belonged to the Aryo-Dravidian race, 12 participants belonged to the Dravidian race and 2 participants belonged to the Indo-Aryan race. Among 21 male participants in Group 2, 4 participants belonged to the Aryo-Dravidian race, 17 participants belonged to the Dravidian race. Out of the 29 female participants in Group 2, 9 participants belonged to the Aryo-Dravidian race, 19 participants belonged to the Dravidian race, and 1 participant belonged to the Indo-Aryan race.
No statistically significant difference was observed in distribution of race between the two groups and also between male and female participants in each group.

**Figure 4: Gender distribution based on the current Tobacco usage of the study population**

**Table 4: Gender distribution based on the current Tobacco usage of the study population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>P value</td>
</tr>
<tr>
<td>Tobacco Usage</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>4</td>
<td>0.129*</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
<td>16</td>
</tr>
</tbody>
</table>

*-Fisher’s Exact Test
Table 4 shows the gender distribution based on the current Tobacco usage of the study population. Among 30 male participants in Group 1, 13 participants had the habit of Tobacco Usage and 17 participants refrained from the habit. Out of the 20 female participants in Group 1, 4 participants had the habit of Tobacco Usage and 16 participants refrained from the habit. Among 21 male participants in Group 2, 6 participants had the habit of Tobacco Usage and 15 participants refrained from the habit. Out of the 29 female participants in Group 2, 8 participants had the habit of Tobacco Usage and 21 participants refrained from the habit. No statistically significant difference was observed in Tobacco usage between the two groups and also between male and female participants in each group.

Figure 5: Gender distribution based on the Alcohol usage of the study population
Table 5: Gender distribution based on the Alcohol usage of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>P value</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Alcohol Usage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>N</td>
<td>N</td>
<td>0.091*</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>18</td>
<td></td>
<td>17</td>
<td>24</td>
</tr>
</tbody>
</table>

*-Fisher’s Exact Test,

Table 5 shows the gender distribution based on the Alcohol usage of the study population. Among 30 male participants in Group 1, 10 participants had the habit of Alcohol Usage and 20 participants refrained from it. Out of the 20 female participants in Group 1, 2 participants had the habit of Alcohol Usage and 18 participants refrained from it. Among 21 male participants in Group 2, 4 participants had the habit of Alcohol Usage and 17 participants refrained from it. Out of the 29 female participants in Group 2, 5 participants had the habit of Alcohol Usage and 24 participants refrained from it. No statistically significant difference was observed in Alcohol usage between the two groups and also between male and female participants in each group.
Results

Figure 6: Gender distribution based on the family history of Type II Diabetes Mellitus of the study population

Table 6: Gender distribution based on the family history of Type II Diabetes Mellitus of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>P Value</td>
<td>Male</td>
</tr>
<tr>
<td><strong>Family History</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>N</td>
<td>N</td>
<td>0.528*</td>
<td>N</td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>15</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>5</td>
<td></td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

*-Fisher’s Exact Test

Table 6 shows the gender distribution based on the family history of Type II Diabetes Mellitus of the study population. Among 30 male
participants in Group 1, 19 participants had a positive family history of Type II Diabetes Mellitus, and 11 participants had a negative history. Out of the 20 female participants in Group 1, 15 participants had a positive family history of Type II Diabetes Mellitus and 5 participants had a negative history. Among 21 male participants in Group 2, 4 participants had a positive family history of Type II Diabetes Mellitus and 17 participants had a negative history. Out of the 29 female participants in Group 2, 6 participants had a positive family history of Type II Diabetes Mellitus and 23 participants had a negative history. The difference in family history of Type II Diabetes Mellitus was statistically significant between Group 1 and Group 2. However no significant difference was observed between male and female participants in each group.

Figure 7: Gender distribution based on the knowledge about Plasma HbA1C test of the study population
Table 7: Gender distribution based on the knowledge about Plasma HbA1C test of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge about Plasma HbA1C test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>Male</td>
<td>Female</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.107*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

*- Pearson Chi-Square Test

Table 7 shows the gender distribution based on the knowledge about Plasma HbA1C test of the study population. Among 30 male participants in Group 1, 17 participants had awareness about Plasma HbA1C test and 13 participants did not have awareness about the test. Out of the 20 female participants in Group 1, 15 participants had awareness about Plasma HbA1C test and 5 participants did not have awareness about the test. Among 21 male participants in Group 2, 9 participants had awareness about Plasma HbA1C test and 12 participants did not have awareness about the test. Out of the 29 female participants in Group 2, 15 participants had awareness about Plasma HbA1C test and 14 participants did not have awareness about the test. There was no statistically significant difference in knowledge about HbA1C test.
between the two groups and also no significant difference was observed between male and female participants in each group.

Figure 8: Gender distribution based on the mean BMI of the study population

Table 8: Gender distribution based on the mean BMI of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (±SD)</td>
<td>N</td>
</tr>
<tr>
<td>BMI</td>
<td>Male</td>
<td>30</td>
<td>24.83(±1.147)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>20</td>
<td>24.90(±1.021)</td>
</tr>
<tr>
<td>P value</td>
<td>0.918**</td>
<td>P value</td>
<td>0.787**</td>
</tr>
</tbody>
</table>

## Mann-Whitney U Test
Table 8 shows the gender distribution based on the mean BMI of the study population. The mean BMI of male and female participants in Group 1 was 24.83(±1.147) and 24.90(±1.021) respectively. In Group 2 male participants had a mean BMI of 22.71(±1.521) and female participants had a mean BMI of 22.55(±1.804). Participants in Group 1 had significantly higher BMI when compared to participants in Group 2, however no significant difference was observed between male and female participants in each group.

Figure 9: Gender distribution based on the mean Plasma Fasting Glucose levels of the study population
Table 9: Gender distribution based on the mean Plasma Fasting Glucose levels of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Fasting Glucose(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>N 30</td>
<td>Mean (±SD) 123.20(±46.643)</td>
<td>Male</td>
<td>N 21</td>
<td>84.14(±6.792)</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>167.60(±68.775)</td>
<td>Female</td>
<td>29</td>
<td>84.55(±5.968)</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.014##</td>
<td>P value</td>
<td></td>
<td>0.738##</td>
</tr>
</tbody>
</table>

## Mann-Whitney U Test

Table 9 shows the gender distribution based on the mean Plasma Fasting Glucose levels of the study population. Male participants in Group 1 had a mean Plasma Fasting Glucose level of 123.20(±46.643) mg/dl and female participants had a mean value of 167.60(±68.775) mg/dl. The mean Plasma Fasting Glucose levels in male and female participants in Group 2 were 84.14(±6.792) and 84.55(±5.968) mg/dl respectively. Participants in Group 1 had significantly higher mean Plasma Fasting Glucose levels, when compared to Group 2, also female participants in Group 1 had significantly higher values when compared to male participants. However in Group 2, no significant difference was observed in the mean Fasting Glucose levels between male and female participants.
Figure 10: Gender distribution based on the mean Plasma Postprandial Glucose levels of the study population

Table 10: Gender distribution based on the mean Plasma Postprandial Glucose levels of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (±SD)</td>
<td>N</td>
</tr>
<tr>
<td>Plasma Postprandial</td>
<td>Male 30</td>
<td>182.77 (±96.524)</td>
<td>Male 21</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>Female 20</td>
<td>259.35 (±105.068)</td>
<td>Female 29</td>
</tr>
<tr>
<td>P value</td>
<td>0.011**</td>
<td>P value</td>
<td>0.288**</td>
</tr>
</tbody>
</table>

**Mann-Whitney U Test
Table 10 shows the gender distribution based on the mean Plasma Postprandial Glucose levels of the study population. Male participants in Group 1 had a mean Plasma Postprandial Glucose level of 182.77(±96.524) mg/dl and female participants had a mean value of 259.35(±105.068) mg/dl. The mean Plasma Postprandial Glucose levels in male and female participants in Group 2 were 110.19(±14.713) and 115.21(±15.486) mg/dl respectively. Participants in Group 1 had significantly higher mean Plasma Postprandial Glucose levels, when compared to Group 2, also female participants in Group 1 had significantly higher values when compared to male participants. However in Group 2, no significant difference was observed in the mean Postprandial Glucose level between male and female participants.

**Figure 11: Gender distribution based on the mean Plasma Glycated Hemoglobin levels of the study population**
Table 11: Gender distribution based on the mean Plasma Glycated Hemoglobin levels of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (±SD)</td>
<td>N</td>
<td>Mean (±SD)</td>
<td></td>
</tr>
<tr>
<td>Plasma HbA1c (%)</td>
<td>Male</td>
<td>30       6.53(±2.097)</td>
<td>Male</td>
<td>21       4.90(±0.539)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>20       8.35(±2.700)</td>
<td>Female</td>
<td>29       4.66(±0.484)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.019**</td>
<td>P value</td>
<td></td>
<td>0.111**</td>
</tr>
</tbody>
</table>

**Mann-Whitney U Test

Table 11 shows the gender distribution based on the mean Plasma Glycated Hemoglobin levels of the study population. Male participants in Group 1 had a mean Plasma Glycated Hemoglobin level of 6.53(±2.097) and female participants had a mean value of 8.35(±2.700). The mean Plasma Glycated Hemoglobin levels in male and female participants in Group 2 were 4.90(±0.539) and 4.66(±0.484) respectively. Participants in Group 1 had significantly higher mean Plasma Glycated Hemoglobin levels, when compared to Group 2, also female participants in Group 1 had significantly higher values when compared to male participants. However in Group 2, no significant difference was observed in the mean Plasma Glycated Hemoglobin levels between male and female participants.
Results

Figure 12: Gender distribution based on the mean Salivary Fructosamine levels of the study population

Table 12: Gender distribution based on the mean Salivary Fructosamine levels of the study population

| Variable | Group 1 | | Group 2 | | P Value |
|----------|---------|----------|---------|----------|
| Salivary Fructosamine (μ mol/lit) | Male | N 30 | Mean (±SD) 158.80(±97.736) | Male | N 21 | Mean (±SD) 80.90(±29.348) | <0.001** |
| | Female | N 20 | 208.10(±72.480) | Female | N 29 | 85.07(±25.209) | 0.036** |
| | P value | | | P value | | | 0.582** |

**Mann-Whitney U Test

Table 12 shows the gender distribution based on the mean Salivary Fructosamine levels of the study population. Male participants in Group 1 had a mean Salivary Fructosamine level of 158.80(±97.736) μ mol/lit and female
participants had a mean value of 208.10 (±72.480) μmol/lit. The mean Salivary Fructosamine levels in male and female participants in Group 2 were 80.90(±29.348) and 85.07(±25.209) μmol/lit respectively. Participants in Group 1 had significantly higher mean Salivary Fructosamine levels, when compared to Group 2, also female participants in Group 1 had significantly higher values when compared to male participants. However in Group 2, no significant difference was observed in the Salivary Fructosamine levels between male and female participants.

**Table 13: Correlation between Salivary Fructosamine, Plasma Fasting and Postprandial Glucose and Plasma Glycated Hemoglobin levels in the two groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variable</th>
<th>r value (Spearman’s rho)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Plasma Fasting Glucose(mg/dl)</td>
<td>0.934</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma Postprandial Glucose(mg/dl)</td>
<td>0.910</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma HbA1C (%)</td>
<td>0.893</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 2</td>
<td>Plasma Fasting Glucose(mg/dl)</td>
<td>0.794</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma Postprandial Glucose(mg/dl)</td>
<td>0.530</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma HbA1C (%)</td>
<td>0.307</td>
<td>0.030</td>
</tr>
</tbody>
</table>
Table 13 shows the correlation between Salivary Fructosamine, Plasma Fasting and Postprandial Glucose and Plasma Glycated Hemoglobin levels in the two groups. Among the Group 1 individuals high correlation was observed between Salivary Fructosamine, Plasma Fasting Glucose levels (r value of 0.934), Plasma Postprandial Glucose levels (r value of 0.910) and, Plasma Glycated Hemoglobin (r value of 0.893). In Group 2 individuals high correlation was observed between Salivary Fructosamine and Plasma Fasting glucose levels (r value of 0.794), moderate correlation was observed between Salivary Fructosamine and Plasma Postprandial Glucose (r value of 0.530) and Plasma Glycated Hemoglobin levels (r value of 0.307).

**Figure 13: Correlation between Salivary Fructosamine and Plasma**

Fasting Glucose levels in Group 1 individuals
Figure 14: Correlation between Salivary Fructosamine and Plasma Fasting Glucose levels in Group 2 individuals

![Graph showing correlation between Salivary Fructosamine and Fasting Glucose levels with regression line and equation: $R^2 = 0.616, Y = 69.105 + 0.183X$.]

Figure 15: Correlation between Salivary Fructosamine and Plasma Postprandial Glucose levels in Group 1 individuals

![Graph showing correlation between Salivary Fructosamine and Postprandial Glucose levels with regression line and equation: $R^2 = 0.753, Y = 33.067 + 1.010X$.]
Figure 16: Correlation between Salivary Fructosamine and Plasma Postprandial Glucose levels in Group 2 individuals

\[ R^2 = 0.186 \]
\[ Y = 92.706 + 0.245X \]

Figure 17: Correlation between Salivary Fructosamine and Plasma Glycated Hemoglobin levels in Group 1 individuals

\[ R^2 = 0.697 \]
\[ Y = 3.170 + 0.023X \]
Figure 18: Correlation between Salivary Fructosamine and Plasma Glycated Hemoglobin levels in Group 2 individuals

\[ R^2 = 0.089 \]
\[ Y = 4.280 + 0.006X \]
Table 14: Correlation between Salivary Fructosamine, Plasma Fasting and Postprandial Glucose and Plasma Glycated Hemoglobin levels in Group 1 individuals

<table>
<thead>
<tr>
<th>Gender</th>
<th>Variable</th>
<th>r value (Spearman’s rho)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Plasma Fasting Glucose(mg/dl)</td>
<td>0.958</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma Postprandial Glucose(mg/dl)</td>
<td>0.941</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma HbA1C (%)</td>
<td>0.880</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>Plasma Fasting Glucose(mg/dl)</td>
<td>0.889</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma Postprandial Glucose(mg/dl)</td>
<td>0.845</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma HbA1C (%)</td>
<td>0.862</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 14 shows the correlation between Salivary Fructosamine, Plasma Fasting and Postprandial Glucose and Plasma Glycated Hemoglobin levels in group 1 individuals. High correlation was observed between Salivary Fructosmine, Plasma Fasting (r value of 0.958 in male participants and 0.889 in female participants), Plasma Postprandial Glucose level (r value of 0.941 in male participants and 0.845 in female participants) and Plasma Glycated Hemoglobin levels (r value of 0.880 in male participants and 0.862 in female participants) in both male and female patients.

**Figure 19: Correlation between Salivary Fructosamine and Plasma Fasting Glucose levels in male participants (Group 1)**

$$R^2=0.861$$

$$Y=52.897+0.443X$$
Results

Figure 20: Correlation between Salivary Fructosamine and Plasma

Fasting Glucose levels in female participants (Group 1)

![Graph showing correlation between Salivary Fructosamine and Plasma Fasting Glucose levels](image)

$R^2 = 0.644$

$Y = 9.097 + 0.762X$

Figure 21: Correlation between Salivary Fructosamine and Plasma

Postprandial Glucose levels in male participants (Group 1)

![Graph showing correlation between Salivary Fructosamine and Plasma Postprandial Glucose levels](image)

$R^2 = 0.764$

$Y = 45.669 + 0.863X$
Figure 22: Correlation between Salivary Fructosamine and Plasma Postprandial Glucose levels in female participants (Group 1)

![Graph showing correlation between Salivary Fructosamine and Plasma Postprandial Glucose levels. The equation is $R^2=0.754$, $Y=1.259X-2.629$.]

Figure 23: Correlation between Salivary Fructosamine and Plasma Glycated Hemoglobin levels in male participants (Group 1)

![Graph showing correlation between Salivary Fructosamine and Plasma Glycated Hemoglobin levels. The equation is $R^2=0.782$, $Y=3.521+0.019X$.]
Results

Figure 24: Correlation between Salivary Fructosamine and Plasma Glycated Hemoglobin levels in female participants (Group 1)

\[ R^2 = 0.639 \]
\[ Y = 2.151 + 0.030X \]
Discussion
DISCUSSION

The present cross sectional study was conducted to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin (HbA1C), Plasma Fasting and Postprandial Glucose levels amongst Type II Diabetes mellitus patients. The study participants were divided into two groups: Group 1 (Type II Diabetes mellitus patients), and Group 2 (non Diabetic healthy individuals). Blood and saliva samples were collected from both the groups and the levels of Salivary Fructosamine, Plasma Glycated Hemoglobin; Plasma Fasting and Postprandial Glucose levels were estimated. The study was conducted over a period of five months.

Monitoring of glycemic status is essential for the management of Diabetes. Asian Indians have a genetic predisposition to developing Diabetes mellitus, due to the polymorphism in Pro 12 Ala gene and Thr 394 gene (which results in increased insulin resistance and central obesity). In India, it has been reported that Ernakulum district of Kerala has the highest prevalence (19.5%), and the state of Kashmir has the lowest prevalence (6.1%) of Diabetes. This is due to the consumption of highly polished rice, with high glycemic index, among South Indian population. In recent times, substantial body of evidence shows alarming levels of increase in prevalence of Diabetes in urban areas when compared to rural areas. This can be attributed to the unhealthy lifestyle and decreased consumption of traditional diet among city
dwellers, with about 32% of the daily energy requirements consumed in the form of fat.\textsuperscript{57}

The high prevalence of Type II Diabetes mellitus, and the associated mortality and morbidity are due to the fact that most people recognize the disease in a state where diabetic complications have already occurred.\textsuperscript{58} Various studies report that Diabetes is the leading cause for blindness, kidney failure and amputations. The complications of Diabetes impose a huge social and financial burden over the community. According to the International Diabetes Federation (IDA), America contributes to 52.7\% of the total Diabetes spending worldwide, on the other hand India, which has largest Diabetes population, spends only 1\% of the global total. In the year 2009 it was reported that the annual expenditure by patients on Diabetes care was, on an average, INR (Indian Rupee value) 10,000 (US $227) in urban areas and INR 6,260 (US $142) in rural areas.\textsuperscript{59} Hence there is the need for early diagnosis of Diabetes, by periodic monitoring of glycemic control.

**DISADVANTAGES OF THE CONVENTIONAL METHODS OF MONITORING GLUCOSE:**

The conventional methods of monitoring glycemic control include the estimation of Plasma Glucose, Plasma Glycated Hemoglobin and Plasma Fructosamine levels. Nevertheless, all these methods are invasive, as they require venous puncture to obtain blood samples. In contrast, the collection of saliva is simple, non-invasive and does not require venous puncture. A few studies have been conducted to estimate the levels of Salivary Glucose, and to
determine the association between Salivary Glucose and Blood Glucose levels. However due to the presence of bacteria and enzymes in the oral cavity, Salivary Glucose is rapidly decomposed, or it may be decomposed during the transfer from blood to saliva. Hence it is difficult to measure Salivary Glucose levels without filtration.²⁸

Campbell MJA observed that the Salivary Glucose levels were significantly higher in Diabetes patients (between 0.44 and 6.33 mg/l00 ml) when compared to non-diabetic healthy individuals (between 0.24 and 3.33 mg/100 ml). However no significant correlation was observed between Blood Glucose and Salivary Glucose levels (r=0.14).³⁹ Similarly in a study conducted by Forbat LN, Collins RE et al the mean Salivary Glucose level was reported as 0.32 mmol/l, but no significant correlation was observed between Blood and Salivary Glucose levels (r=0.18).⁴²

Nakamoto I et al, compared the levels of Salivary Glucose levels among healthy controls, patients with Impaired Glucose Tolerance Test and diabetic patients. The mean Salivary Glucose level was reported to be 1.5±2.0 mg/dl. No correlation was observed between Salivary Glucose and Plasma Glucose levels (r= 0.032, p value = 0.826).This was attributed to the decomposition of Salivary Glucose by oral bacteria and enzymes.²⁸

Manjrekar PA, Hedge A et al, compared the levels of Salivary Glucose levels among healthy controls (Group 1), non-diabetic first degree relatives of Type II diabetic patients (Group 2) and Type II diabetic patients (Group 3). The mean Salivary Glucose levels in Group 1, 2, and 3 were reported to be
9.4±6.8, 9.2 ± 5.9, 8.8 ±4.4 mg/dL respectively. However no significant difference was observed in the glucose levels between the three groups and also Salivary Glucose levels did not correlate with Plasma Glucose levels, in all the three groups (Group 1 r=0.063, Group 2 r=0.170, Group 3 r= –0.039). This was attributed to the micro angiopathy and fatty infiltration of the salivary glands that occurs in diabetic patients.53

In the present study Salivary Glucose levels were not assessed as extensive literature review shows no correlation between Salivary and Plasma Glucose levels, and as Salivary glucose is metabolized by Salivary bacteria and enzymes.28

**IMPORTANCE OF GLYcation:**

**GLYCATED HEMOGLOBIN (HbA1C):**

Glycation of Hemoglobin occurs when, valine residue of beta chain, epsilon amino groups of lysine (in the alpha and beta chain) and the N terminus (in the alpha beta chain) reacts with glucose. The half life of Hemoglobin is 60 days; hence it reflects the glycemic control over a period of six to eight weeks.15

**PLASMA FRUCTOSAMINE:**

Carbonyl groups of reducing sugars such as glucose react, non-enzymatically with amino group of proteins and results in the formation of Schiff base (aldimines). The Schiff base (labile aldimine) undergoes Amadori rearrangement to form stable Fructosamine. Further the straight chain
Fructosamine, if formed from glucose undergoes cyclization to a hemiketal furanose or pyranose ring structure for added stability. The half-life of albumin is about 14-20 days; hence it reflects the glycemic control over a period of two to three weeks.

**ADVANTAGES OF PLASMA FRUCTOSAMINE:**

Extensive literature review shows that Plasma Fructosamine levels correlate with plasma HbA1C levels.

1. Since Plasma Fructosamine reflects glycemic control over a period of two weeks, it is an earlier objective evidence of metabolic response to therapeutic interventions, and can be regarded as an intermediate index of Diabetes control.

2. Unlike HbA1C, Plasma Fructosamine levels are not altered in conditions such as anemia and blood transfusions.

3. Studies suggest that Plasma Fructosamine is a better indicator of glycemic control in hemodialysis patients, as it predicts the risk of developing infection and hospitalization.
ADVANTAGES OF SALIVARY FRUCTOSAMINE:

Salivary Fructosamine is a glycated protein formed by the non-enzymatic reaction between glucose and plasma proteins (mostly albumin). Since the protein combines with glucose, it is relatively stable against bacteria when compared to Salivary Glucose, and also reflects the glycemic control over a period of two weeks. Substantial body of evidence shows that, Plasma Fructosamine is correlated with Plasma Glucose and HbA1C levels, however a very few studies have been conducted to estimate the levels of Salivary Fructosamine in diabetic patients. Hence the present study was contemplated to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin (HbA1C), Plasma Fasting and Postprandial Glucose levels amongst Type II Diabetes mellitus patients.

Zhang -Ru gen et al, conducted a study among diabetic patients and healthy controls. The study results reported that Salivary Fructosamine levels were significantly higher in diabetic individuals (0.61±0.19 mmol DMF/L) when compared to healthy controls (0.38±0.07 mmol DMF/L). Salivary Fructosamine was positively correlated with Plasma Fasting Glucose (r=0.449), Plasma Fructosamine (r=0.526) and Plasma Glycated HbA1C (r=0.411).

Nakamoto I et al, compared the levels of Salivary Fructosamine levels among healthy controls, patients with Impaired Glucose Tolerance Test and diabetic patients. The mean Salivary Fructosamine levels of the study
population was reported as $25.2\pm11.6 \, \mu\text{mol/g protein}$. The study reported positive correlation between Salivary Fructosamine, Plasma HbA1C and Plasma Blood Glucose levels ($r=0.449; \ p=0.001$ and $r=0.445; \ p=0.001$, respectively). The study suggests that, since Salivary Fructosamine is formed by the combination of proteins and glucose, it is relatively stable against oral bacterial and enzymatic degradation, hence a positive correlation was observed between Salivary Fructosamine and Plasma HbA1C. No correlation was observed between Salivary Fructosamine and Plasma Fructosamine ($r=0.260; \ p=0.065$). This was attributed to the protein compensation that occurs in the oral cavity (by the combination of salivary proteins and glucose); however such protein compensation was not observed in Plasma Fructosamine. The study suggests that, since no correlation was observed between Salivary Glucose and Plasma Glucose, the correlation between Salivary Fructosamine and Plasma Glucose and HbA1C reflects that the Salivary Fructosamine was produced in the blood or in saliva over a short period of time.\textsuperscript{28}

Morenkova SA, compared the levels of Salivary Fructosamine among healthy controls, Type I and Type II Diabetes patients. The study reported that there was an increase in Salivary Fructosamine concentration with an increase in Plasma Glucose levels. The levels of Salivary Fructosamine in healthy controls, Type I diabetic patients and, Type II diabetic patients were $3.7 \pm 0.7 \, \text{mmol/l}$, $6.2 \pm 1.7 \, \text{mmol/l}$ and $7.3 \pm 2.0 \, \text{mmol/l}$ respectively.\textsuperscript{49}
Manjrekar PA, Hedge A et al, compared the levels of Salivary Fructosamine among healthy controls (Group 1), non-diabetic first degree relatives of Type II diabetic patients (Group 2) and Type II diabetic patients (Group 3). Individuals in Group 3 had significantly higher levels of Salivary Fructosamine (202.1 ±103.4 mg /dl) when compared to individuals in Group 2 (130 ± 71.6 mg/dl) and Group 1 (99.8 ± 50.1mg/dl). However no significant correlation was observed between Salivary Fructosamine and Plasma Fasting Glucose levels in all the three groups (Group 1 r value=0.061, Group 2 r value=0.171, Group 3r value =-0.078).53

In the present study Type II Diabetes patients had significantly higher levels Salivary Fructosamine when compared to non-diabetic healthy individuals (p value< 0.001). The mean levels of Salivary Fructosamine among Group 1 male, and female participants was observed to be 158.80 (±97.736) and 208.10 (± 72.480) u mol/lit respectively. Among Group 2 male and female participants the mean levels of Salivary Fructosamine was observed to be 80.90 (±29.348) and 85.07 (±25.209) u mol/lit respectively. It was also observed that female participants in Group 1 had significantly higher levels of Salivary Fructosamine, when compared to male participants (p value =0.036). However no significant difference was observed in Group 2 between male and female participants (p value =0.582). The higher levels of Salivary Fructosamine among Group 1 female participants can be attributed to
the significantly higher levels of Plasma Fasting, Post Prandial Glucose levels in them, which in turn leads to increased glycation of plasma proteins.

**PLASMA FASTING GLUCOSE LEVELS:**

The mean Plasma Fasting Glucose levels among Group 1 male and female participants were observed to be 123.20 (±46.643) mg/dl and 167.60 (±68.775) mg/dl respectively. Among Group 2 male and female participants the mean levels of plasma fasting glucose was observed to be 84.14 (±6.792) and 84.55 (±5.928) mg/dl respectively. Participants in Group 1 had significantly higher levels of Plasma Fasting Glucose when compared to Group 2 (p value < 0.001), also in Group 1 female participants had significantly higher levels of Plasma Fasting Glucose when compared to male participants (p value =0.014). However in Group2, no significant difference was observed in the levels of Plasma Fasting Glucose between male and female participants (p value =0.738). In a study conducted by Willer AK et al, it was observed that women had less insulin secretion and sensitivity when compared to men, also insulin secretion and sensitivity was observed to decrease with increase in BMI. Increased BMI in women was also associated with increased insulin resistance, and increased hepatic glucose production. Hence the significantly higher Plasma Fasting Glucose levels among female participants in Group 1 may be attributed to the difference in insulin sensitivity, insulin secretion, hepatic glucose production, between male and female diabetic patients.
PLASMA FASTING GLUCOSE AND SALIVARY FRUCTOSAMINE LEVELS:

A high correlation was observed between Salivary Fructosamine and Plasma Fasting glucose levels in Group 1 (r =0.934, p value <0.001) and Group 2 (r=0.794, p value <0.001) individuals. Also among Group 1 male and female participants, a high correlation was observed between Salivary Fructosamine and Plasma Fasting Glucose levels (r=0.958, p value<0.001, r = 0.889, p value <0.001 respectively).

PLASMA POSTPRANDIAL GLUCOSE LEVELS:

The mean Plasma Post Prandial Glucose levels among Group 1 male and female participants were observed to be 187.77(±96.524) mg /dl and 259.35(±105.068) mg /dl respectively. Among Group 2 male and female participants the mean levels of Plasma Postprandial Glucose was observed to be 110.19(±14.713) and 115.21(±15.486) mg /dl respectively. Participants in Group 1 had significantly higher levels of Plasma Postprandial Glucose when compared to Group 2 (p value <0.001), also in Group 1 female participants had significantly higher levels of Plasma Postprandial Glucose when compared to male participants (p value =0.011). However in Group2, no significant difference was observed in the levels of Plasma Postprandial Glucose between male and female participants (p value =0.288). The significantly higher Plasma Postprandial Glucose levels among female...
participants in Group 1 may be attributed to the prolonged gut absorption of glucose in women.\textsuperscript{55}

**PLASMA POSTPRANDIAL GLUCOSE AND SALIVARY FRUCTOSAMINE LEVELS:**

A high correlation was observed between Salivary Fructosamine and Plasma Post Prandial Glucose levels in Group 1 (r = 0.910, p value < 0.001). In Group 2, a moderate correlation was observed between Salivary Fructosamine and Plasma Postprandial Glucose levels (r = 0.530, p value < 0.001) individuals. Also among Group 1 male and female participants, a high correlation was observed between Salivary Fructosamine and Plasma Post Prandial Glucose levels (r = 0.941, p value < 0.001, r = 0.845, p value < 0.001 respectively).

**PLASMA GLYCATED HEMOGLOBIN (HbA1C) LEVELS:**

The mean Plasma HbA1C levels (in %) among Group 1 male and female participants was observed to be 6.53(±2.097) and 8.35(±2.700) respectively. Among Group 2 male and female participants the mean levels of Plasma HbA1C was observed to be 4.90 (±0.539) and 4.66 (±0.484) respectively. Participants in Group 1 had significantly higher levels of Plasma HbA1C when compared to Group 2 (p value < 0.001), also in Group 1 female participants had significantly higher levels of Plasma HbA1C when compared to male participants (p value = 0.019). However in Group 2 participants no significant difference was observed in the levels of Plasma HbA1C levels.
among male and female participants (p value= 0.111). Glycemic and metabolic control in women are affected by Post Prandial Glucose levels.\textsuperscript{55} Hence the significantly higher Plasma HbA1C levels among female participants in Group 1 may be attributed to the high levels of Plasma Fasting and Post Prandial Glucose levels, which in turn leads to increased glycation of hemoglobin.\textsuperscript{55}

**PLASMA HbA1C AND SALIVARY FRUCTOSAMINE LEVELS:**

A high correlation was observed between Salivary Fructosamine and Plasma HbA1C levels in Group 1 (r =0.893, p value <0.001) and in Group 2, a moderate correlation was observed (r=0.307, p value =0.030) . Also among Group 1 male and female participants, a high correlation was observed between Salivary Fructosamine and Plasma HbA1C levels (r=0.880, p value <0.001, r = 0.862, p value <0.001 respectively).

**ADVANTAGES OF SALIVARY BIOMARKERS:**

1. Saliva collection apart from being simple and non invasive, can be utilized in instances where collection of blood is not ideal due to patient’s age (especially in pediatric and elderly people), attitude towards venous puncture, and hygiene issues in rural areas.\textsuperscript{56}

2. Collection of saliva does not require any special training or equipments. Hence salivary biomarkers can be used in large-scale population assessments and screening programs.\textsuperscript{56}
ADVANTAGES OF THE PRESENT STUDY:

1. To the best of our knowledge, the present study is one of the few studies that have been conducted in an Indian scenario.

2. Literature review shows that earlier studies have been carried out only with limited participants however in the present study, a total of 100 participants were included in the study (with 50 participants in each group)

LIMITATIONS OF THE PRESENT STUDY:

1. Convenience sampling technique was used for the study.

2. The present study has a cross sectional design; hence further longitudinal studies are required to validate the use of Salivary Fructosamine for the diagnosis and monitoring of glycemic control in diabetic patients.
SUMMARY

The present cross sectional study was conducted to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin (HbA1C), Plasma Fasting and Postprandial Glucose levels amongst Type II Diabetes mellitus patients. The study participants were divided into two groups: Group 1 (Type II Diabetes mellitus patients), and Group 2 (non-Diabetic healthy individuals). Blood and saliva samples were collected from both the groups and the levels of Salivary Fructosamine, Plasma Glycated Hemoglobin; Plasma Fasting and Postprandial Glucose levels were estimated. The study was conducted over a period of five months.

Ethical clearance was obtained from the Institutional Review Board of Ragas Dental College and Hospital, Chennai. Blood and saliva samples were collected as a part of routine medical checkup, from patients visiting the Department of Diabetology at, Aringar Anna Government Hospital of Indian Medicine, Arumbakkam, Chennai. Blood samples were analyzed at Aringar Anna Hospital to estimate the levels of Plasma Fasting, Post Prandial Glucose and Plasma HbA1C levels. Salivary samples were analyzed at Anderson Laboratories P.H Road to estimate the level of Salivary Fructosamine.

A total of 100 participants with 50 patients in each group were included, based on the inclusion and exclusion criteria. The nature and purpose of the study was explained to all the subjects. Only subjects who were
willing to participate voluntarily, and gave consent were recruited for the study. A pre validated questionnaire (validated by Pan American Health Organization and World Health Organization) was administered to collect the socio demographic factors, family history of non-communicable disease, tobacco usage and alcohol consumption, before the collection of blood and saliva samples.

Statistical analyses were performed using Statistical Package for Social Sciences software (SPSS version 20, USA). Pearson- Chi square Test and Fisher’s Exact Test were used to analyze all the qualitative data (Demographic details). T- Test was used to compare the age among the two groups and Mann-Whitney U Test was used to compare the other quantitative variables (BMI, Salivary Fructosamine, Plasma Fasting and Postprandial Glucose and Plasma Glycated Hemoglobin levels). To test for any positive association between Salivary Fructosamine and, Plasma Glycated Hemoglobin, Plasma Fasting and Postprandial Glucose levels, simple linear regression was carried out.

The study results showed that

1. Type II Diabetes patients had significantly higher levels of Salivary Fructosamine, when compared to non-diabetic healthy individuals (p value < 0.001).

2. Among the Group1 individuals high correlation was observed between Salivary Fructosamine, Plasma Fasting Glucose levels (r
value of 0.934), Plasma Postprandial Glucose levels (r value of 0.910) and, Plasma Glycated Hemoglobin (r value of 0.893).

3. In Group 2 individuals high correlation was observed between Salivary Fructosamine and Plasma Fasting Glucose levels (r value of 0.794), moderate correlation was observed between Salivary Fructosamine and Plasma Postprandial Glucose (r value of 0.530) and Plasma Glycated Hemoglobin levels (r value of 0.307).

The study concluded that Salivary Fructosamine levels were significantly higher in diabetic patients when compared to healthy individuals. Also positive correlation was observed between Salivary Fructosamine, Plasma Fasting, Postprandial Glucose and Plasma Glycated Hemoglobin levels. Hence further longitudinal studies are required to validate the clinical utility of Salivary Fructosamine as a non-invasive bio marker for the diagnosis of Diabetes and monitoring of glycemic control in diabetic patients.
Conclusion
CONCLUSION

India has become the Diabetes capital of the world, with about 66.8 million Indians suffering from Diabetes and it may raise to 109 million by the year 2025. Substantial body of evidence shows that the onset of Diabetes can be prevented or delayed greatly in individuals at high risk (people with impaired glucose regulation), by adopting a comprehensive preventive approach, which includes healthy lifestyle and by periodic monitoring of glycemic control.

Early diagnosis of Diabetes and periodic monitoring of glycemic control is necessary to prevent diabetic complications such as such as nephropathy, retinopathy, neuropathy, cardiovascular disease, stroke, and death.

The conventional methods of monitoring glycemic control, which includes Plasma glucose, HbA1C and Fructosamine are invasive as they require venous puncture. Since the collection of saliva is simple and non-invasive, significant research is being carried out on the development and validation of salivary biomarkers in recent times. Hence the present study was contemplated to determine the association between Salivary Fructosamine and Plasma Glycated Hemoglobin (HbA1C), Plasma Fasting and Postprandial Glucose levels amongst Type II Diabetes mellitus patients.
Conclusion

The present study throws light on the potential use of Salivary Fructosamine for the diagnosis and post treatment monitoring of glycemic control among diabetic patients. The overall study results showed that Salivary Fructosamine levels were significantly higher in diabetic patients when compared to healthy individuals. Also a positive correlation was observed between Salivary Fructosamine, Plasma Fasting, Plasma Postprandial and Plasma Glycated Hemoglobin.
Recommendations
RECOMMENDATIONS

1. Longitudinal studies are required to validate the clinical utility of Salivary Fructosamine for the diagnosis of Diabetes and monitoring of glycemic control among diabetic patients.
2. Development of standardized method of estimation of Salivary Fructosamine is required, for uniform reporting and comparison among various studies.
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Annexures
ANNEXURE – I

TO WHOMSOEVER IT MAY CONCERN

10.7.2015

From
Institutional Review Board,
Ragas Dental College and Hospital,
Uthanadi,
Chennai - 600 119.

INSTITUTIONAL REVIEW BOARD APPROVAL

The dissertation topic titled “Association between salivary fructosamine, plasma glycated hemoglobin and plasma glucose levels amongst diabetes mellitus patients – a cross sectional study” submitted by Dr Sadhana K., has been approved by the Institutional Review Board of Ragas Dental College and Hospital on 17th November 2014.

Dr S.Ranachandran
Member Secretary,
Ragas, IRB.
ANNEXURE – II

TO WHOM IT MAY CONCERN

This is to certify that Dr. Sadhana K., a Post Graduate Student, Department of Public Health Dentistry, Ragas Dental College and Hospital, Chennai was granted permission to collect saliva samples from diabetic patients visiting our hospital outpatient department, as a part of her thesis titled “Association Between Salivary Fructosamine, Plasma Glycated Hemoglobin And Plasma Glucose Levels Amongst Diabetes Mellitus Patients – A Cross Sectional Study”. She has successfully completed it over a period of five months (August 2015 – December 2015).

[Signature]

Hospital Superintendent
Pondicherry Government Dental College & Hospital
Puducherry, India

02.01.2016
Chennai 600 106
ANNEXURE – III

01/02/2016
CHENNAI-600084.

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Dr. Sahana K, Post Graduate student, Department of Public Health Dentistry, Ragas Dental College and Hospital, Chennai was granted permission to conduct the laboratory procedures for her thesis titled "Association between Salivary Fructosamine, Plasma Glycated Hemoglobin and plasma Glucose levels amongst Diabetes mellitus patients – a cross sectional study" at Anderson Diagnostics and labs P.H Road, Chennai. She has successfully completed it over period of five months. (August 2015- December 2015).

DR. MALLIKA RAVIDRAN, Ph.D.
LAB DIRECTOR.

Dr. Mallika Ravindran, Ph.D.
Laboratory Director
Anderson Diagnostics & Labs
ANNEXURE – IV

RAGAS DENTAL COLLEGE AND HOSPITAL
DEPARTMENT OF PUBLIC HEALTH DENTISTRY

TITLE OF THE STUDY:
ASSOCIATION BETWEEN SALIVARY FRUCTOSAMINE, PLASMA GLYCATED HEMOGLOBIN AND PLASMA GLUCOSE LEVELS AMONG TYPE II DIABETES MELLITUS AND NON DIABETIC INDIVIDUALS – A CROSS SECTIONAL STUDY

INVESTIGATOR 1: 
Dr. Sadhana K.
Post graduate student,
Department of Public Health Dentistry,
Ragas Dental College and Hospital

INVESTIGATOR 2:
Dr. P. D. Madan Kumar M.D.S.,
Professor
Department of Public Health Dentistry,
Ragas Dental College and Hospital

INFORMED CONSENT FORM

UNDERTAKING BY THE INVESTIGATOR:

Your consent for the above study is sought. We undertake to maintain complete confidentiality regarding the information obtained from you during the study. If you have any doubts regarding the study, please feel free to clarify the same. The investigator and contact number is given below:

Dr Sadhana K.
Mob no- 9840228092
CONSENT FORM

I ______________________________, S/O, ______________________________, residing at ______________________________

__________________________________________________________

I hereby solemnly and state as follows.

I am the deponent herein; as such I am aware of the facts stated here under.

I have been informed about the purpose and procedures of the study that is to be conducted. I understand that if I give my consent for the study, I will have to provide the necessary details required for the study and co-operate.

I ______________________________ give my consent to be a part of this investigation.

Signature of the investigator

Signature of the Participant

Date:

Place:
ANNEXURE – V

Association between Salivary Fructosamine, Plasma Glycated Haemoglobin and Plasma Glucose levels among Type II Diabetes Mellitus and Non Diabetic Individuals: A Cross Sectional Study

Q1: What was the aim of the study?
Q2: What were the significant findings of the study?
3. இந்த அனுமானமாக விளக்கமாற்றத்தை பொருளையனுக்கும் கல்வி குறிப்பிட்டு அளிக்கவேண்டும்.

4. வேளாயிற்று வரலாற்றுக்கு பராமரிக்கப்பட்டது என்று நம்பப்படுகின்றது, இது அவர்களுடன் வாழ்ந்து வந்தபோது அவ்விலையானது வந்துவிட்டது.

5. இந்த அனுமானமாக விளக்கமாற்றத்தை பொருளையனுக்கும் கல்வி குறிப்பிட்டு அளிக்கவேண்டும்.