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THE USE OF SALIVARY GLUCOSE CONCENTRATION AS AN INDICATOR FOR GLYCEMIC CONTROL IN DIABETIC PATIENTS

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A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Oral Medicine

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The Use of Salivary Glucose Concentration as an Indicator for Glycemic Control in Diabetic Patients

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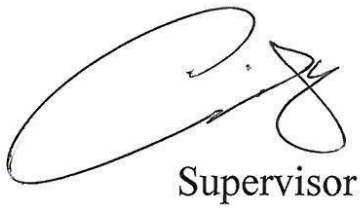
CERTIFICATE

This is to certify that the work presented in this thesis represents original research carried out by **Loai. A. F. Ben Saod** Submitted in partial fulfillment of requirements for the degree of Master of Science in Oral Medicine according to the regulations of university of Benghazi.



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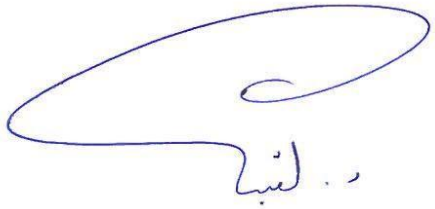
DECLARATION

This is to declare that I have not submitted the research work embodied in this thesis **"The use of salivary glucose concentration as an indicator for glycemic control in diabetic patients"** to any other university before.



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DEDICATION

This thesis is dedicated to my beloved **mother** and my **wife**. Their supports, encouragements and infinite sacrifices have sustained me throughout my life.

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ATP	Adenosine Triphosphate
BMS	Burning Mouth Syndrome
DM	Diabetes Mellitus
FBS	Fasting Blood Sugar
Hb_{A1c}	Glycated Haemoglobin
IDDM	Insulin Dependent Diabetes Mellitus
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
NAD	Nothing Abnormality Detected
OGTT	Oral Glucose Tolerance Test
OLP	Oral Lichen Planus
RAU	Recurrent Aphthus Ulcer
SD	Standard Deviation
SGC	Salivary Glucose Concentration
GDH	Glucose Dehydrogenase
NAD^+	Nicotinamide Adenine Dinucleotide
NADH	Reduced Form of NAD^+
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
$NADPH^+$	Reduced Form of $NADP^+$

Chapter 1

INTRODUCTION

INTRODUCTION

People with diabetes mellitus (DM) have an increased incidence of both microvascular and macrovascular complications. Long-term sequelae of DM may include retinopathy with potential loss of vision and nephropathy leading to renal failure. Hypertension, hyperlipidemia, atherosclerotic cardiovascular disease, peripheral vascular disease and cerebrovascular disease also are common. Some people experience peripheral and autonomic neuropathies such as numbness and tingling of extremities. People with poorly controlled DM also may have impaired wound healing and increased susceptibility to infections [(1)].

Although there are several diabetic complications that may occur in the body, several soft tissue abnormalities have been reported to be associated with DM in the oral cavity. These complications include periodontal diseases (periodontitis and gingivitis), salivary dysfunction leading to a reduction in salivary flow and changes in saliva composition, and taste dysfunction. Oral fungal and bacterial infections have also been reported in patients with DM. There are also reports of oral mucosal lesions in the form of stomatitis, geographic tongue, fissured tongue, oral lichen planus (OLP), lichenoid reaction and angular chelitis [(2, 3)]. In addition, delayed mucosal wound healing, mucosal neuro-sensory disorders, dental carries and tooth loss have been reported in patients with DM [(4)].

The prevalence and the chance of developing oral mucosal lesions were found to be higher in patients with DM compared to healthy controls [(5)].

Dentists have a role in the diagnosis of DM throughout the detection of associated oral complications and they have an opportunity and responsibility to educate patients with DM about the oral complications of the disease, and to promote proper oral health behaviors that limit the risks of tooth loss, periodontal disease and soft-tissue pathologies [(6)].

Hb_{A1c} has become the preferred clinical measure of glycaemic control. Hb_{A1c} is a measure of the non-enzymatic glycation of adult haemoglobin. The quantities of these glycated hemoglobins are much higher in diabetic patients than in healthy people and are more risky for complications of DM [(7)].

Several studies and researches have been done in order to evaluate the benefit of the use of saliva as a diagnostic tool for some diseases via its analysis which is potentially valuable for children and older adults, since whole saliva can be collected non-invasively, easily and with no special equipments compared with the collection of blood.

Saliva needs to be more investigated by researchers to evaluate its benefits to measure glycemic control.

Chapter 2

REVIEW OF LITERATURES

REVIEW OF LITERATURES

2.1 Diabetes Mellitus

Diabetes mellitus designates a group of metabolic diseases characterized by hyperglycemia due to insufficient insulin secretion and/or reduced insulin sensitivity and associated with abnormal carbohydrate, lipid and protein metabolism [(8)]. Kadiki and Roaeid studied a randomly selected group of 868 subjects the prevalence of diabetes in people over 20 years of age was 14.1% in the year 2000 in Benghazi [(9)].

2.2 Etiologic Classification of DM:

The American Diabetes Association approved recent etiologic classification of diabetes mellitus. Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. For example, a female diagnosed with gestational diabetes mellitus (GDM) may continue to be hyperglycemic after delivery and may be determined to have, in fact, type 2 diabetes [(10)].

Thus, for clinicians and patients, it is less important to label the particular type of diabetes than it is to understand the pathogenesis of the hyperglycemia and to treat it effectively [(10)].

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

A. Immune mediated:

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes or juvenile onset diabetes, results from a cellular mediated autoimmune destruction of the β-cells of the pancreas. Usually normal body weight or thin, mainly adolescents aged less than 30 years. Often abrupt onset with symptoms of insulin deficiency (polyuria, polydipsia, weight loss and fatigue), may present with ketoacidosis. Exogenous supply of insulin is vital [(10)].

These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia [(10)].

B. Idiopathic:

Some forms of type 1 diabetes have no known etiologies. A minority of patients with type 1 diabetes fall into this category. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not human leukocyte antigen (HLA) associated [(10)].

2.2.2 Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

This form of diabetes, which accounts for 90–95% of those with diabetes, previously referred to as non–insulin dependent diabetes or adult-onset diabetes, mostly individuals aged over 40 years, often overweight/obese, few classical symptoms of hyperglycemia, not prone to ketoacidosis except in stressful

periods. Exogenous insulin supply is not vital, but as the disease progresses and endogenous insulin decreases, most patients will require insulin therapy, either alone or in combination with oral hypoglycemic agents [10].

2.2.3 Other specific types

2.2.3.1 Genetic defects of β-cell function

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) [10].

2.2.3.2 Genetic defects in insulin action

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. This include Type A insulin resistance, Leprechaunism, Rabson-Mendenhall syndrome and Lipoatrophic diabetes [10].

2.2.3.3 Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion [10].

2.2.3.4 Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved [(10)].

2.2.3.5 Drug or chemical induced

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. Examples include vacor, pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, β-adrenergic agonists, thiazides, dilantin and g-interferon [(10)].

2.2.3.6 Infections

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella. In addition, coxsackie virus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease [(10)].

2.2.3.7 Uncommon forms of immune-mediated diabetes

In this category, there are two known conditions; Stiff-man syndrome and Anti-insulin receptor antibodies [(10)].

2.2.3.8 Other genetic syndromes sometimes associated with diabetes

Many genetic syndromes are accompanied by an increased incidence of diabetes. These include the chromosomal abnormalities of Down syndrome, Klinefelter syndrome, Turner syndrome, Wolfram syndrome, Friedreich

ataxia, Huntington chorea, Laurence-Moon-Biedl syndrome, Myotonic dystrophy, Porphyria and Prader-Willi syndrome [10].

2.2.4 Gestational diabetes mellitus

Gestational diabetes mellitus has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Usually disappears after delivery. Mostly affects women with familial predisposition to diabetes and/or are overweight. They are at increased risk of later developing type 2 diabetes in life. It produces few symptoms and is usually discovered by routine screening [10].

Although most cases resolve with delivery, the definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy [10].

2.3 Diagnosis of DM

In 2003, the American Diabetes Association's Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [11] approved new criteria for the diagnosis of DM.

Criteria of the diagnosis:

1. A casual plasma glucose level of ≥200 mg/dl (11.1 mmol/l) when the symptoms of diabetes are present (Classic symptoms of diabetes include polydipsia, polyuria and unexplained weight loss). Casual is defined as any time of day without regard to time since last meal.
2. A fasting plasma glucose level of ≥126 mg/dl (7.0 mmol/l). The normal fasting plasma glucose level is now defined as <110 mg/dl. Fasting is defined as no caloric intake for at least 8 hours.

3. An oral glucose tolerance test (OGTT) value in the blood of ≥200 mg/dl when measured at the two-hour interval. In normal glucose tolerance test, blood glucose <140 mg/dl after 2 hours using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

These criteria are expected to lead to a further increase in the number of patients diagnosed as having DM. Included in the current classification system are the terms “impaired glucose tolerance (IGT) “ and " impaired fasting glucose (IFG) ", which refer to a metabolic stage between the stages of normal glucose homeostasis and DM .

A person with impaired fasting glucose (IFG) has fasting plasma glucose levels of (110 mg/dl ≤ IFG < 126 mg/dl). In impaired glucose tolerance (IGT), the 2 hours blood glucose is (140 mg/dl ≤ IGT ≤ 199 mg/dl).

Because early detection and prompt treatment may reduce the burden and complications of type 2 DM, the American Diabetes Association recommends that all people older than age 45 years be screened every three years, and that screening should be earlier and more frequent in high-risk people, including those with previously identified IGT or IFG [(12)].

2.4 Glycemic Control and Hb_{A1c}

Glycated hemoglobin (Hb_{A1c}) is a marker of evaluation of long-term glycemic control in diabetic patients and predicts risks for the development and/or progression of diabetic complications. Glycosylation process depends on the exposure to glucose, so on the half-life of erythrocyte. Hb_{A1c} is the product of non-enzymatic reaction between glucose and free beta terminal valines amino groups of hemoglobin (Figure 1) [(13)].

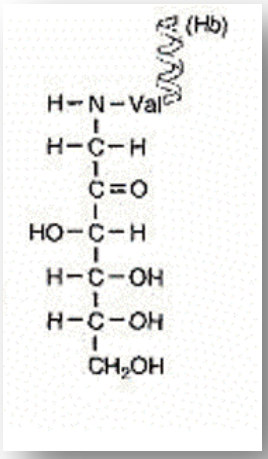


Figure 1: structure of glycated hemoglobin (Hb_{A1c}).

Concentration of Hb_{A1c} is an indicator of average blood glucose concentration over the preceding 2- 3 months. Hb_{A1c} is currently considered the best index of metabolic control for diabetic patients in clinical setting and participants in epidemiological studies as well as a measure of risk for the development of micro and macro vascular complications [(13)].

Criteria of evaluation of the metabolic control for (the value of) Hb_{A1c} were defined as:

- Hb_{A1c} < 6.3%: very good glycemic control.
- Hb_{A1c} between 6.3 and 7.1%: good glycemic control.
- Hb_{A1c} between 7.1 and 9%: poor glycemic control.
- Hb_{A1c} > 9%: bad glycemic control.

Protective values of Hb_{A1c} to chronic complications are considered < 7% [(13)].

An International Expert Committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the Hb_{A1c} test to diagnose diabetes, with a threshold of 6.5%, and ADA affirms this decision [(14)].

2.5 The methods of estimation the reducing sugar content in solution in clinical laboratories

Enzymatic method is the most commonly used in clinical laboratories for glucose estimation. Glucose is commonly measured using an enzyme to convert the glucose to a product that can be easily detected. Common enzymes used are glucose oxidase, glucose dehydrogenase and hexokinase [(15)].

2.5.1 Glucose oxidase (GO)

It requires both water and oxygen to oxidise glucose to gluconic acid and hydrogen peroxide. This first reaction step needs the cofactor flavin adenine dinucleotide (FAD). Oxygen consumption is measured by polarographic methods or the production of hydrogen peroxide which detected by chromogenic methods. The different chromogens (C) used (e.g., o-dianisidine, p-aminophenazone/phenol) act as hydrogen donors (Cred), which are oxidised (Cox) by H2O2 from the glucose reaction, with the colour change being proportional to the glucose concentration [(15)]. However, the peroxidase coupling reaction used in the glucose oxidase method is subject to positive and negative interference. Increased levels of uric acid, bilirubin, and ascorbic acid can cause falsely decreased values as a result of these substances being oxidized by peroxidase, which then prevents the oxidation and detection of the chromogen. An oxygen consumption electrode can be used to perform the direct measurement of oxygen by the polarographic technique, which avoids this interference (Figure 2) [(15)].

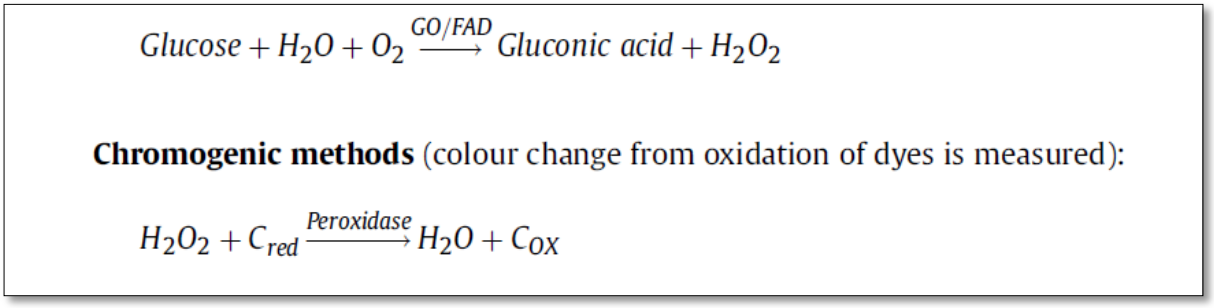


Figure 2: principles of glucose oxidase method.

2.5.2 Glucose dehydrogenase (GDH)

It requires the cofactor NAD, pyrrolochinolin-chinone (PQQ) or FAD as first electron. The resulting NADH could be measured directly or used for a chromogenic reaction (Figure 3) [15].

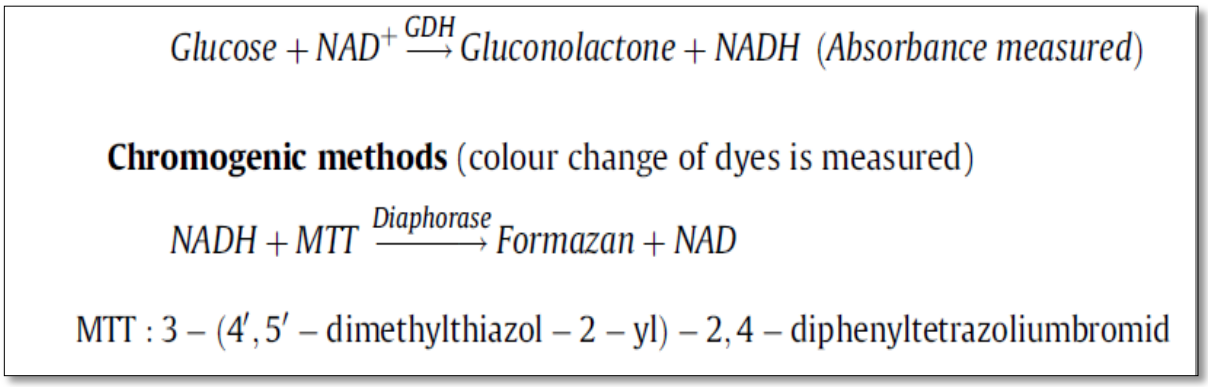


Figure 3: principle of glucose dehydrogenase method.

It's less specific than glucose oxidase or the combination of hexokinase / glucose-6-phosphate dehydrogenase. GDH also reacts with mannose, maltose, xylose, glucosamine and 2-desoxyglucose. Systems using GDH-based methods are, in general, less prone to interferences from exogenous substances and blood oxygen content [15].

2.5.3 Hexokinase (Hk)

It turns glucose into glucose-6-phosphate and also reacts, for example, with fructose, mannose and glucosamine. It is only the second step that yields the necessary specificity for glucose by using glucose-6-phosphate dehydrogenase to oxidase the intermediate to the final product, gluconate-6-phosphate. In addition to the two enzymes, the complete reaction requires ATP and NADP (Figure 4) [15] .

$$Glucose + ATP \xrightarrow{HK} Glucose - 6 - P + ADP$$

$$Glucose - 6 - P + NADP^{+} \xrightarrow{Glc\text{-}6\text{-}p\text{-}DH} Gluconat - 6 - P + NADPH + H^{+}$$

Figure 4: principle of hexokinase method.

2.6 The alterations in salivary compositions and excretions with different diseases

The main salivary glands are in the form of pairs and include the parotid, the submandibular and the sublingual glands. These glands secrete saliva into the oral cavity. The secreted saliva is composed of a combination of serous and mucosal components. A total amount of 1-1.5L of non-stimulated saliva is produced daily by these glands. The share of each gland in secretion is different, with the submandibular glands having 65%, parotid 23% and sublingual glands having 4% of the share; the minor salivary glands secrete only a small amount 8% of saliva [(16)].

Several factors affect the flow of secretion and the physiological characteristics of saliva. These factors include the circadian rhythm and physical activity [(17)]. Saliva is a clear, relatively acidic liquid (pH=6.0-7.0) containing electrolytes, immunoglobulins, proteins and different enzymes that play important roles in oral health. The primary roles of saliva include protecting the integrity of mucosal membrane, lubrication, buffering and cleaning the oral cavity, antimicrobial effects, gustatory sense and primary digestion of food [(16)]

The advent of new sensitive technologies such as molecular diagnostic techniques and nanotechnology has facilitated the analysis of salivary proteins and peptides that may be used as diagnostic biomarkers [(18)].

Analysis of saliva may be useful for the diagnosis of infectious diseases, autoimmune diseases, malignancy, hereditary disorders and endocrine disorders, as well as in the assessment of therapeutic levels of drugs [(19)].

A reduction in salivary epithelial growth factor (EGF) in patients with high frequency of RAU has been found [(20)]. This marker has also been measured in patients with RAU and Behcet's disease and the results, similar to the above-mentioned study, showed a decrease in the level of this factor [(21)].

In one study, a significant increase in salivary nitrates in patients with oral candidiasis was observed [22]. Also, a reduction in antimicrobial proteins in the saliva causes an increase in oral candidiasis [23]. The salivary concentration of IL10, INF-G and INF-K in patients with erosive lichen planus was found to be high. The concentration of these markers decreased significantly after treatment with prednisone [24, 25].

Saliva has also been used for evaluation of BMS. The amount of Nerve Growth Factor (NGF), substance P and products of degranulation of mast cells and neutrophils in the saliva was measured in the control and BMS patients. The results showed a significant increase in NGF and mast cell products, a significant decrease in substance P and no change in neutrophil markers. Thus, these factors can be used for diagnosis and monitoring of BMS [26].

Certain proteome changes in the saliva indicate dental caries. For example, proteins with high amounts of proline (PRP1 and PRP3), histatin1 and statin decrease in high-caries patients while the level of these proteins is high in caries-free individuals. Histatin S and Statin are useful predictors for caries [18, 27, 28]. Comparison of the salivary proteome of patients with invasive periodontitis and healthy subjects revealed that eleven salivary proteins such as alpha amylase, albumin and carbonic anhydrase 6 were different [18].

Some systematic diseases affect the salivary glands either directly or indirectly and thus, change the components of the secreted saliva. Therefore, it is possible to use such changes for early diagnosis, determination of severity and even prognosis of diseases.

Saliva has also been used for detection of renal diseases. Some studies measured the salivary creatinine level and predicted the renal disease with high sensitivity and specificity [29].

Sialochemistry has been useful in diagnosis of Sjögern's syndrome. The results of saliva analysis in people with this disorder have shown an increase in

inflammatory proteins, H-microglobulin, lactoferrin, lysozyme C, sodium, chloride, IgA, IgG, albumin and cystatins and decrease in high proline containing proteins and amylase. Also, there is a reduction in the saliva flow in Sjögern's syndrome; which is not a pathognomonic feature of this syndrome but will cause oral symptoms like caries, fungal infections, dysphagia and oral pains [30, 31].

In Celiac disease, the results of several studies indicate that the amount of IgA Antigliadin Antibody (IgA-AGA) is elevated in the serum of these patients and this increase is in perfect alignment with salivary IgA Antigliadin Antibody. Therefore, it is possible to use this factor with a good sensitivity and specificity for screening of these patients. Some studies have even suggested the use of this test before performing intestinal villi biopsy [27, 32, 33].

Breast cancer was explored for the presence of proteomic signatures in the saliva. Streckfus et al. [34] has reported that c-erbB-2 protein and CA 15-3 levels are elevated in cancer respect to control saliva.

Wu et al. [35] explored the expression of saliva proteins in patients affected by gastric cancer. Other studies detected a significant increase in different makers like IL-6 and IL-8 and also salivary glutathione of patients with oropharyngeal squamous cell carcinoma (SCC) compared with healthy controls [36, 37].

Collection of saliva facilitates determination of hormonal level and diurnal variation [38]. The amount of salivary cortisol in some studies indicated the level of serum cortisol. Based on the literature, the morning concentration of salivary cortisol can be used for the diagnosis of Addison's disease and its nocturnal concentration can be used for diagnosis of Cushing's disease [39-42].

Regarding Diabetes Mellitus, Salivary dysfunction is one of the most dental complications that may contribute to disturbance of oral health state. A lot of studies were carried out on saliva composition and excretion in order to use it as a diagnostic fluid.

Salivary dysfunction has been reported in patients with DM [43, 44]. A cross sectional epidemiological study was conducted in 2003 to look at the prevalence of hyposalivation and xerostomia (dry mouth) and to determine the relationship between salivary dysfunction and diabetes complications. This study was conducted in type 1 diabetics and control subjects without diabetes. They found that symptoms of reduced salivary flow rate and xerstomia were more frequently reported by patients with DM than the controls, especially by those diabetics who had developed neuropathy [45].

Saliva composition has been reported to be altered in diabetic patients. Significant concentrations of glucose were reported in saliva in children IDDM [46]. In diabetics in relation to healthy individuals was different; in diabetic urea and potassium level increased and sodium level decreased [47]. Also, glucose, urea and total proteins were found greater in diabetic children than controls, while calcium values were decreased in such a study [48].

The salivary samples of IDDM patients showed significantly lower salivary flow rate, pH value, and calcium concentration when compared with control group, while total proteins and potassium concentration were significantly higher. The plaque indexes (PI), gingival index (GI) and calculus index (CI) were significantly higher in IDDM Patients [49].

Recent study confirmed that the glucose concentration in saliva is higher in diabetic patients than in control subjects. It also confirmed that in normal subjects and diabetic patients, the salivary flow is higher in stimulated as compared to unstimulated saliva [50]. Another study also revealed that salivary glucose concentration (SGC) was significantly higher in diabetic patients. The total salivary flow was significantly reduced in diabetic patients [51].

One of the researches reported that glucose could only be detected in the salivary samples with DM whereas none of the salivary samples from the non-diabetic control subjects showed the presence of glucose [52].

Many researchers conducted studies about relation of saliva glucose with glycemia in the blood. Jurysta et al. in 2009 [50] found no significant correlation in the diabetic patients between glycemia and SGC. Vasconcelos et al. in 2010 [51] concluded that there was no correlation between salivary and blood glucose concentrations in diabetic patients. Also, Vaziri et al. in 2010 [53] did not observe any relation between FBS and SGC.

In contrast, this conflict with Amer et al. in 2001 [52] in which they revealed positive significant relation between SGC and FBS in diabetic patients. In 2003, López et al. [48] have supported the significant relation between SGC and FBS. In 2012, Panchbhai [54] revealed that significant positive correlation between salivary glucose levels and the fasting blood glucose levels was seen in group with uncontrolled diabetes. In 2013, Agrawal et al. [55] revealed that a correlation was observed between SGC and FBS of diabetic as well as non-diabetic subjects.

2.7 Oral Manifestations of DM

There is a plethora of oral manifestations in patients with DM, many related to the degree of glycemic control. Mucosal conditions include oral dysesthesia, including burning mouth, altered wound healing, increased incidence of infection and candidal infections (particularly acute pseudomembranous candidiasis of the tongue, buccal mucosa, and gingiva). Xerostomia and Sialosis are often related to poor glycemic control [56].

Sialosis is defined as asymptomatic, non-inflammatory, non-neoplastic, bilateral chronic diffuse swelling mainly affecting the parotid glands. Sialosis has been found to be more prevalent in patients with DM [57].

Medications taken by DM patients for related or unrelated systemic conditions may produce salivary hypofunction. Thus, the xerostomia seen in these patients may result more from medications than from the diabetic

condition itself. Dry mucosal surfaces caused by insufficient salivary output are easily irritated, causing minor mucosal ulcerations, oral burning sensations, and increased likelihood of overgrowth of fungal organisms.

Neuropathy of the autonomic system can also cause changes in salivary secretion since salivary flow is controlled by sympathetic and parasympathetic pathways.

The high incidence and severity of dental caries in diabetic mellitus patients have been associated with xerostomia, increased gingival crevicular fluid glucose levels, and increase in dental plaque accumulation [(56)].

DM is also an established risk factor for the prevalence and severity of gingivitis and periodontitis. Depending on the level of glycemic control, DM promotes gingival inflammation in response to bacterial plaque to a greater extent than seen in well-controlled DM or non-diabetic individuals. Hyperglycemia increases glucose levels in gingival crevicular fluid. This may alter periodontal wound healing significantly by changing the interaction between cells and their extracellular matrix within the periodontium. Vascular changes of poorly controlled DM patients also occur in the periodontium; hence, progressive destructive periodontitis is more common in patients with poor glycemic control. In addition to hyperglycemia, poor oral hygiene and smoking contribute to the increased incidence and severity of periodontitis in DM [(56)].

2.8 Role of the Dentists in the diagnosis and monitoring of diabetic patients

It is worth mentioning that, DM is one of the diseases that disturb the oral health state by implication of its complications. Dentists can detect undiagnosed patients and monitoring the glycemic control for the known diabetic patients that may enhance the proper oral health maintenance.

Well-controlled diabetics can be treated in the dental office similarly to non-diabetic patients, but morning appointments are preferable, and patients should be instructed not to fast, in order to reduce the risk of the occurrence of hypoglycemia [58].

A DM patient's response to dental treatment depends on factors that are specific to each individual. These include glycemic control, concomitant medical problems, diet, oral hygiene, and habits such as excessive use of alcohol and tobacco [56].

It is imperative that dentists have an updated assessment of each patient's level of glycemic control prior to initiating treatment and maintain a close working relationship with the patient's physician. A patient presenting with signs and symptoms of undiagnosed or poorly controlled DM should be referred to a physician for diagnosis and treatment [56].

Oral health practitioners should also be familiar with the common tests used to diagnose and monitor DM. They should have in-office glucose monitoring devices or glucometers to readily obtain immediate information about glycemic status if needed [56].

The important steps in treating the dental patient with DM is determining the type of DM, methods of treatment (diet, oral hypoglycemics, insulin, a combination of these), level of glycemic control, and presence of DM complications. Diabetes-associated medical complications require assessment by the patient's physician since they could impact the provision of dental care [56].

Chapter 3

AIMS OF THE STUDY

AIMS OF THE STUDY

The present study was conducted to:

1. Evaluate the presence of glucose in whole unstimulated saliva in diabetic and healthy groups of Libyan population.

2. Assess the possibility of using salivary glucose concentration in unstimulated whole saliva in diabetic patients as a non-invasive monitoring tool reflecting the glycemic control instead of using glycated hemoglobin in blood.

Chapter 4

SUBJECTS AND METHODS

SUBJECTS AND METHODS

4.1 Study design

This is a Case-Control study.

4.2 Study setting

The study was conducted at Benghazi Diabetic Center.

4.3 Study period

This study was conducted during the period starting from February 2010 until the end of the year 2010.

4.4 Ethical considerations

Approval was taken from the director of Benghazi Diabetic Center. After explanation to each participant the nature of the study work, written informed consent was taken (Appendix A).

4.5 Tools of the study

Case record sheet used to register the clinical data of each participant which include name, age, gender, medical history, type of diabetic treatment and duration of the DM as well as clinical biochemistry data such as Hb_{A1c}, SGC and FBS (Appendix B).

4.6 Patients' selection

Fifty two (52) diabetic patients were selected by disproportional stratified random sampling method. The sample design consists of two equal strata depending on gender (26 male and 26 female). Each stratum divided into two equal subgroups depending on type of therapy (insulin and oral hypoglycemic agents). Each Final subgroup composed of 13 patients.

Patients excluded only if they have impairment in motor and cognitive skills.

4.7 Control healthy group selection

Twenty five (25) healthy non diabetic individuals of both sexes were selected randomly.

4.8 Materials and Equipments:

4.8.1 (Cobas c 111) Roche.co device

Hb_{A1c} level was determined by using of hemolyzing reagent (Tina-quant Hemoglobin A1c Gen.2 – Whole blood and Hemolysate Application) and (Cobas c 111) Roche.co device (Figure 5) [(59)].

The Hb_{A1c} determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

Reagent Constituents:

- Sample and addition of R1 (buffer/antibody)

  Hb_{A1c} in the sample reacts with anti-Hb_{A1c} antibody to form soluble antigen-antibody complexes [(59)].

- Addition of R2 (buffer/polyhapten)

  The polyhaptens react with excess anti-Hb_{A1c} antibodies to form an insoluble antibody-polyhapten complex which can be measured turbidimetrically [(59)].

Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during (sample + R1) of the above immunological reaction [(59)].

The final result is expressed as percent $Hb_{A1c} = (Hb_{A1c} / Hb) \times 100$ [(59)].

Note: Whole blood sample was taken without centrifugation for Hb_{A1c}.

Figure 5: (Cobas c 111) Roche.co.

4.8.2 Beckman Glucose Analyzer II device

The samples of saliva and blood were centrifuged in the lab at speed of 3000 rpm for 5 minutes in order to get clear saliva and serum (Figure 6). Serum and saliva were assayed by the use of Beckman Glucose Analyzer II (Figure 7).

It uses the measurement of the rate of oxygen depletion during the oxidation of glucose to gluconic acid by glucose oxidase. The rate of change is proportional to the glucose concentration [(60)].

GLUKAR reagent was used in Beckman Glucose Analyzer II to determine glucose concentration by an oxygen rate method employing a Beckman Coulter Oxygen Electrode (Figure 8) [(61)]. Sensitivity of oxygen rate method was found even at levels as low as 10 mg/dl [(62)].

- Reagent Constituents [(60)]

A. Bottle 1

Glucose Oxidise >75 U/mL
Denatured Ethanol 5%
Potassium Iodide 10 mmol/L
Preservative and other ingredients.

B. Bottle 2

Glucose standard: 150 mg/dl for calibration.

10 micro liters (1µL) is injected in a reaction cup containing a glucose oxidase solution (Figure 9). The ratio used is one part sample to 76 parts reagent (765 µL) [(62)].

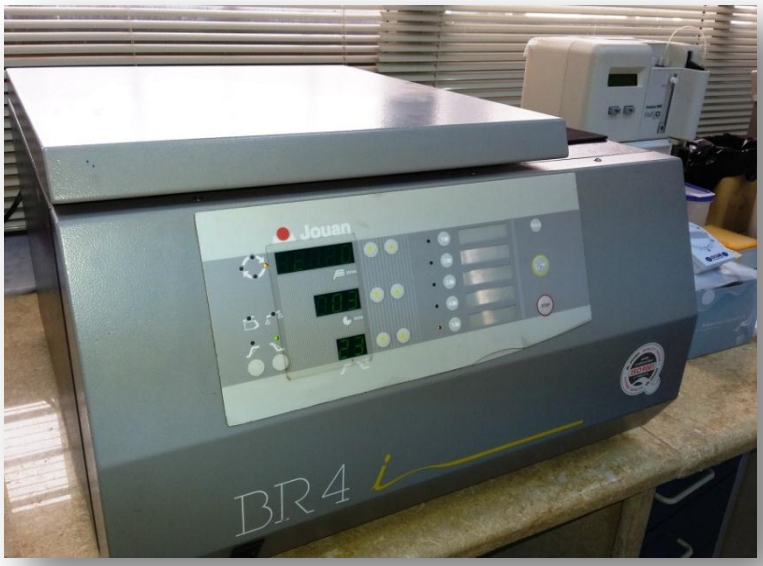


Figure 6: Centrifuging machine for blood and saliva samples.

Figure 7: Beckman Glucose Analyzer II.

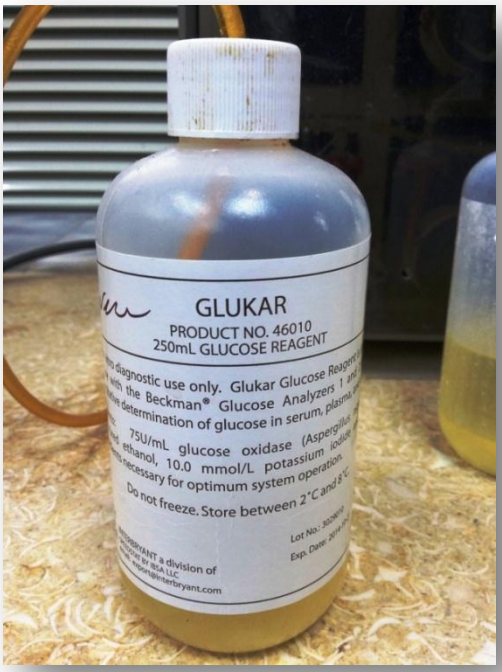
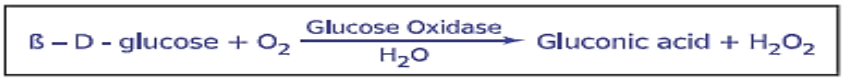


Figure 8: GLUKAR reagent for blood and saliva glucose determination.

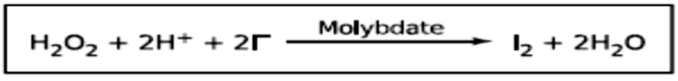
Figure 9: A precise volume of sample 10 micro liters (10µL) is injected in a reaction cup containing a glucose oxidase solution.

Oxygen is consumed at the same rate as glucose reacts to form gluconic acid [62].



Because oxygen consumption rather than peroxide formation is measured, the only requirement for peroxide is that it must be destroyed by a path not leading back to oxygen. The addition of ethanol to the reagent causes peroxide to be destroyed in the presence of catalase without yielding oxygen, according to the following reaction [62]:

To ensure complete destruction of the peroxide, iodide and molybdate are added to the enzyme reagent. This reaction is effective even after the catalase activity has diminished with length of storage, as shown in the following [62]:



Every O2 molecule is detected by an oxygen electrode sensor coupled to an electronic system and it corresponding to glucose molecule [61]. Calibration is performed with aqueous glucose standards. Glucose standard (150 mg/dl glucose) was measured after every 10 unknown samples [60].

4.8.3 Plain test tubes

These tubes are used for collection of saliva samples by participants (Figure 10).

Figure 10: Plain test tubes used for collecting of saliva sample by the participants.

4.8.4 Special tubes for blood sampling

Specialized tubes for blood sampling provided from the laboratory (Figure 11).

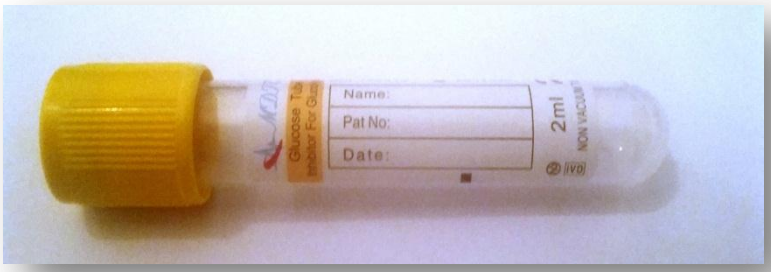


Figure 11: specialized tubes used for blood sampling.

4.9 Steps of clinical procedures

- All participants were given appointments for collection of samples at 8:30 a.m.
- The following instructions were given for all participants:

1. They must be fasting 8 hours before coming to the appointment.
2. The brushing of the teeth was ordered from all participants the night before coming.
3. Do not use dental brush, floss or mouth wash in the day of sample collection.
4. The participant may rinse with water before saliva collection.

- On sitting in the dental chair, the subjects were asked to bend their heads forward and after an initial swallow, allow saliva flowing into the floor of the mouth. Subjects will expectorate the saliva collected in the floor of the mouth into a test tube (Figure 12).
- During collecting the sample of saliva, the subject should be seated in a quiet room, not to cough or clear the throat into the collection tube.
- A sample of 1 cc unstimulated saliva was collected and blood samples were taken from each participant in the same visit.
- The samples collected were transferred to the lab for analyses after collecting immediately.
- Laboratory technicians have done all the analyses of samples collected.

Figure 12: The way of collecting saliva sample in the test tube by the participant.

4.10 Statistical Analysis

All collected data were fed to PC and checked. Statistic Package for Social Science version 18 (SPSS) was used for analysis of data.

Clinical data were presented as charts, scatter plots, histograms, or tables. All variables were assessed for normality of distribution, using skewness and kurtosis. (means ± standard deviations) of parametric variables were calculated.

Independent samples t-test was used for the comparison between means of the same variables presented in two independent normally distributed samples.

Pearson correlation coefficient (r) analysis and Regression line drawn on the scatter plot diagram were used to determine possible associations between variables. Pearson correlation coefficient (r) values were considered to be significant when $p<0.05$. One-way analysis of variance (ANOVA) and F statistic and t-test were applied for parametric data to evaluate the strength of regression equation.

Chapter 5

RESULTS

RESULTS

5.1 Descriptive characteristics of the groups of study

The groups of study composed of two main groups; diabetic and healthy control. The control group was composed of 16 females and 9 males that were selected randomly. Demographic data such as ages for both groups, and medical history of diabetic group were presented in tables 1 and 2 and figure 13.

Table 1: Descriptive statistics of ages of diabetic and healthy control group.

Age	N	Minimum	Maximum	Median	Mean	Std. Deviation
Diabetic	52	23	75	56.5	53.52	11.487
Healthy	25	12	76	35	37.96	18.798

Table 2: Descriptive statistics of duration of the disease in diabetic group in years.

	N	Minimum	Maximum	Median	Mean	Std. Deviation
Duration	52	.30	25.00	6	7.8904	5.98322
Valid N (listwise)	52					

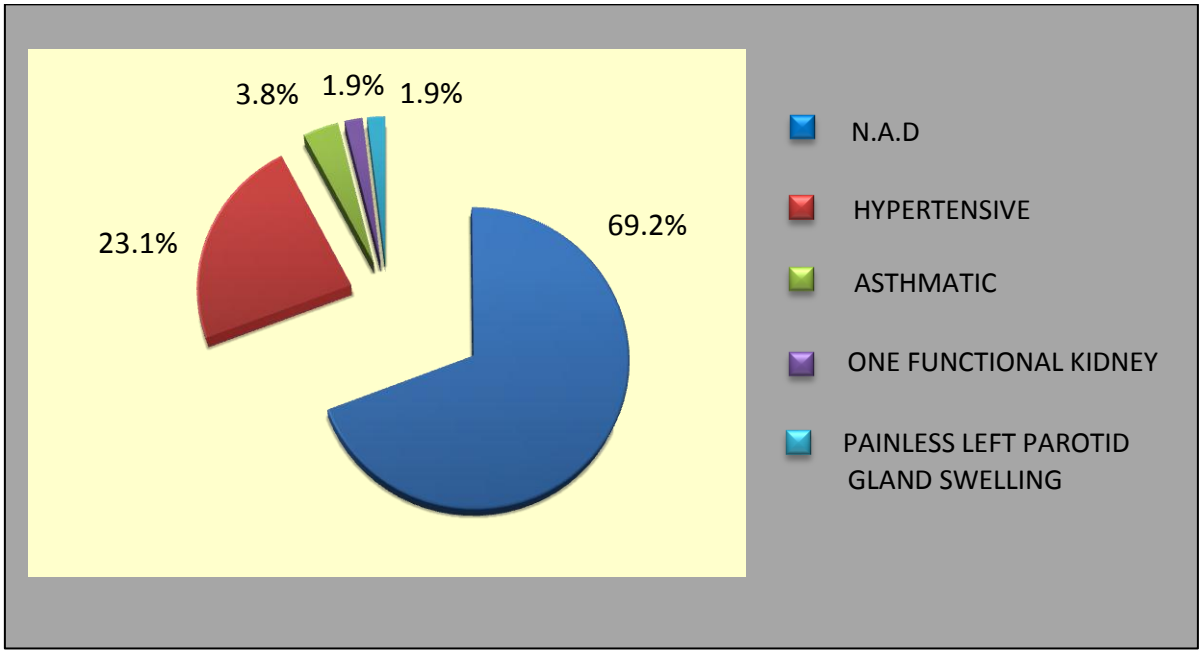


Figure 13: Summary of medical history of diabetic patients.

5.2 Checking the normality of distribution of the data (Hb_{A1c}, SGC and FBS) for diabetic and control groups

In this study, a visual inspection of their histograms and scatter plots showed that Hb_{A1c}, SGC and FBS were approximately normally distributed for both groups as shown in figures 14-25. All the data in both groups were approximately normally distributed in terms of skewness tests which presented in table 3. Since skewness values were less than one, data would be equally distributed [(63)]. Also in terms of Skewness and Kurtosis Z-value, we can assume that our data are approximately normally distriuted. Z-value can be measured by dividing Skewness and Kurtosis on their (Std.Error) which should be somewhere between -1.96 and +1.96, i.e. our data are little skewed and kurtotic.

Table 3: Descriptive statistics of Hb_{A1C}, SGC and FBS for diabetic and control groups.

sample		Hb_{A1c}	SGC	FBS
Diabetic	N	52	52	52
	Mean	8.7038	17.1346	142.7308
	Std. Deviation	1.66686	4.69054	46.58036
	Std. Error of Mean	.23115	.65046	6.45953
	Minimum	6.20	10.00	80.00
	Maximum	12.00	31.00	270.00
	Kurtosis	-.988-	.756	.362
	Std. Error of Kurtosis	.650	.650	.650
	Skewness	.388	.826	.971
	Std. Error of Skewness	.330	.330	.330
Healthy	N	25	25	25
	Mean	5.7000	14.0800	80.8400
	Std. Deviation	.38079	1.63095	4.97226
	Std. Error of Mean	.07616	.32619	.99445
	Minimum	5.00	11.00	73.00
	Maximum	6.40	17.00	91.00
	Kurtosis	-.406-	-.691-	-.718-
	Std. Error of Kurtosis	.902	.902	.902
	Skewness	-.266-	.049	.325
	Std. Error of Skewness	.464	.464	.464

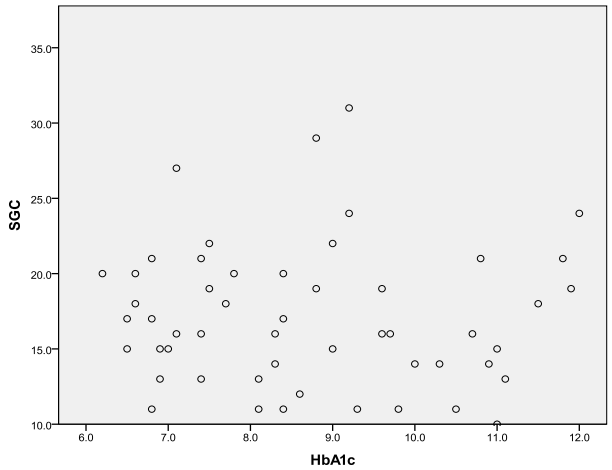


Figure 14: Presentations of data of SGC and Hb_{A1c} for diabetic group in scatter plot diagram.

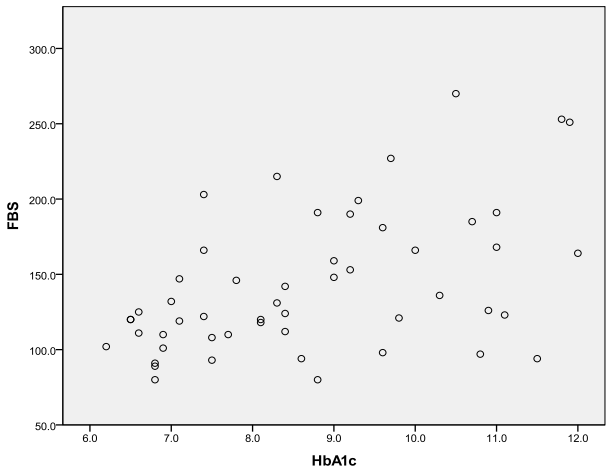


Figure 15: Presentations of data of FBS and Hb_{A1c} for diabetic group in scatter plot diagram.

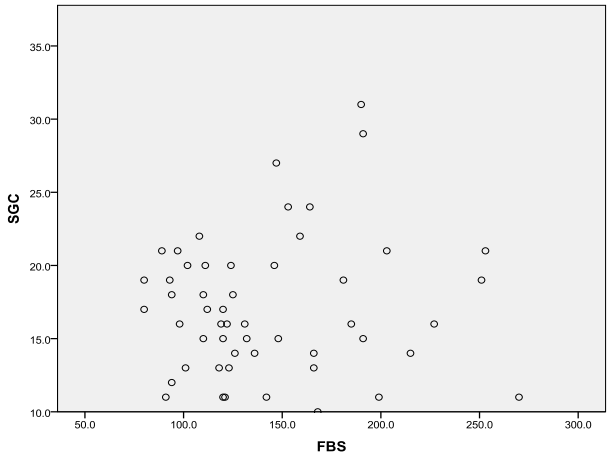


Figure 16: Presentations of data of SGC and FBS for diabetic group in scatter plot diagram.

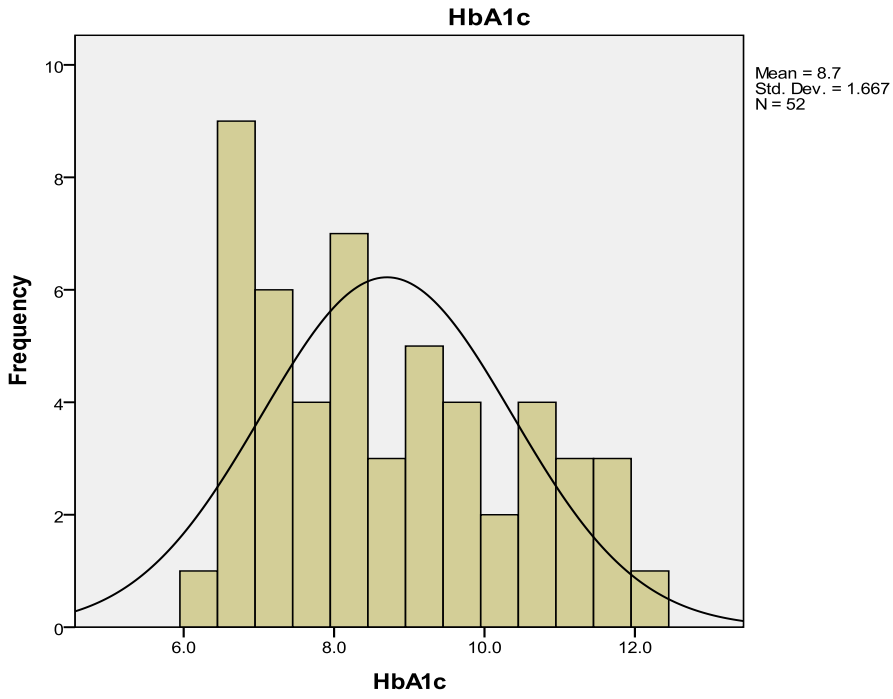


Figure 17: The distribution of Hb_{A1c} data obtained from diabetic patients.

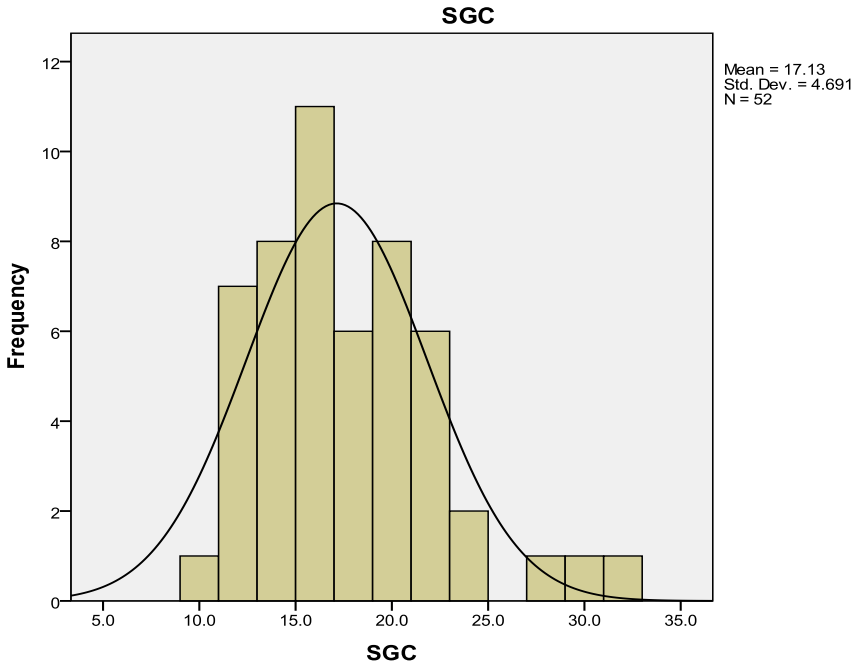


Figure 18: The distribution of SGC data obtained from diabetic patients.

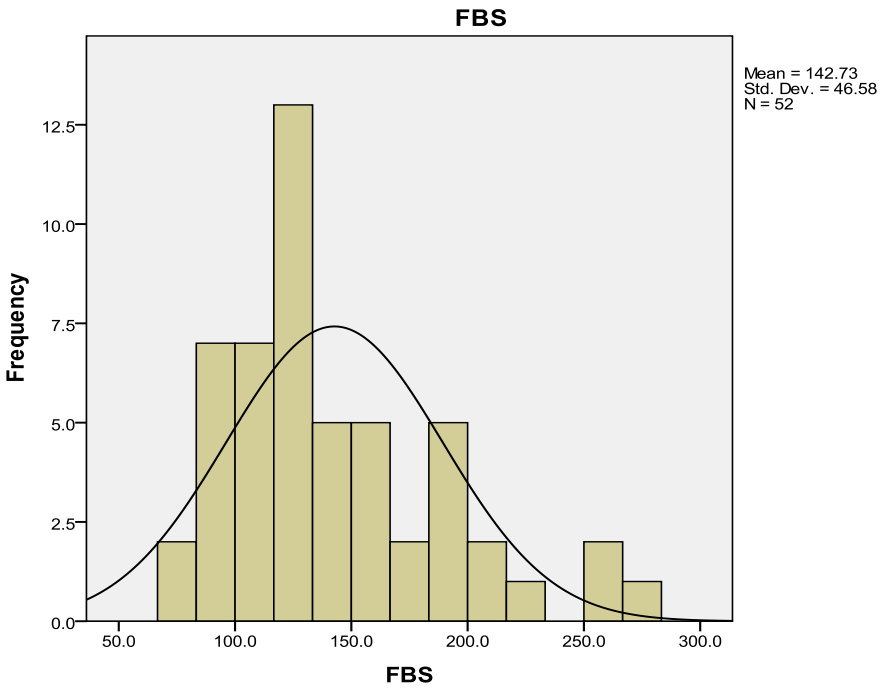


Figure 19: The distribution of FBS data obtained from diabetic patients.

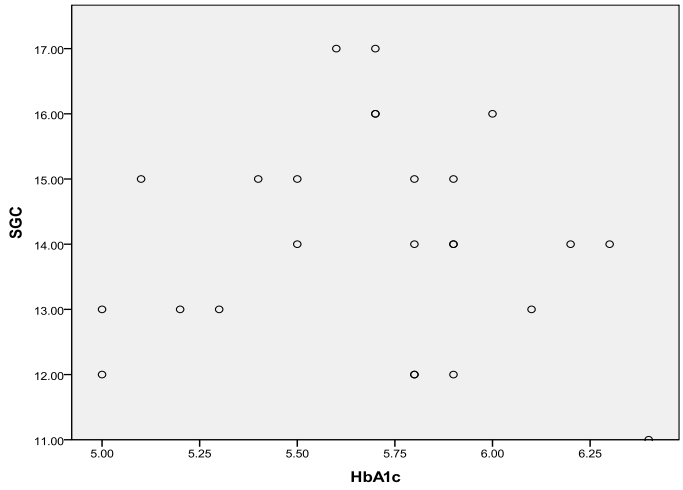


Figure 20: Presentations of data of SGC and Hb_{A1c} for control healthy group in scatter plot diagram.

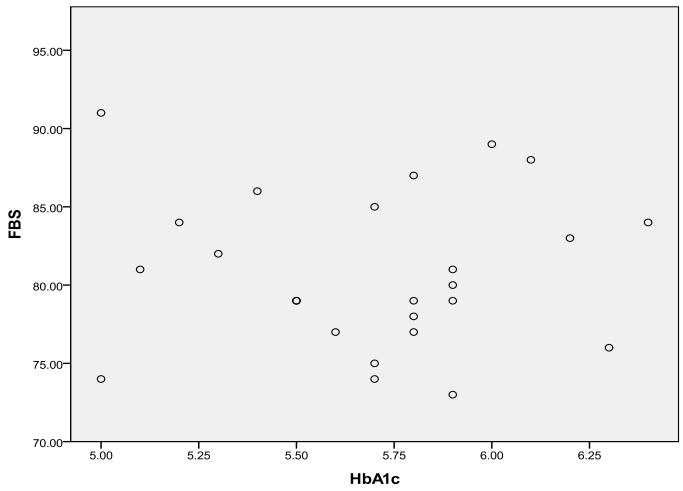


Figure 21: Presentations of data of FBS and Hb_{A1c} for control healthy group in scatter plot diagram.

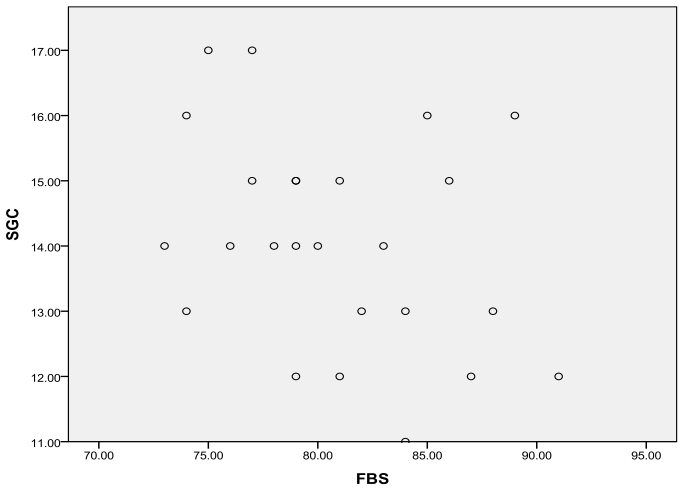


Figure 22: Presentations of data of SGC and FBS for control healthy group in scatter plot diagram.

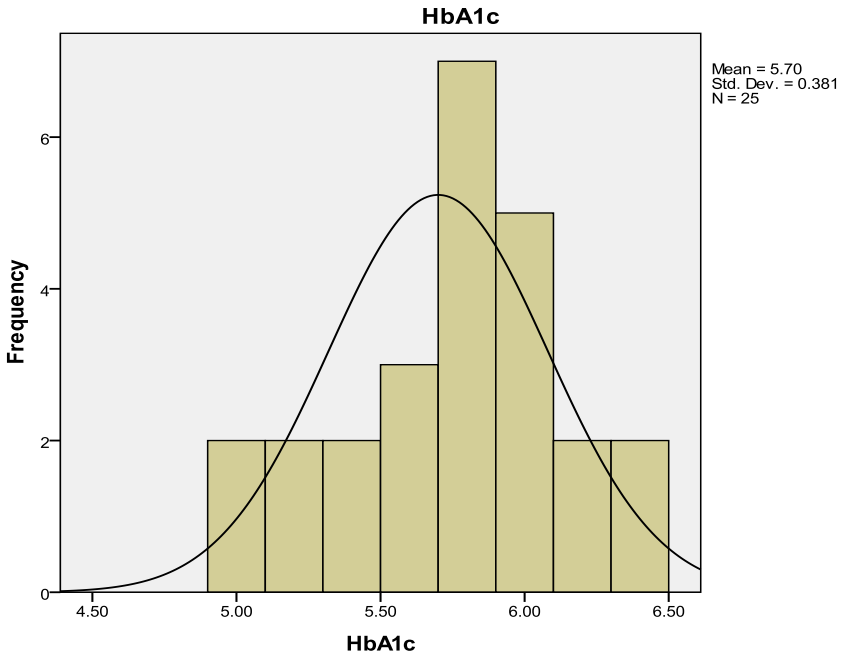


Figure 23: The distribution of Hb_{A1c} data obtained from control healthy group.

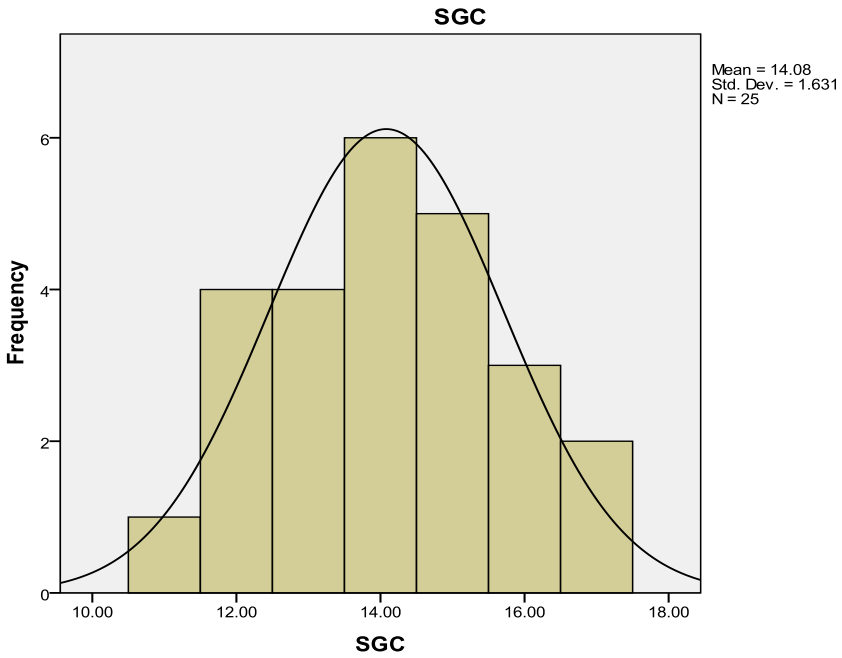


Figure 24: The distribution of SGC data obtained from control healthy group.

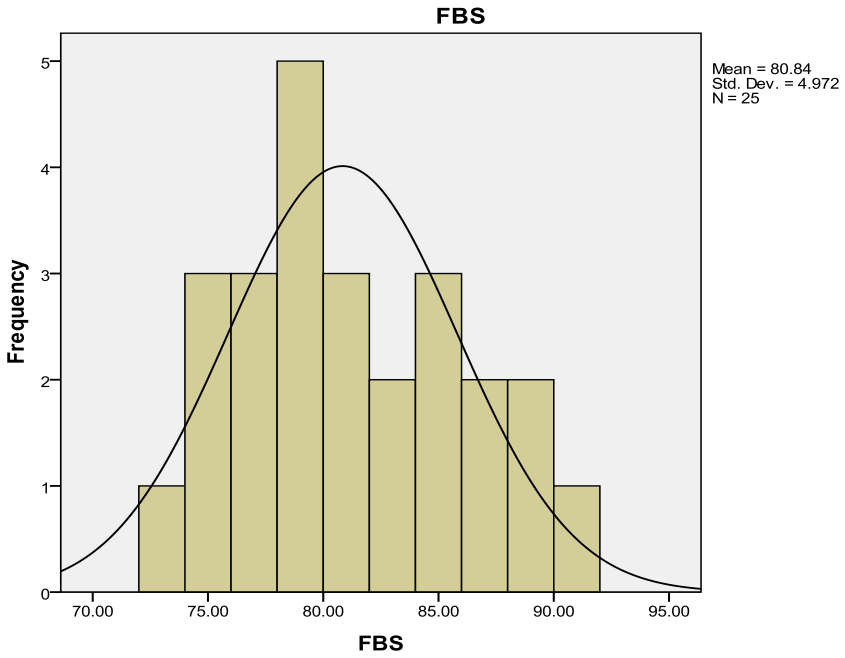


Figure 25: The distribution of FBS data obtained from control healthy group.

5.3 The comparison between means of SGC in diabetic and control healthy groups

An independent samples t-test was conducted to examine whether there was a significant difference in mean SGC between diabetic and control healthy groups. Because of Levene's Test was verified no equality of variances in the samples ($P<0.05$), we'll take into account the bottom raw results of table 5. The test revealed statistically significant difference between diabetic and healthy groups ($t=4.198$, $p=0.000$) (Table 5). The average SGC levels (17.14 ± 4.69) in diabetic group reported significantly higher levels than non-diabetic group (14.08 ± 1.63), (Table 4 and Figure 26).

Table 4: Mean of SGC in both diabetic and control healthy groups.

	study groups	N	Mean	Std. Deviation	Std. Error Mean
SGC	**Diabetic**	52	17.1346	4.69054	.65046
	healthy	25	14.0800	1.63095	.32619

Table 5: Independent samples t-test for the comparison of mean SGC in both groups.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
SGC	Equal variances assumed	16.758	.000	3.156	75	.002 ***	3.05462	.96776	1.12674	4.98249
	Equal variances not assumed			4.198	70.413	.000 ***	3.05462	.72767	1.60348	4.50575

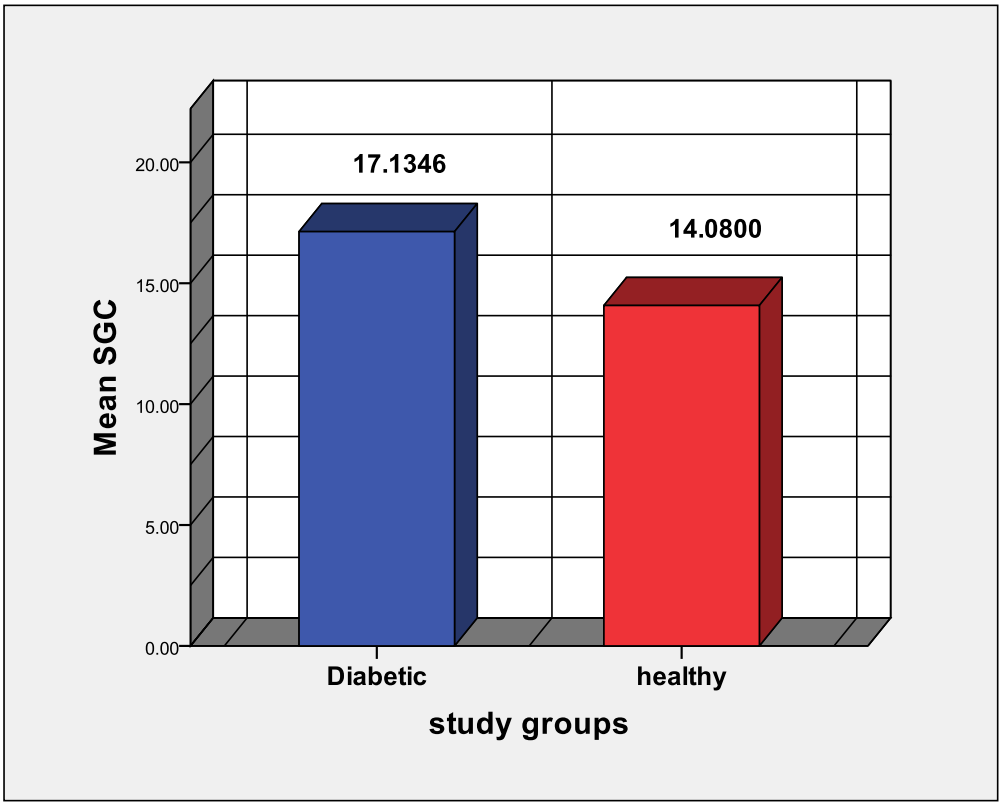


Figure 26: The comparison between mean SGC in diabetic and control healthy groups.

In addition, within diabetic group, independent samples t-test was conducted to compare mean SGC in many subgroups including males and females, patients on insulin and oral hypoglycemic, patients aged less than or equal 60 and over 60 years, and patients that have onset of diabetes since 10 years with those over 10 years, respectively (Tables 6-13 and Figures 27-30). The tests revealed no statistically significant differences in mean SGC ($p>0.05$) in all these subgroups within diabetic group since p-value equals (0.579, 0.430, 0.140 and 0.827), respectively.

Table 6: Mean of SGC in males and females within diabetic group.

Diabetic group		N	Mean	Std. Deviation	Std. Error Mean
SGC	male	26	17.5000	4.74342	.93026
	female	26	16.7692	4.70155	.92205

Table 7: Independent samples t-test for the comparison of mean SGC between males and females within diabetic group.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
SGC	Equal variances assumed	.035	.852	.558	50	.579	.73077	1.30979	-1.90003-	3.36157
	Equal variances not assumed			.558	49.996	.579	.73077	1.30979	-1.90004-	3.36157

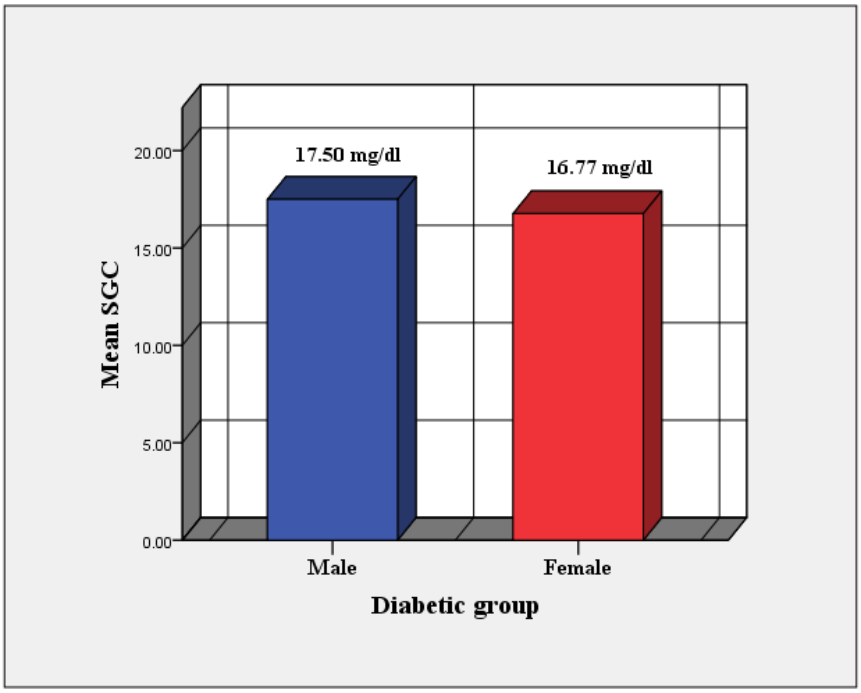


Figure 27: The comparison of mean SGC between males and females within diabetic group.

Table 8: Mean of SGC in patients under insulin therapy and oral hypoglycemic agents within diabetic group.

	Diabetic group	N	Mean	Std. Deviation	Std. Error Mean
SGC	Insulin	26	16.6154	4.31812	.84685
	oral hypoglycemic	26	17.6538	5.06709	.99374

Table 9: Independent samples t-test for the comparison of mean SGC between patients under insulin therapy and oral hypoglycemic agents within diabetic group.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
SGC	Equal variances assumed	.100	.753	-.795-	50	.430	-1.03846-	1.30563	-3.66090-	1.58398
	Equal variances not assumed			-.795-	48.773	.430	-1.03846-	1.30563	-3.66254-	1.58561

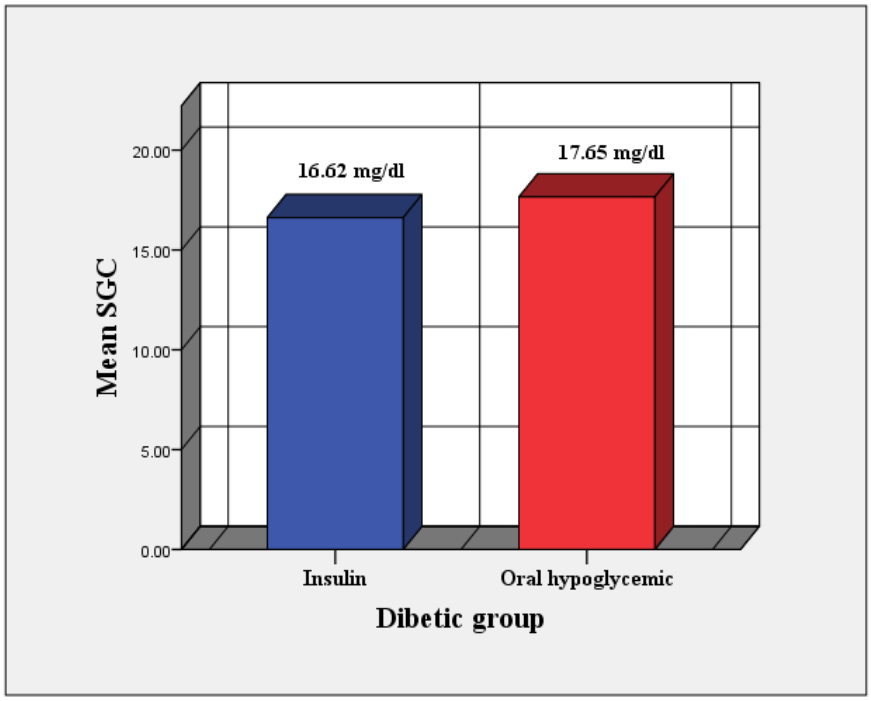


Figure 28: The comparison of mean SGC between patients under insulin therapy and oral hypoglycemic agents within diabetic group.

Table 10: Mean of SGC of patients aged less/equal 60 years and over 60 years within diabetic group.

	sample	N	Mean	Std. Deviation	Std. Error Mean
SGC	up to 60 years	36	17.7778	4.96336	.82723
	over 60 years	16	15.6875	3.75444	.93861

Table 11: Independent samples t-test for the comparison of mean SGC between patients aged less/equal 60 years and over 60 years within diabetic group.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
SGC	Equal variances assumed	.877	.354	1.501	50	.140	2.09028	1.39232	-.70628-	4.88683
	Equal variances not assumed			1.671	37.624	.103	2.09028	1.25112	-.44331-	4.62386

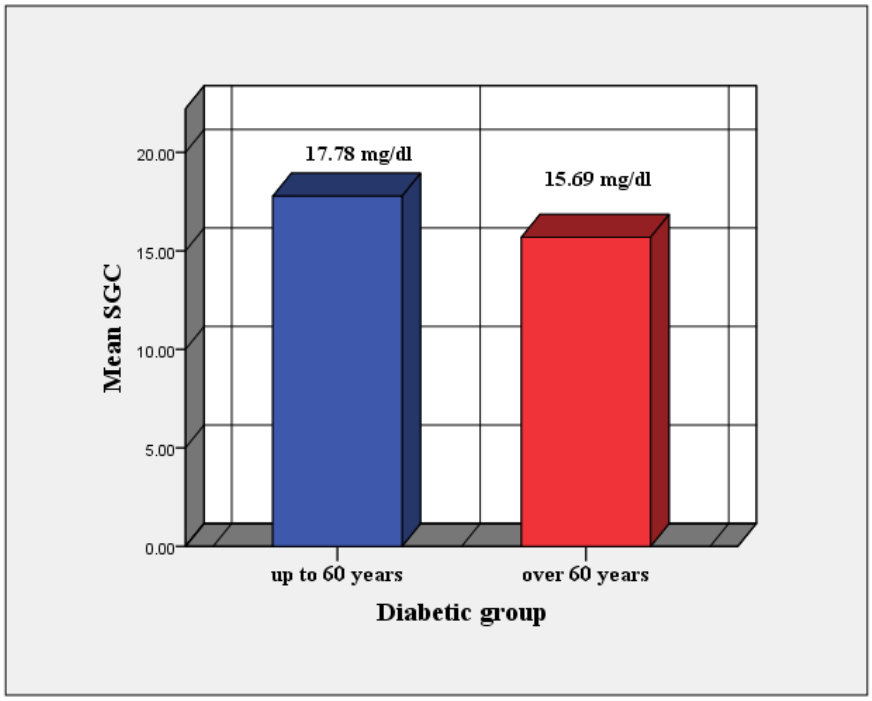


Figure 29: The comparison of mean SGC between patients aged less/equal 60 years and over 60 years within diabetic group.

Table 12: Mean of SGC patients that have onset of diabetes since 10 years and those over 10 years within diabetic group.

	sample	N	Mean	Std. Deviation	Std. Error Mean
SGC	up to 10 years	39	17.0513	4.66199	.74652
	over 10 years	13	17.3846	4.95880	1.37532

Table 13: Independent samples t-test for the comparison of mean SGC between patients that have onset of diabetes since 10 years and those over 10 years within diabetic group.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
SGC	Equal variances assumed	.697	.408	-.220	50	.827	-.33333	1.51639	-3.37909	2.71242
	Equal variances not assumed			-.213	19.576	.834	-.33333	1.56487	-3.60212	2.93546

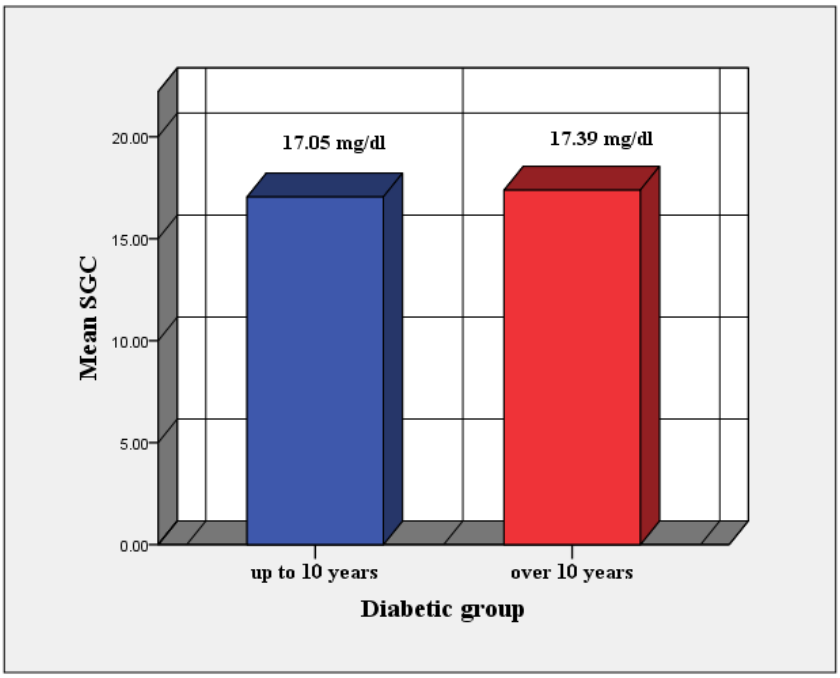


Figure 30: The comparison of mean SGC between patients that have onset of diabetes since 10 years and those over 10 years within diabetic group.

5.4 The relationships between (Hb_{A1c}, SGC and FBS) in both diabetic and healthy groups by use of Pearson's r correlation coefficient test and regression analysis

5.4.1 The relationship between SGC and Hb_{A1c}

The null hypothesis is that the SGC and Hb_{A1c} are not linearly related. By the use of Pearson's r correlation coefficient, r = - 0.031, p (0. 825) and r = - 0.054, p (0.799) for diabetic and control groups, respectively, as presented in tables 14 and 15. Regression lines were drawn on scatter diagram relating SGC with Hb_{A1c} data in diabetic and control healthy groups, respectively, as shown in figures 31 and 32. Because of (p>0.05), we cannot reject the null hypothesis. Hence, there is no evidence of significant relation between them in both groups.

Table 14: The correlation coefficient between (SGC- Hb_{A1c}) in diabetic group.

		Hb_{A1c}	**SGC**
SGC	**Pearson Correlation**	-.031-	
	Sig. (2-tailed)	.825	
	N	52	52

Table 15: The correlation coefficient in between (SGC- Hb_{A1c}) control healthy group.

		Hb_{A1c}	**SGC**
SGC	**Pearson Correlation**	-.054-	
	Sig. (2-tailed)	.799	
	N	25	25

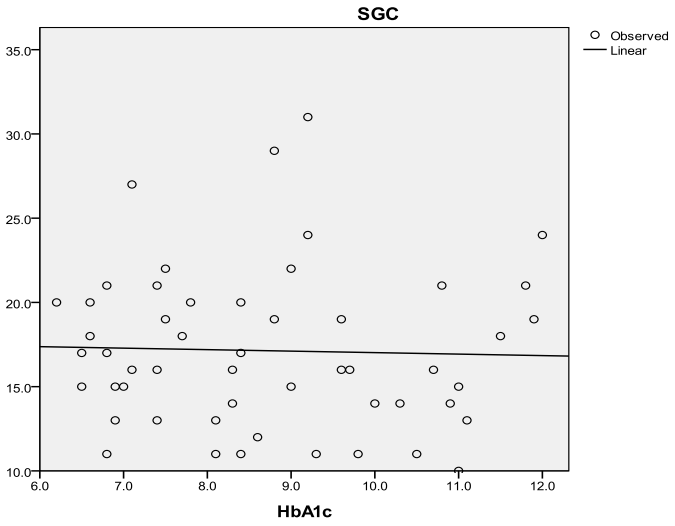


Figure 31: Regression line drawn on scatter diagram relating SGC with Hb_{A1c} data in diabetic group.

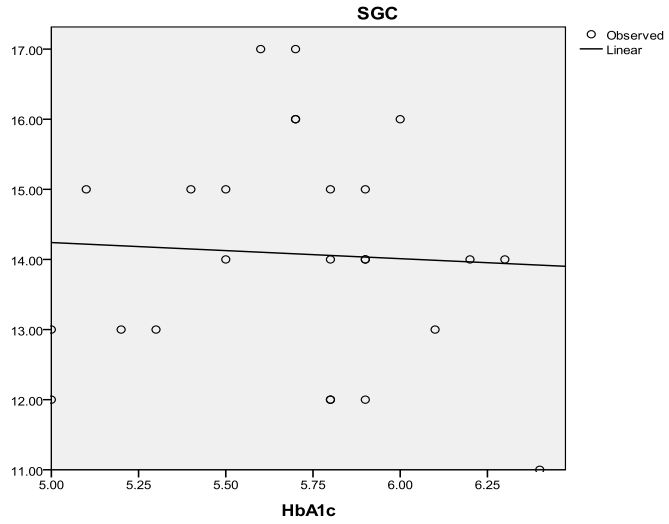


Figure 32: Regression line drawn on scatter diagram relating SGC with Hb_{A1c} data in control healthy group.

5.4.2 The relationship between SGC and FBS

Similarly, the null hypothesis here is that the SGC and FBS are not linearly related. The Pearson's r correlation coefficient was measured between SGC and FBS and revealed that r = 0.088, (p=0.534) and r = - 0.327, (p=0.110) for diabetic and control groups, respectively, as presented in tables 16 and 17. Regression lines were drawn on scatter diagram relating SGC with Hb_{A1c} data of diabetic and control healthy groups, respectively, as shown in figures 33 and 34. The null hypothesis could not be rejected because of (p>0.05) and there is no evidence of significant correlation in both groups.

Table 16: The correlation coefficient between (SGC-FBS) in diabetic group.

		SGC	FBS
SGC	Pearson Correlation		.088
	Sig. (2-tailed)		.534
	N	52	52

Table 17: The correlation coefficient between (SGC-FBS) in control healthy group.

		SGC	FBS
SGC	Pearson Correlation		-.327-
	Sig. (2-tailed)		.110
	N	25	25

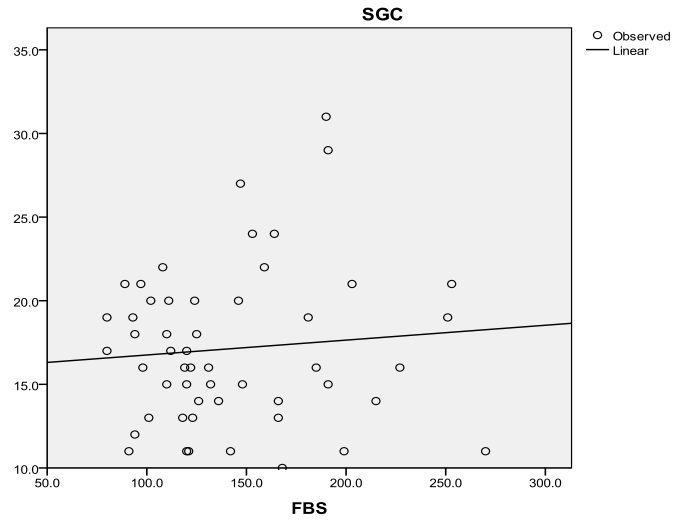


Figure 33: Regression line drawn on scatter diagram relating SGC with FBS data in diabetic group.

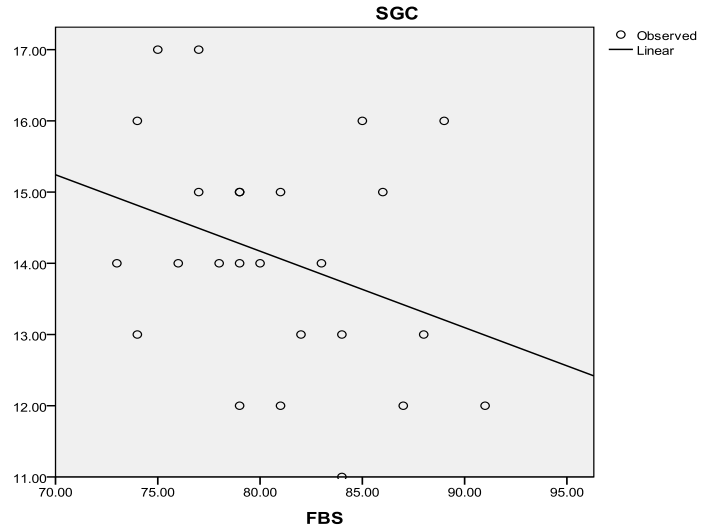


Figure 34: Regression line drawn on scatter diagram relating SGC with FBS data in control healthy group.

5.4.3 The relationship between FBS and Hb_{A1c}:

The null hypothesis here is that the FBS and Hb_{A1c} are not linearly related. The Pearson's r correlation coefficient between FBS and Hb_{A1c} in diabetic and control groups revealed that r = 0.492, (p=0.000) and r = - 0.033, (p=0.876), respectively, as presented in tables 18 and 19. Regression lines were drawn on scatter diagram relating SGC with Hb_{A1c} data in diabetic and control healthy groups, respectively, as shown in figures 35 and 36.

In term of Pearson's r test, FBS and Hb_{A1c} had a moderate positive significant correlation in diabetic group ($p<0.05$), so the null hypothesis was rejected. In control group, we cannot reject the null hypothesis and there was no evidence of significant correlation ($p>0.05$).

Table 18: The correlation coefficient between (FBS- Hb_{A1c}) in diabetic group.

		FBS	Hb_{A1c}
FBS	**Pearson Correlation**		.492
	Sig. (2-tailed)		.000 ***
	N	52	52

Table 19: The correlation coefficient between (FBS- Hb_{A1c}) in control healthy group.

		FBS	Hb_{A1c}
FBS	**Pearson Correlation**		-.033-
	Sig. (2-tailed)		.876
	N	25	25

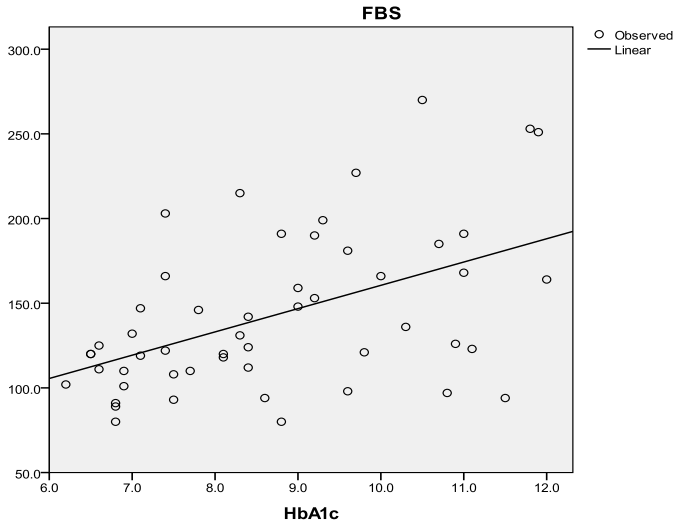


Figure 35: Regression line drawn on scatter diagram relating FBS with Hb_{A1c} data in diabetic group.

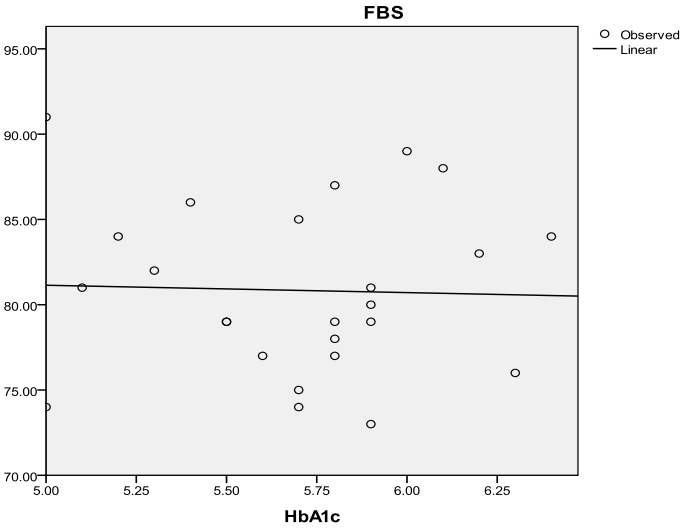


Figure 36: Regression line drawn on scatter diagram relating FBS with -Hb_{A1c} data in control healthy group.

A significant regression equation was found in diabetic group regarding the correlation between FBS and Hb_{A1c}. R^2 (coefficient of determination) was measured (R^2=0.242) to evaluate the strength of regression equation and (Adjusted R^2=0.227) was measured (Table 20). R^2 range should be ($0 \leq R^2 \leq 1$).

Due to R^2 (0.242) is small, we can use ANOVA F-test or t-test to evaluate the usefulness of regression equation for predicting FBS on Hb_{A1c}. Since the computed p value equals (0.000) for both t-test and F test as presented in tables 21 and 22, the null hypothesis was rejected. From table 22, a regression equation formula was derived as ($y_{dependent}$ (FBS) =13.751× $x_{independent}$ (Hb_{A1c}) +23.041) for predicting FBS from a given known value of Hb_{A1c} and vice versa.

Table 20: The coefficient of determination R^2 (FBS-Hb_{A1c}) in diabetic group.

R	R Square	Adjusted R Square	Std. Error of the Estimate
.492	.242	.227	40.954
The independent variable is Hb_{A1c}.			

Table 21: ANOVA table for simple linear regression of FBS and Hb_{A1c} in diabetic group**.**

	Sum of Squares	df	Mean Square	F	Sig.
Regression	26795.220	1	26795.220	15.976	.000 ***
Residual	83861.011	50	1677.220		
Total	110656.231	51			
The independent variable is Hb_{A1c}.					

Table 22: T-test Statistic one way for (FBS-Hb_{A1c}) regression equation in diabetic group.

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
Hb_{A1c}	13.751	3.440	.492	3.997	.000 ***
(Constant)	23.041	30.479		.756	.453

Chapter 6

DISCUSSION

DISCUSSION

The goal of achieving new techniques by using saliva to evaluate glycemic control in diabetic patients is saving time and creating a comfortable way instead of needle puncture to measure glycemic control which may lead to future development of such devices using saliva in dental and medical clinics.

Hb_{A1c} is used for the measurement of glycemic control. Hb_{A1c} is currently considered as the best index of metabolic control for diabetic patients in clinical setting and participants in epidemiological studies as well as a measure of risk for the development of micro and macro vascular complications [(13)].

Saliva plays an important role in the maintenance of oral health state by its contributing as antibacterial, antiviral, buffering action, digestion, taste, and lubrication and repair of oral mucosa. Frequent monitoring of DM through blood sampling was inconvenient to the patients due to accompanied pain during the procedure. Researchers had confirmed that some organic and inorganic components of saliva are modified in DM (glucose, lipids, proteins and electrolytes) [(64)].

The present study is an observational case-control study and it was undertaken with the aim of suggesting saliva as a diagnostic fluid to measure glycemic control instead of using blood in diabetic patients at Benghazi Diabetes center.

The level of Hb_{A1c}, FBS and SGC were determined in 52 diabetic patients and 25 randomly healthy control individuals. We used a disproportional stratified random sampling method for selecting diabetic patients. This type of sample selection was mandatory to ensure representation of subgroups of interest or desired elements, including gender and each type of therapy. Since exact proportions of these elements in our study population were unknown,

equal proportions of numbers selected to provide a desired balance of representation in the study [65].

In the present study, all salivary samples in both diabetic and healthy groups reflected presence of glucose in saliva with SGC means equal (17.14±4.69) mg/dl and (14.08±1.63) mg/dl, respectively, and this conflict with Amer et al. [52] in which they reported the SGC level was only detected in diabetic patients.

Our study rely on the oxygen rate method (Sensitivity range ≥ 10mg/dl) with the use of glucose oxidase (Glukar reagent) to measure glucose levels in both blood and saliva samples which was capable of glucose detection in lower concentrations even in non-diabetics. Mean SGC in Healthy control group in our study ranged from 11 mg/dl to 17 mg/dl which can be easily detected by using this method.

Amer et al. [52] have used a method that was not sensitive in case of lower glucose level, but greater level of the glucose (Sensitivity range > 20mg/dl). Therefore, it is suitable for glucose detection in blood but not in saliva [66] that makes the oxygen rate method used more specific and sensitive.

In this study, the average of SGC level in diabetic group was higher than non-diabetic subjects and this difference was statistically significant (p=0.000) by using independent samples t-test as also reported by many studies [46, 48, 50, 51, 54, 66-68].

Belazi et al. [46] showed that the glucose levels in the unstimulated whole saliva as well as in serum of the IDDM group were higher than in healthy subjects (P<0.05). López et al. [48] showed diabetic saliva glucose values were higher than in controls. Jurysta et al. [50] confirmed that the glucose concentration in saliva is higher in diabetic patients than in control subjects. It extends this knowledge to both unstimulated and stimulated saliva. Panchbhai [54] demonstrated that the significantly higher mean SGL was found in group with diabetes compared to healthy non-diabetic group. Mahdavi et al. [66] had shown

that the average of SGC level in diabetic group was statistically higher than non-diabetic subjects (p=0.0001). Lasisi and Fasanmade [67] proved that mean salivary glucose levels was significantly elevated (p=0.002) in diabetic subjects when compared with non-diabetic subjects. Also, Aydin [68] has shown that salivary glucose was significantly higher in obese diabetic subjects than in controls (p< 0.05); and salivary glucose levels in non-obese diabetic subjects were also significantly higher than those of control. Conversely, Vaziri et al. [53] found no significant differences in salivary glucose concentrations between type 1 and healthy control group 1 (p=0.88) as well as type 2 diabetic patients and healthy control group 2 (p=0.19).

This may be explained that glucose is a small molecule could easily diffuse through semipermeable membranes and DM is often associated with increased basement membrane permeability, which could be attributed to the increased passage of molecules from serum into exocrine glands secretions and through gingival crevicular fluids [51, 69-71].

By dividing the diabetic group in our study into subgroups, the salivary glucose concentration (SGC) failed to differ significantly (p>0.05) in mean by using independent samples t-test between males and females (p=o.579), patients on insulin and oral hypoglycemic (0.430), patients aged less than or equal 60 years and over 60 years (p=0.140), and patients that have onset of diabetes since 10 years with those over 10 years (p=0.827). Therefore, we can consider that there were no intra-group differences in mean SGC with change of gender, type of therapy, age of the patients and duration of the disease.

In agreement with our study, Gupta et al. [72] did not find any significant differene of SGC with change of gender in diabetic group (p=0.867) and Soares et al. [73] did likewise. Nevertheless, Darwazeh et al. [74] found higher levels of salivary glucose in males as compared to females.

Gupta et al. [72] proved no significant effect on mean SGC with change of age (p=0.439), Darwazeh et al. [74] and Sashikumar and Kannan [75] did not find a statistically significant effect of age on SGC levels in their studies in diabetic group.

Regarding to the effect of duration of diabetes on salivary glucose levels, our results revealed no significant difference in mean SGC that were similar to the findings reported by Gupta et al. [72] and Darwazeh et al. [74].

Overall, there were rich data of studies on the estimation of glucose in saliva of both diabetic and healthy and a lot of researchers have studied the correlation between FBS and SGC. But, few studies have been found concerning SGC and its relation with glycemic control (Hb_{A1c}).

As shown in our results, irrespective of periodontitis, the salivary glucose concentration (SGC) had no significant relation with Hb_{A1c} level by the use of Pearson's test, r = - 0.031, (p=0.825) and r = - 0.054, (p=0.799) for diabetic and control groups, respectively, that contrast with the aim of using SGC instead of Hb_{A1c}. In line with our results, Sashikumar and Kannan [75] did not find a correlation between both SGC and $Hb_{A1c.}$ Thus, we have substantiated their findings.

Our findings were in contradiction with recent reports. Mahdavi et al. [66] study showed a strong correlation between Hb_{A1C} and SGC in diabetic group r = 0.516, (p=0.0001) and no significant correlation in non-diabetic r = - 0.112, (p=0.454). Gupta et al. [72] revealed that a significant correlation was found in SGC and HbA1c levels in diabetic subjects (P<0.001). Abhikshyeet et al. [76] also reported a significant correlation between HbA1c levels and SGC. The criteria of exclusion of diabetics in these studies was based on excluding any associated diseases, smoking, radiotherapy and non-diabetics drugs may have a role in the discrepancies with our result.

Because of obtaining of FBS levels as an extra routine analysis for all participants at the same time when SGC and Hb_{A1C} were measured, we've attempted proving correlations of FBS with other parameters.

Our results have revealed that no significant relation between serum glucose (FBS) and salivary glucose concentration (SGC), r = 0.088, (p=0.534) and r = - 0.327, (p=0.110) for diabetic and control groups, respectively. These results are in agreement with other studies [50, 51, 53]. On the other hand, some studies were conflicted with these results [48, 52, 54, 55].

In our study, a moderate positive correlation between FBS and Hb_{A1c} in diabetic group was found with r = 0.492, (p=0.000) and (R^2 = 0.242). It was confirmed by using ANOVA F-test and t-test as p value ($p<0.05$). From adjusted R^2, (22.7%) of Hb_{A1c} values could estimate or predict FBS values, In contrast, there was no significant correlation in control healthy group since r = - 0.033, (p=0.876), and these results were supported by recent study of Khan et al. in 2015 [77].

There are some limitations associated with the present study should be noted when analyzing the results. The sample size of study groups is not very big that has been restricted by the cost of saliva and blood investigations; especially there were no sources of funding. Thus, availability of sources of funding would give a chance of increasing sample size with taking into account the use of another randomized method would show representation of study population to good advantage.

Secondly, collecting of whole unstimulated saliva regardless of the degree of periodontitis may be associated with increase in glucose concentration from gingival crevicular fluids. More explorations and researches regarding the effect of gingival crevicular fluids on salivary glucose concentration by determining the amount of effect would estimate more accurate levels of glucose molecules in saliva.

Chapter 7

SUMMURY AND CONCLUSION

SUMMARY AND CONCLUSION

The aim of our study is to evaluate the benefit of using salivay glucose concentration instead of Hb_{A1c} in blood for measuring glycemic control.

This case-control study composed of two main groups; fifty two diabetic patients group and twenty five non-diabetic healthy control group. Diabetic subjects were recruited from those patients attending Benghazi Diabetes center. The diabetic patients were selected by stratified sampling method. The sample design consists of two equal strata depending on sex (26 male and 26 female). Each stratum divided into two equal subgroups depending on type of therapy (insulin and oral hypoglycemic agents). Each final subgroup composed of 13 patients that were selected randomly up to the desired number. Patients excluded only if they have impairment in motor and cognitive skills. Non-diabetic subjects were randomly selected.

Informed consent, demographic data and medical history were taken from all participants. All participants were given appointments for collection of samples at 8:30 a.m. They must be fasting 8 hours before coming to the appointment. The brushing of the teeth was ordered from all participants the night before coming. Do not use dental brush, floss or mouth wash in the day of saliva sample collection. The participant may rinse with water before saliva collection.

Serum and saliva samples were collected from all subjects. On sitting in the dental chair, the subjects were asked to bend their heads forward and after an initial swallow, allow saliva flowing into the floor of the mouth. Subjects will expectorate the saliva collected in the floor of the mouth into a test tube.

A sample of 1 cc of whole unstimulated saliva was collected and blood samples were taken from each participant in the same visit to measure SGC,

Hb_{A1c} and FBS. The samples collected were transferred to the lab for analyses after collecting immediately.

The collected data were fed to PC and checked. Statistic package for social science (SPSS), version 18, was used for statistical analysis of data.

All data in both groups were approximately normally distributed in terms of skewness. A visual inspection of their histograms and scatter plots showed that Hb_{A1c}, SGC and FBS were approximately normally distributed for both groups.

Our results proved that all salivary samples of the participants in both groups reflected presence of glucose in saliva and revealed that Hb_{A1c} mean (8.70±1.67 mg/dl), SGC mean (17.14±4.69 mg/dl) and FBS mean (142.731±46.58) for diabetic group. And Hb_{A1c} mean (5.70±0.38 mg/dl), SGC mean (14.08±1.63 mg/dl) and FBS mean (80.84±4.97 mg/dl) for healthy control group.

In terms of Independent Samples Test, the average of SGC (17.14±4.69 mg/dl) level in diabetic group was significantly higher than healthy control group (14.08±1.63 mg/dl), (p=0.002). And within the diabetic group, no differences were found in mean SGC with change of gender, type of therapy, age of the patients and duration of the disease.

Pearson's *r* was used to evaluate the direction of the correlation among the three parametric variables in both groups.

There was no significant relation between SGC and Hb_{A1c} in diabetic and healthy control groups as r =- 0.031, (p=0.825) and r =- 0.054, (p=0.799), respectively. Also there was no significant relation between SGC and FBS in diabetic and healthy control groups as r =.088, (p=0.534) and r =-.327, (p=0.110), respectively.

In contrast, there was only a positive moderate correlation between FBS and Hb_{A1c} in diabetic group, r =.492, (p=0.000), whereas there was no significant relation in control healthy group, r = -.033, (p=0.876). In terms of regression

equations' statistics for diabetic group, there was statistically significant relation between FBS and Hb_{A1c} by the use of ANOVA F test and t-test ($p<0.05$).

In conclusion, the study has reported the following findings:

1. All the salivary samples in both diabetic and healthy groups reflected presence of glucose in saliva and the average of salivary glucose concentration (SGC) levels in diabetic patients was significantly higher than non-diabetic healthy subjects.
2. Changes of gender, type of therapy, age of the patients and duration of the disease have no significant effect on the value of salivary glucose concentration in diabetic patients.
3. The present study cannot support the use of saliva as an indicator for monitoring glycemic control because salivary glucose concentration (SGC) is not directly influenced by change in glycated hemoglobin (Hb_{A1c}) level.

Chapter 8

RECOMMENDATIONS

RECOMMENDATIONS

1. Further researches to be done using cohort study using large sample size of diabetic cases and taking into consideration that some of diabetics may receive antihypertensive drugs which may affect the salivary glands function.

2. Effect of gingival crevicular fluids on salivary glucose concentration required to be more investigated in future.

3. To evaluate the benefits of obvious significant higher levels of glucose in saliva constituents in diabetic patients.

Chapter 9

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APPENDICES

طلب موافقة للإشتراك فى برنامج الدراسة العلمية بعنوان

استخدام تركيز جلوكوز اللعاب كمؤشر لمراقبة
مدى التحكم فى مستوى السكر فى الدم عند مرضى السكرى

فى هذه الدراسة نسعى لإختبار ملائمة استخدام جلوكوز اللعاب عند مرضى السكرى لقياس مدى التحكم فى مستوى السكر فى الدم وذلك كوسيلة أسهل لاستخدامها مستقبلاً كبديل لاستخدام الحقن لسحب الدم وما تخلفه من اثر نفسي وجسدي . ونحن نتكفل بسحب عينة من الدم لقياس سكر الصيام و السكر التراكمي وكذلك أخذ عينة من اللعاب بطريقة سهلة جداً تحت إشراف الطبيب القائم بهذا البحث ، فإذا لم يكن لديكم اى مانع من الاشتراك فى هذه الدراسة نرجو منكم مشكورين كتابة الاسـم و التوقيع بالموافقة على ذلك .

الأســـــــم : ..... رقم البطاقة : .....

التاريخ : ..... التوقيع : .....

University of Benghazi
Faculty of Dentistry
Department of Oral Medicine
Oral Pathology, Diagnosis and Radiology

The use of salivary glucose concentration as an indicator for glycemic control in diabetic patients

(INTERVIEW QUISTIONARE)

Demographic data Date : …. /…. /…….

Diabetic patient Yes ☐ No ☐ Case number: ……..

Name: ……..... Age: …… Sex: ……

Nationality: ……Occupation: …… Tel: …….

- **Reasons for attending dental clinic:**

……...……

……...……

……...

- **General health:**

……...…

……...……

……...……

- **Diabetic control method :**

	Yes	No	Remarks
Diet	☐	☐	……
Excersize	☐	☐	……..…
Others	☐	☐	specify ……..……...……

- **Drug therapy :**

	Yes	No	Remarks
Oral hypoglycemic	☐	☐	……
Insuline	☐	☐	……
Both	☐	☐	……

Dose ……

- **Medical checkup :**

	Yes	No	Remarks
Regular	☐	☐	……
Irregular	☐	☐	……

- **Previous diabetic coma :**

	Yes	No	Remarks
hyperglycemic	☐	☐	……
hypoglycemic	☐	☐	……

Frequency of diabetic coma ……

- **Lab investigations :**

→ Fasting blood sugar = ……. mg/dl

→ Hb_{A1c} = …… %

→ Saliva glucose concentration = ……. mg/dl

Remarks

……

……

……

ARABIC SUMMURY

بعد التأكد من ان متغيرات الدراسة موزعة توزيع طبيعى أظهرت الدراسة النتائج الأتية:

(1) وجود جزيئات الجلوكوز فى عينة اللعاب تزامناً مع وجودها فى عينة الدم عند جميع المشاركين فى الدراسة وكان متوسط كمية جزيئات الجلوكوز فى اللعاب فى مجموعة مرضى السكرى (17.14±4.69 mg/dl) أعلى من مجموعة الغير مصابين بمرض السكرى (14.08±1.63 mg/dl) وذلك لمصلحة مرضى السكرى (p(0.002).

(2) لايوجد اى تأثير على تركيز جزيئات الجلوكوز فى اللعاب مع التغيير فى العمر والجنس ونوع علاج السكرى وطول الفترة الزمنية من تشخيص مرض السكر.

(3) عدم وجود اى علاقة ارتباطية بين تركيز جزيئات الجلوكوز فى اللعاب مع معدل السكر التراكمى وكذلك عدم وجود اى ارتباط مع تركيز الجلوكوز فى الدم أثناء الصيام فى كل من مجموعتى الدراسة ، ولكن تم ظهور علاقة ارتباط ذو قيمة احصائية جيدة أثناء الدراسة بين قيمة التحليل اليومى للسكر أثناء الصيام مع معدل السكر التراكمى لدى مرضى السكرى فقط بمعامل ارتباط بيرسون (r =.492) مع (p (0.000) وقد تم تأكيد هذا الارتباط باستعمال اختبار F (ANOVA) و اختبار T.

- بعد دراسة وتحليل هذه النتائج نستخلص أنه تم اثبات وجود جزيئات الجلوكوز فى اللعاب لكل من مرضى السكرى والأشخاص الذين لا يعانون من مرضى السكرى مع زيادة فى التركيز لصالح مرضى السكرى ، وأنه لايوجد اى ارتباط بين قيمة متغيرات تركيز الجلوكوز فى اللعاب مع قيمة متغيرات معدل السكر التراكمى ولهذا فإن تركيز الجلوكوز فى اللعاب لايمكن أن يستعمل كلينيكياً كبديل لمعدل السكر التراكمى لمراقبة مدى التحكم فى مستوى السكر فى الدم لدى مرضى السكرى.

- **وقد أوصت الدراسة** بالمزيد من العمل على دراسة مدى علاقة تركيز جلوكوز اللعاب مع معدل السكر التراكمى.

بســـم الله الرحمن الرحيم

(الملخص العربي)

استخدام تركيز جلوكوز اللعاب كمؤشر لمراقبة مدى التحكم في مستوى السكر في الدم عند مرضى السكرى

- أجريت هذه الدراسة فى العيادة المركزية لمرضى السكرى (سيدى حسين – بنغازى) لدراسة امكانية استخدام وجود جزيئات الجلوكوز فى اللعاب كبديل لفحص معدل السكر التراكمى نظراً لسهولة استعمال وتجميع اللعاب فى مقابل التعب والألم فى سحب عينة الدم ، حيث استهدف البحث تقييم مدى وجود جزيئات الجلوكوز فى اللعاب فى كل من مجموعة مرضى السكرى والمجموعة المقارنة وكذلك دراسة مدى ارتباط قيمة الجلوكوز فى اللعاب مع قيمة معدل السكر التراكمى لمريض السكرى، اضافةً الى أنه تم فحص كلاً من، مدى تأثر جلوكوز اللعاب مع التحليل اليومى للسكر أثناء الصيام ومدى تأثر قيمة التحليل اليومى للسكر أثناء الصيام مع معدل السكر التراكمى كدراسة جانبية.

- تمت فى هذه الدراسة تسجيل التاريخ الطبى للمشارك ومن ثم سحب عينة الدم من المشارك فى الدراسة وذلك لفحص معدل السكر التراكمى و قيمة السكر أثناء الصيام، وكذلك قام كل مشارك بتجميع كمية من اللعاب فى انبوبة مناسبة.

- تم اختيار 52 مريض من مرضى السكرى الذين يترددون على العيادة وتقسيمهم وفقاً لمتطلبات الدراسة الى مجموعتين بالتساوى حسب الجنس (26 مريض فى كل مجموعة) ، وكل مجموعة قسمت بالتساوى الى مجموعتين (13 مريض فى كل مجموعة) اعتماداً على نوع علاج السكر باستخدام حقن الانسولين أو العلاج بتناول الأدوية بالفم بحيث يتم اختيارهم عشوائياً.

- بالنسبة للأشخاص الغير مصابين بالسكرى والذين لايعانون من اى مشاكل صحية أخرى ، فقد تم اختيار 25 شخص عشوائياً للاستعانة بهم كمجموعة مقارنة للدراسة حيث تم تجميع نفس العينات واجراء نفس التحاليل لكل مشارك فى الدراسة.

المشـرف

أ.د. عزام أحمد سلطان

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كلية طب وجراحة الفم والأسنان

جامعة بنغازى

جامعة بنغازى
كلية طب وجراحة الفم والأسنان
قسـم طب وأمراض الفـم
والتشخيص والأشعة

وزارة التعليم
ليبيا – بنغازى
ادارة الدراسات العليا

استخدام تركيز جلوكوز اللعاب كمؤشر لمراقبة مدى التحكم في مستوى السكر في الدم عند مرضى السكرى

إعداد
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إشراف
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قدمت هذه الرسالةإستكمالاً لمتطلبات درجة الماجستير فى تخصص طب الفم

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