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Cytomorphometric Analysis of Oral Mucosal Epithelium in Type 2 Diabetic Patients

A Thesis
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Dedication

To the pure soul and the kindest hearted man, who will stay forever in my memory… To my beloved , departed father.

To my great mother with love and respect

To my beloved brother

To all my lovely sisters

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Abstract

Background: Diabetes mellitus is an extremely common endocrine metabolic disorder that results in chronic hyperglycemia . It has effects on various tissues of the body. Due to this increased blood glucose levels considerable cellular changes occur in oral cavity as well. Oral exfoliative cytology is a simple, non-invasive clinical technique which can be used to determine the morphology and cytomorphometric changes in exfoliated cells.

Objectives: The aims of the study were to analyze the changes in cytomorphometric measurements of the oral epithelial cells by using exfoliated cytology smears in type 2 diabetic patients and healthy control subjects, to compare the cytoplasmic diameter, nuclear diameter, and nucleus: cytoplasm ratio between type 2 diabetics and healthy control subjects and to assess the oral manifestations in type 2 diabetic patients.

Materials and Methods: The total sample composed of 75 adult, aged 30-60 years. (50 patients with type 2 diabetes mellitus divided into controlled and uncontrolled on the bases of HbA1c levels and 25 non-diabetic healthy persons as a control group).Smears were obtained from (buccal mucosa and lateral border of tongue) from each subject.The freshy obtained specimens streaked on clean dry glass slide and immediately fixed in 96% ethyl alcohol then stained with Papanicolaou method for cytomorphometric analysis. An eyepiece

micrometer was used to measure mean values of nuclear diameter cytoplasmic diameter, and nuclear to cytoplasmic ratio.

Results: The results showed that statistically significant increase in nuclear diameter (*P*=0.001 for buccal mucosa and tongue), with statistically significant decrease in cytoplasmic diameter *(P=0.001* for both tongue and buccal mucosa) were found in diabetic patients compared to controls. Degree of glycemic control significantly affected nuclear diameter (*P*=0.007 for buccal mucosa, P=0.003 for tongue) and nuclear to cytoplasmic ratio (*P*=0.001 for both tongue and buccal mucosa). In general, as the severity of diabetes increased, nuclear diameter and nuclear to cytoplasmic ratio raised gradually. The results displayed no statistical significant difference in the cytoplamsic diameter, nuclear diameter and nuclear cytoplasmic ratio between the two sites. Nuclear diameter and cytoplasmic diameter varied significantly with advancing age. There was no significant variation in either criterion between males and females.

Conclusion: The results associated with clinical observations suggest that type 2 diabetes mellitus can produce definite cytomorphometric changes in oral epithelial cells, detectable by microscopic and cytomorphometric analysis using exfoliative cytology which can be used in diagnosis of oral complications. In addition the main oral manifestations found in type 2 diabetic patients were periodontal disease and oral dryness.

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List of Abbreviations

Abbreviations	Full name
%	Percentage
ADA	American Diabetes Association
ADH	Antidiuretic hormone
BM	Buccal mucosa
BMS	Burning mouth syndrome
CyD	Cytoplasmic diameter
DI	Diabetes Insipidus
DM	Diabetes mellitus
DPX	Distyrene, plasticizer and toluen-xylene
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
HbA	haemoglobin-A
HbA1c	Glycosylated Hemoglobin
Hr	Hour
L	Liter
L/d	Liter Per day
mg/dL	milligrams per decilitre
min	Minute
mmol	millimole
MOH	Ministry of health
N:C ratio	Nuclear to Cytoplasmic ratio
ND	Nuclear diameter
OGTT	Oral glucose tolerance test
PAP	Papanicolaou
PMN	Polymorphonuclear
RBC	Red blood cell
ß-cell	beta cell
T	Tongue
UCD	Uncontrolled diabetes
µg	microgram
µm	micrometer

Introduction

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by inappropriate hyperglycemia, resulting from the failure of the pancreatic beta cells to produce insulin and/or inability of the body to employ the insulin produced because of insulin deficiency in the body cells. In the body, insulin is the only hormone that reduces blood glucose levels while; other hormones such as thyroid hormone, glucagon, growth hormone, catecholamine (epinephrine and norepinephrine) and glucocorticoids all elevate the blood glucose levels (World Health Organization, 2009).

The broad categories of DM are designated as Type1 known as insulin dependent diabetes mellitus (IDDM) and Type 2 known as non-insulin dependent diabetes mellitus (NIDDM). Type 2 DM is a heterogeneous group of disorder usually characterized by insulin resistance, impaired insulin secretion and increase glucose production. Distinct genetic and metabolic defect in insulin action and secretion give rise to common phenotype of hyperglycemia in type2 DM (Braunwald *et al*., 2003; Shoback *et al*., 2011).

The main complications associated with DM are retinopathy, nephropathy and micro/macro angiopathy. It damages tissue repair processes and cause stomatologic problems of dental interest. Several studies suggest a higher prevalence and severity of oral pathologies like gingivitis, periodontitis, candidiasis and other manifestations such as alteration of salivary flow and burning sensation (Alberti, *et al*.,2003).

Oral health plays valuable role in the general health status; the community is precluding from many diseases by an optimum oral health that important not only at oral cavity level but also systemic level of the body (Sultan Meo, 2002).

However, patients with diabetes mellitus are said to expose poorer oral health (Sandberg *et al*., 2000).

A significant indicator of this common condition and possible diabetes complications is metabolic control, which is monitored through the blood glucose level (BGL) and glycosylated hemoglobin level (HbA1c). The glycosylated hemoglobin (HbA1c) reflects average plasma glucose over the previous 2-3 months in a single measure which can be performed at any time of the day and does not require any special preparation such as fasting. (HbAlc) test is currently considered to be the best measure and the gold standard test for assessing glycemic control, In healthy individuals, the proportion of HbA1c ranges between (4% -6%) of total hemoglobin, while in the hyperglycemic patients can reach more . Since the severity of DM and the efficacy of metabolic are control by the HbA1c test which measures the glucose amount that bounds to haemoglobin A1C molecules (Delong & Burkhart, 2008). In 2009, an international committee for the diagnosis of diabetes recommended use of glycated hemoglobin (HbA1c), an index of average plasma glucose over several weeks, as a marker for the disease (American Diabetes Association, 2010) .This recommendation was also made by the American Diabetes Association (ADA) in 2010, which suggested that HbA1c $\geq$ 6.5% (48 mmol/mol) be considered diagnostic of diabetes (Selvin *et al*; 2010*)*. HbA1c is considered equal to fasting plasma glucose (FPG) as a predictor of diabetes (D'Emden *et al*; 2012).

Several studies have examined the deleterious effects of diabetes on oral mucosa. It was reported that diabetes adversely affects the morphology of cheek mucosa, which may compromise tissue function to favour the occurrence of oral infections and neoplasia (Eduardo *et al*.,2004 ; Auluck,2007) .

Oral exfoliative cytology may be more appropriate in condition like DM where the invasive techniques lose viability. The morphologic and functional changes in oral mucosa can be studied at cellular level by using exfoliative cytology which can help in diagnosis with better patient acceptability.

Exfoliative cytology is the study of superficial cells which have been exfoliated from mucous membrane or which have been scraped or pulled from surface. The rationale of exfoliative cytology lies in epithelial physiology. Normal epithelium undergoes continuous exfoliation or shedding of superficial cells, and it is replenished by a new crop of cells from the basal layer. These exfoliated cells are stained by various stains for example Papanicolaou (PAP) according to need. It is painless, non invasive and less time consuming procedure.With the advancement in the field of quantitative exfoliative cytology there has been a reemergence of oral exfoliative cytology as a powerful diagnostic tool. By using cytomorphometric analysis various parameters such as cytoplasm diameter (CyD), nuclear diameter (ND) and nuclear to cytoplasmic ratio (NCR) can be evaluated manually or collectively using image analysis system. CyD, ND, and NCR ratio have shown to be significant in diagnosis of oral and systemic diseases (Nayar and Sundharam, 2003).

Aims of the study

The aims:

1. Determination the cytomorphometrical changes in oral epithelial cells using exfoliative cytology from patients with type 2 diabetes and compared to healthy patients.
2. Assessment of oral manifestations in type 2 diabetic patients

Chapter One

Review of Literature

Chapter One

Literatures Review

1.1. Diabetes Mellitus (DM):

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. (American Diabetes Association, 2007; Guyton and Hall, 2012). Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage retinopathy, nephropathy and neuropathy. It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life (World Health Organization, 2006).

1.2. Epidemiology of diabetes mellitus:

The worldwide prevalence of DM, it is estimated that 366 million people have diabetes, with half unaware they have the disease. Of the ten countries with the highest prevalence of diabetes, six are in the Middle East (Qatar, Kuwait, Bahrain, Lebanon, the United Arab Emirates and Saudi Arabia). In the 20 Arab countries for which data are available, nearly 20.5 million people are living with diabetes and another 13.7 million are in the prediabetes stage, having impaired glucose tolerance. In contrast with developed countries, in which most people with diabetes are over the age of retirement, nearly three quarters (73.4%) of diabetics in Arab countries are under 60 years of age and hence in their most productive years, further increasing the burden of disability due to diabetes (International Diabetes Federation, 2011).

It has been found in Iraq there is no effect of gender on occurrence of diabetes when only one parent is type 2 diabetic (Saeed, 2004). While Ministry of Health in Iraq (MOH) (2006) showed that prevalence of diabetes appears relatively low in male (21.6 per 1000) than in female (22.1 per 1000) (Ministry of health in Iraq, 2006). Diabetes mellitus is one of the most serious disease of metabolism and produces a developing medical complications, with concomitant morbidity and mortality that involve people of all ages (Ship, 2003), but many studies reported that diabetes is possibly underreported as a cause of death. The modern evaluation submits the diabetes mellitus was responsible for almost 3 million deaths annually (1.7–5.2% of deaths worldwide) and it is the fifth leading cause of death (Fauci *et al.,* 2008).

Diabetes is shortening people's lives; the estimated decrease in life expectancy for a person diagnosed with diabetes at the age of 40 is about 12 years for men and 14 years for women (Skamagas *et al*., 2008).

Prevalence of type 2 diabetes had been estimated at 4 percent for the world population in 1995 and is expected to rise to 5.4 percent by 2025. Most of this increased disease burden will occur in developing countries. Among the possible reasons for this estimated increase are shifts toward a modern western lifestyle with high calorie diets, decreased physical activity and greater obesity (Paul *et al*., 2003).

1.3. Etiologic classification of diabetes mellitus: (ADA, 2007)

I. Type 1 diabetes (ß-cell destroys, usually leading to insulin lack).

A. Immune mediated

B. Idiopathic

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

III. Other specific types.

A. Genetic defects of beta cell function

1. Chromosome 12, HNF-1 alpha (MODY3)

2. Chromosome 7, glucokinase (MODY2)

3. Chromosome 20, HNF-4 beta (MODY1)

4. Mitochondrial DNA

5. Others

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipoatrophic diabetes

5. Others

C. Diseases of the exocrine pancreas

1. Pancreatitis

2. Trauma/pancreatectomy

3. Neoplasia

4. Cystic fibrosis

5. Hemochromatosis

6. Fibrocalculous pancreatopathy

7. Others

D. Endocrinopathies

1. Acromegaly

2. Cushing’s syndrome

3. Glucagonoma

4. Pheochromocytoma

5. Hyperthyroidism

6. Somatostatinoma

7. Aldosteronoma

8. Others

E. Drug- or chemical-induced

1. Pyrinuron

2. Pentamidine

3. Nicotinic acid

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5. Thyroid hormone

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8. Thiazides

9. Phenytoin

10. alpha-Interferon

11. Others

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3. Others

G. Uncommon forms of immune-mediated diabetes

1. “Stiff-man” syndrome

2. Anti-insulin receptor antibodies

3. Others

H. Other genetic syndromes sometimes associated with diabetes

1. Down’s syndrome

2. Klinefelter’s syndrome

3. Turner’s syndrome

4. Wolfram’s syndrome

5. Friedreich’s ataxia

6. Huntington’s chorea

7. Laurence-Moon-Biedl syndrome

8. Myotonic dystrophy
9. Porphyria
10. Prader-Willi syndrome
11. Others

IV. Gestational diabetes mellitus.

1.3.1. Type 1 diabetes or (Insulin-Dependent Diabetes Mellitus-IDDM)

The diagnosis of type 1 diabetes may occur at any age, the majority of the patients with type 1 diabetes are diagnosis before the age of twenty which means that it usually manifests in childhood, adolescence or early adulthood. At birth the individuals with a genetic susceptibility have normal beta cell mass but start to lose beta cells secondary to autoimmune destruction that occurs over months to years. The percentage of this type account for 10 to 15% of all cases of DM (Beers *et al*., 2006).

The autoimmune devastation of pancreatic beta cells of the islets of Langerhans lead to impair the secretion of insulin in type 1 diabetes mellitus. This type of diabetes can be further classified as idiopathic or immune mediated. The main cause of type 1 diabetes is immunemediated nature, and the beta cell loss is a (T- cell) mediated autoimmune attack (Rother, 2007).

1.3.2. Type 2 diabetes or (Non-Insulin Dependent-NIDDM):

Type 2 diabetes mellitus is characterized by insulin resistance which may be associated with relatively decreased insulin secretion (Shoback *et al*., 2011). In spite of the initial defect is controversial, the largest studies support the picture that the insulin resistance is preceding the insulin secretory defect but the development of diabetes occurs only when insulin secretion becomes deficient. The disease is polygenic and multifactorial since the environmental

factors (e.g. obesity, physical activity and nutrition) in addition to genetic susceptibility modulate the phenotype. The most common form is called adult-onset diabetes. In people, the development of type 2 diabetes occurs at any age, even during childhood, but usually occurs in people > 40 years of age. However, type 2 DM is being increasingly seen in the teenager years and occurs without loss of beta islet cell function but the peripheral tissue is resist to insulin action; (a condition in which the liver , muscle, and fat cells use the insulin incorrectly) (Shoback *et al*., 2011) .

The glucose tolerance remains near normal in the early stages of the disorder due to compensate the pancreatic beta cells by increasing insulin output; in spite of the insulin resistance is present. As a result, the body requires more insulin to give aid the glucose enters cells to be applied for energy. In the beginning the pancreas balances with the extra demand by generating more insulin. By the time the pancreas loses its capability to secrete adequate insulin in response to meals. These patients are not ketosis prone, but may develop it under conditions of stress (Defronzo *et al*., 2004).

The predominant abnormality in early stage of type 2 diabetes is diminished the sensitivity of insulin. The hyperglycemia regression in this stage by a kind of measures and medications that reduce glucose production by the liver or enhance the sensitivity of insulin (Horn *et al*., 2007).

The obesity accompanying type 2 DM is contribute to insulin resistance through elevation of circulating free fatty acids levels which derived from the adipocytes; when the levels of circulating free fatty acids and other fat cell products are increased, the glucose uptake, glycogen synthesis and glycolysis become inhibit. In many obese individuals, insulin resistance is compensated by increased insulin production. However, in one-third of obese individuals, ß-cell mass is reduced by a marked increase in ß-cell apoptosis, which results in insufficient creation of insulin (Mealey & Oates, 2006).

The type 2 DM has a strong genetic background more than type 1diabetes, but the risk gene or genes still remain unknown and less clarion. Individuals with a parent with type 2 DM have high susceptibility of diabetic incidence and when the both parents have type 2 DM, the risk approaches 40%. The etiology of type 2 diabetes is heterogeneous, but the majority of patients with type 2 diabetes are believed to result from a combination of hyperinsulinemia/ insulin resistance and ß-cell failure, the deficiency of insulin resulting from the impairment of the reaction of cell, tissue and peripheral organs to insulin activity (Abrairaa *et al*., 2003).

1.3.3. Gestational diabetes (GDM)

The Gestational diabetes mellitus first occurs during pregnancy and insulin resistance is associated to the metabolic differences of late pregnancy. When women are pregnant, the requirement for insulin becomes increased, and several pregnant women can progress gestational diabetes during the late stages of pregnancy (Lawrence *et al*., 2008).

Gestational diabetes mellitus (GDM) resemble type 2 diabetes in many considerations concerning the combination of insulin secretion deficient and responsiveness. During pregnancy the detection of GDM gives a convenience to diagnose the women at risk of short term and long term complications (Crowther, *et al*., 2005; Horvath, *et al*., 2010). The GDM take place (~ 2%-5% of all pregnancies) and may recover or disappear after delivery. It needs accurate medical supervision during pregnancy in order to reach entirely treatable, but (~ 20%-50%) of affected female establish type 2 diabetes later in life. The gestational diabetes may be transient but can impair the health of the mother or fetus if it untreated (Lawrence *et al*., 2008).

1.4. Physiological action of insulin

Insulin is "a hormone, produced by the pancreas, which is essential to regulating carbohydrate and fat metabolism in the body". Proinsulin is structurally correlated to insulin-like growth factors I and II, which bind weakly to the insulin receptor and makes the cells in the muscle, liver and fat tissue to assume the glucose from the blood, storing in as glycogen inside these tissues (Benedict *et al.,* 2004).

The mechanism action of insulin is breaks the employ of fat as an energy source by arresting the glucagon release. With the exclusion of the metabolic syndrome and metabolic disorder diabetes mellitus, insulin is maintained in the body in stable proportion to eliminate the excess glucose from the blood, which any other way would be toxic. The body initiates to utilize the stored sugar as an energy source by glycogenolysis process, when the blood glucose levels decline below a normal level, the glycogenolysis includes the breakdown of the glycogen that stored in the muscles and liver into glucose which can then be used as an energy source. As its level is a fundamental metabolic control mechanism, also its status is utilized as a regulation signal to other body systems (such as amino acid uptake by body cells) (Bergamini *et al*., 2007).

Insulin also affect other body functions like the cognition and vascular compliance and consider the most important significant regulator of metabolic equilibrium, when insulin arrived the human brain, it's improve memory and learning and helps verbal memory in particular (Benedict *et al.,* 2004)**.** Enhancing the acute thermoregulatory and glucoregulatory response to food intake also enhances brain insulin signaling by means of intranasal insulin administration, referring that central nervous insulin accommodates the control of whole-body energy homeostasis in humans (Benedict *et al.*, 2010).

1.5. Diagnostic Criteria for Diabetes Mellitus: (James *et al*., 2003)

1. Classic symptom of diabetes mellitus including polydipsia, polyuria and unexplained weight loss which is appreciate for the diagnosis of DM and causal plasma glucose concentration of > 200 mg/dl (11.1 mmol/L), or:

2. A fasting plasma glucose test (FPG Test): The FPG test is the most predictable and beneficial test for diagnosing DM in a symptomatic individuals because of its accessibility and low cost. The FPG test is defined as "no caloric intake for at least 8 hour and it is most reliable when done in the morning". People with a fasting glucose level of 110 to 125 milligrams per deciliter (mg/dL) have a pre-diabetes pattern called impaired fasting glucose (IFG). A level of 126 mg/dL or above, explained by repeating the test on another day, means a person has diabetes (Silvio, 2012), or:

3. Random plasma glucose test

A random (casual) "is defined as without concern to the time since the last meal". The blood glucose level of < 180 mg/dl, plus the presence of the classic symptoms (mention above), other symptoms like fatigue, blurred vision, increased hunger, and sores without repair are associated with diabetic patients (Silvio, 2012).

4. Oral glucose tolerance test (OGTT)

Research has shown that the most sensitive test is OGTT rather than the FPG test that used for diagnosing pre-diabetic individuals, but it is less acceptable to carry out. The OGTT requires fasting for at least eight hours before the test. The measurement of plasma glucose level act immediately before and 2 hours after a person drinks a liquid containing 75 grams of glucose dissolved in water. The person of pre-diabetes pattern called impaired glucose tolerance (IGT) when the blood glucose level is (> 100 mg/dL) after drinking the liquid. A person has diabetes is accepted by repeating the test on another

day (2-hour glucose level of 200 mg/dL or higher) (Hanson *et al*., 1995; Silvio, 2012).

5. Glycated hemoglobin (HbA1c)

The patient's measurement of hemoglobin A1C provides a picture of long-term glycemic control and reflects average glycemic control over the previous 2–3 months. The term 'glycosylated' (nomenclature) was used initially, but it has been pointed out that this term strictly refers to glycosides. Therefore, the Joint Commission on Biochemical Nomenclature has that the term 'glycation' is appropriate for any reaction that links a sugar to a protein, or in the particular case of a reaction with hemoglobin, the term "glycated hemoglobin" (Abrairaa *et al*., 2003).

In the American Diabetes Association ADA, (2007), HbA1c has been referred to as A1C. It is considered as agood indicator of average glycemic concentrations during the previous 90 to120 days and it is the standard method for assessing long-term glycemic control (Sacks, 2005; Sultanpur *et al*., 2010).

American Diabetes Association ADA, (2007) recommend that A1C should be performed at least twice a year in patients who are meeting their treatment goals (and who have stable glycaemic control), and quarterly in patients whose therapy has changed or who are not meeting their glycemic goals. When plasma glucose is consistently elevated, there is an increase in nonenzymatic glycation of hemoglobin; this alteration reflects the glycemic history over the previous 2–3 months, since erythrocytes have an average life span of 120 days (glycemic level in the preceding month contributes about 50% to the A1C value). The normal HbA1c level among individuals without diabetes falls between 4and 6 percent. Among adult with diabetes, the target is to maintain HbA1c levels of 7 percent or lower. The levels of HbA1c more than 8 present reflect poorly controlled diabetes, which will need more aggressive management so the glycated haemoglobin has been used as a bio-marker of

long-term glycemic control (Hanas and John, 2010). HbA1c values between 6% and 7% were consider as a sign of good control of the diabetes, HbA1c values between 7.1% and 8% indicated moderated control, and HbA1c value >8% were designated as poor control of the diabetes (Marshall, 2010). The glycated hemoglobin levels (HbA1c) is convenient, reflect long-term hypoglycemia and reliable when standardized, this features are screening and diagnosing criteria for diabetes mellitus which is recommended for it **(**Saudek *et al.,* 2008).

The diagnostic criteria for diabetes and pre-diabetic are shown in Table 1-1. In many instances, the various forms of glycated hemoglobin are measuring by many laboratory methods and these have significant interassay variations. Usually the glycated hemoglobin measurements are compared to prior measurements, it is essential for the assay results to be comparable.

"HbA1c is a minor component of total haemoglobin-A (HbA) and it's a glycated from of haemoglobin, formed by the attachment of sugars to the molecule when glucose levels are elevated''. There are several methods of glycated hemoglobin measurement like ion-exchange, chromatography and immunoassay (Marshall, 2010; Nitin, 2010).

HbA1c result will be misleading in certain situations (change in RBC lifespan) for example in hematological conditions where there is abnormal hemoglobin, abnormal red cell turnover, or in nutritional anemia's such as iron deficiency anemia or in renal or liver disease and in hemolytic anemia's. In pregnancy, HbA1c may be slightly lower, the presence of abnormal hemoglobin or in conditions with altered red cell survival rates may interfere with the A1C result and HbA1c results may not be reliable (Nitin, 2010).

Table (1-1): Major Diagnostic Criteria for Diabetes and Pre diabetic or At-Risk States.*

Measure	American Diabetes Association		World Health Organization	
	Diabetes	Prediabetes	Diabetes	Impaired Glucose Regulation
Fasting plasma glucose	≥126 mg/dl	100–125 mg/dl (IFG)	≥126 mg/dl	110–125 mg/dl (IFG)
2-Hr plasma glucose (during an OGTT with a loading dose of 75 g).	≥200 mg/dl	140–199 mg/dl (IGT)	≥200 mg/dl	140–199 mg/dl (IGT)
Casual (or random) plasma glucose (in a patient with classichyperglycemic symptoms)	≥200 mg/dl		≥200 mg/dl	
Glycated hemoglobin	≥6.5%	5.7–6.4%	≥6.5%	

*American Diabetes Association (2010); American Diabetes Association,(2012)

1.5.1. Advantages of proposal to utilize HbA1c for Diagnosis of Diabetes Mellitus :(Diabetes Care, 2009)

• Acceptable for patients that means no fasting or other test preparation needed.

• Significant international attempts to standardize assays.

• The coefficients of variation between & within person become lower and the possibility of pre-analytic errors become lower in compared with glucose.

• Scientific and definite measure of chronic glycemic levels.

• It is already used to guide therapeutic determination. (Familiar test parameter).

• Correspond to the risk of diabetes defining complications like retinopathy.

1.5.2. Disadvantages of Proposal to utilize HbA1c for Diagnosis of Diabetes Mellitus : (Diabetes Care, 2003)

- Although the actual glucose based diagnostic criteria remain accurate, the current proposal does not promoter a confirmatory checkup of glucose level at any stage.
- Directly the point of care instruments is considered deficient for diagnostic purposes.
- Although the relation between HbA1c and glucose is good, but it is not ideal.
- It is not applicable for gestational diabetes diagnosis.
- The technique ways undergo numerous interferences that make the clinicians may not be attuned.
- Impact of reporting HbA1c in molar units.
- The uppermost limit of normal HbA1c (6.0%) leaves a diagnostic interval between (6.1% and 6.5%), in addition to a differences between the ordinary treatment target of <7% and the diagnostic level (Diabetes Care, 2009).

1.6. Diabetic organ complications

In diabetic patients the clinical signs and symptoms result from affect many organ systems. Chronic complications can be divided into vascular (micro vascular/macro vascular) and non-vascular complication. The micro vascular disease including retinopathy and blindness, nephropathy, neuropathy while the macro vascular disease (a coronary artery disease, cerebrovascular disease) and impaired wound healing may all be caused by sustained action of hyperglycemia (American Diabetes Association, 2003).

The chronic hyperglycemia resulted in micro vascular complications of both type 1 and type 2 DM. Retinopathy is common in both type 1 and type 2

diabetes . The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic glycaemia which is measured most reliably with glycated hemoglobin assay (ADA, 1998a; Stratton *et al.,* 2002).

Nephropathy arises from glomerulosclerosis, which is characterized by thickening of glomerular basement membrane and arteriosclerosis of small arterioles. The diabetic retinopathy occurs more commonly in individuals with diabetic nephropathy. The mechanisms proposed to induce glomerulosclerosis include hyperglycemia, a hyperfilteration-related increase of glomerular pressure and increased blood viscosity. The clinical manifestation of nephropathy is proteinuria, mostly albuminuria and the early phases of nephropathy are characterized by micro albuminuria that are associated with increased risk of cardiovascular disease in DM patients, during micro albuminuria, the protein contented in urine is so high (>200μg/min) that can be recorded by sensitive laboratory measurements (Mogensen, 1997; ADA, 1998b).

The other microangiopathic complication of diabetes is neuropathy, as with other complications of DM, the duration of diabetes and glycemic control are correlates with the development of neuropathy correlates. The main histological finding is thickening of the basement membranes of nerve sheets and the capillaries that supply blood to the nerves. The most important factor in the etiology of neuropathy is reduced nerve perfusion (Greene *et al.*, 1990; Thomas, 1997).

1.7. Oral manifestations of diabetes mellitus

Several soft tissue pathologies and various inflammatory diseases have been reported to be associated with diabetes mellitus in the oral cavity. (Vernillo 2003; Baldwin 2009). The decreased polymorphonuclear (PMN)

leukocyte function and abnormal collagen metabolism are the main factors that contribute to oral manifestations in diabetes and the PMN dysfunction leads to impaired resistance to infections which may facilitate bacterial persistence in the tissue and predispose diabetic patients to greater risk of disease. Altered protein metabolism resulting from impaired utilization of glucose can contribute to increased breakdown of collagen in the connective tissues. In addition, impaired macrophage function and neutrophil chemotaxis may add to the impaired wound healing responses in diabetic patients (Straka, 2011).

These manifestations 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 diabetes. There are also reports of oral mucosa lesions in the form of stomatitis, geographic tongue, benign migratory glossitis, fissured tongue, traumatic ulcer, lichen planus, lichenoid reaction and angular chelitis . (Sandberg *et al*., 2000; Collin *et al*., 2000; Chomkhakhai *et al*., 2009)

In addition, delayed mucosal wound healing, mucosal neuro- sensory disorders, dental carries and tooth loss has been reported in patients with diabetes. (Lamster *et al*., 2008; Saini *et al*., 2010).

1.7.1. Periodontal Diseases

1.7.1.1. Periodontitis and diabetes mellitus

Periodontitis is one of the most widespread diseases in the world affecting the oral cavity, and is highly prevalent in both developed and developing countries (Poul, 2005). The most consistent finding in poorly controlled diabetic patients is the periodontal disease. Approximately 75% of these patients have periodontal disease that characterized by increased alveolar bone resorption and inflammatory gingival changes (Deshmukh *et al.,* 2011). Periodontitis is a chronic inflammatory disorder affecting the gingiva and the

periodontal tissue initiated by bacteria. The daily formation of micro-flora in the dental plaque which is adjacent to the teeth causes this inflammatory process (Kuo *et al.,* 2008).

Numerous risk factors have been reported like poorly metabolic control, poor oral hygiene ,longer duration of diabetes, and smokers make the patients with diabetes more susceptible to periodontal disease (Irwin *et al.,* 2007; Kibayashi *et al.,* 2007).

The micro-flora in the dental plaque that forms daily adjacent to the teeth causes this inflammatory process. Eventually, the toxins that are released by the microorganisms in the dental plaque will start the gingival inflammation as a result of failure to remove the dental plaque on a daily basis. A periodontal pocket is formed as a result of the progression of the gingival inflammation causing the gingiva to detach from the tooth surface. This periodontal pocket is filled with bacteria and its toxins. As the disease worsens, the pocket will get deeper carrying the dental plaque until it reaches the alveolar bone that will eventually be destroyed with the periodontal attachment. This process is very common and causes destruction of periodontal tissues, loss of alveolar bone and, finally, tooth loss. There are many factors contributing to this type of inflammation beside the presence of bacteria in dental plaque; a susceptible host is one of them.

The link between diabetes mellitus and periodontal disease is not well recognised by the medical community. Periodontal disease has been reported with increased prevalence and severity in patients with type 1 and type 2 diabetes. (Preshaw, 2009).

The mechanism by which hyperglycaemia can induce periodontal destruction is not yet fully understood. However, there are many theories which propose factors such as advanced glycation end products, changes in collagen statue, and altered immune function that causes impaired polymorphonuclear leukocyte function which may facilitate bacterial persistence in the tissue and

the accumulation of advanced glycation end products, which results from prolonged and chronic hyperglycaemia and increased secretion of pro-inflammatory cytokines such as tumour necrosis factor-α and prostaglandin E-2 (Ritchie, 2009). The increase in collagenase activity together with the reduction in collagen synthesis will adversely influence collagen metabolism. This would result in compromised wound healing as well as periodontal tissue destruction.

Recent studies indicate that periodontitis has a bidirectional effect on glycaemic control in patients with diabetes (Teeuw, 2008). There is a cluster of research studies, which support the hypothesis of periodontitis occurring more frequently in patients with diabetes with poor glycaemic control (Taylor,1999 ;Tsai *et al*., 2002; Pihlstrom *et al*., 2005; Davies and Davies, 2005; De Silva *et al*., 2006; Teeuw, 2008).

In addition, there is enough evidence to support the hypothesis that poor periodontal conditions could worsen glycaemic control as well. Many studies report that diabetes is a risk factor for gingivitis and periodontitis and it is more severe with poor glycaemic control (Taylor and Borgnakke,2008). The risk of developing periodontitis in patients with diabetes has been reported to be three times higher than the general population (Ryan *et al*., 2003).

Several studies showed that the treatment of periodontal disease has an influence on glycaemic control in both type 1 and type 2. A recent meta analysis of the efficacy of periodontal treatment on glycaemic control in patients with diabetes suggested that such treatment could lead to a significant reduction in HbA1c (Darr *et al*., 2008; Taylor and Borgnakke,2008).

1.7.2. Salivary Dysfunction

Saliva has a major role in maintaining a healthy oral cavity. Saliva is produced by major salivary glands (parotid, sub-mandibular and sub-lingual) and numerous minor salivary glands distributed in the oral cavity. Salivary dysfunction has been reported in patients with diabetes (Lin *et al*., 2002).

A cross sectional epidemiological study was conducted in 2001 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 diabetes than the controls, especially by those diabetics who had develop neuropathy (Moore *et al*.,2001).

Other studies conducted in type 2 diabetics also confirmed that xerostomia and hyposalivation were more prevalent in this group of patients (Sandberg and Wikblad,2003). It has been shown that poorly controlled type 2 diabetics have a lower stimulated parotid gland flow rate compared to well-controlled patients and patients without diabetes (Chavez *et al*., 2001). An increase in salivary pathogens was also reported in these patients (Chavez *et al*.,1989; Chomkhakhai *et al*., 2009).

Patients with diabetes usually complain of xerostomia and the need to drink very often (polydypsia and polyuria). The constant dryness of the mouth would irritate the oral soft tissues, which in turn will cause inflammation and pain.

Patients with diabetes with xerostomia are more predisposed to periodontal infection and tooth decay. The cause of this is not yet fully understood in patients with diabetes, but may be related to polydypsia and polyuria or alternation in the basement membrane of the salivary glands. It is known that diabetes mellitus is associated with chronic complications such as neuropathy, microvascular abnormalities and endothelial dysfunction that lead to deterioration of microcirculation and this may play a role in reduction of the salivary flow rate and composition **(**Khovidhunkit *et al*.,2009). 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 diabetes mellitus (Scully *et al*., 2008).

1.7.3 .Taste dysfunction

There are many factors that have been implicated in altered taste sensation in the oral cavity. Metabolic and endocrine diseases were proposed as causative factors for this disturbance; nevertheless, salivary dysfunction can contribute to altered taste sensation or elevation of detection thresholds.(Ship and Chavez, 2001; Negrato and Tarzia, 2010).Taste dysfunction has been reported to occur more frequently in patients with poorly controlled diabetes compared to healthy controls (Lalla and Ambrossio, 2000) .Diabetic patients who suffer the consequences of neuropathy have a higher taste threshold that occur more frequently in patients with poorly controlled diabetes compared to healthy controls because of inhibit the ability to maintain a good diet. The descriptive of taste alteration in diabetic patients is salty, peculiar and altered taste sensation that are associated with salivary flow rate reduction, low production rate of gustin, buccal breathing with dryness of mucosa, zinc insufficiency (that leads to reduced gustin synthesis) and coated tongue (due to the production of sulfide compounds that introduce the sour taste) (Mese ,2007; Negrato and Tarzia, 2010). The deficiency or loss of gustin production with low salivation results in taste papillae maturation and cause taste alteration (Heckman *et al.*, 2003; Negrato and Tarzia, 2010).

1.7.4.Oral Infection

1.7.4.1. Fungal infections

Oral candidiasis is an opportunistic infection frequently caused by candida albicans species. Many predisposing factors can lead to this infection; these include smoking, xerostomia and endocrine and metabolic diseases (Mclntyre, 2001). Other factors were also implicated such as old age, medications, Cushing's syndrome, malignancies, and the use of dentures (Samaranayake, 1990). Oral candidiasis has been classified into primary and

secondary. Primary oral candidiasis is subclassified into acute (pseudomembranous and erythematous), chronic (pseudomembranous, erythematous and hyperplastic) and candida associated lesions.

Pseudomembranous candidiasis is also known as oral thrush. It is characterised by the presence of a creamy white patch which, when wiped, reveals underlying erythematous and bleeding oral mucosa. The soft palate is the most commonly affected area followed by the cheek, tongue and gingiva. It could be chronic in immuno-compromised patients. Erythematous candidiasis is can present as acute or chronic infection. It is believed to result from the usage of steroid and broad spectrum antibiotics and mainly affects the tongue (Akpan and Morgan, 2002) .Hyperplastic candidiasis is known as candidal leukoplakia. It appears as an irregular whitish raised plaque like lesion commonly seen in the buccal mucous membrane near the commissures. Candida associated lesions include denture induced stomatitis, angular chelitis and median rhomboid glossitis which have mixed bacterial and fungal etiology. Denture induced stomatitis is mainly seen in full denture wearers in the underlying surface of the upper denture. Angular chelitis is seen in the lip commissures as an erythematous crusting lesion. The lesion has been reported to occur in diabetics with poor glycaemic control. Median rhomboid glossitis is seen on the dorsal surface of the tongue as adepopulated erythematous diamond-shaped patch at the midline. The incidence of fungal infections in patients with diabetes mellitus has been recognised for many years (Lamey *et al*., 1988). Candidal infection is reported to be more prevalent in patients with diabetes especially in those patients have poor glycaemic control , wear dentures, who smoke and use steroids and broad spectrum antibiotics (Willis *et al*., 1999). In addition, salivary dysfunction in patients with diabetes can also contribute to higher carriage of fungi in this group of patients. It is clear from these studies that both local and systemic predisposing factors might increase candidal carriage rate

and hence increase the risk of oral candidal infection in patients with diabetes (Soysa *et al*.,2006 ; Khosravi *et al*.,2008).

1.7.4.2. Bacterial infections

Patients with diabetes are more susceptible to developing oral bacterial infections. They are well known to have an impaired defense mechanism hence considered to be immuno-compromised. Diabetics with diabetic complications and poor metabolic control are more prone to spreading and recurrent bacterial infection. Several studies have reported that patients with diabetes are more prone to deep neck bacterial infection compared to patient without diabetes (Huang *et al*.,2005; Uthkarsh and Shrinath,2007). A four year prospective study by Rao et al. investigated the severity of maxillofacial space infection of odontogenic origin, the type of micro-organism, the sensitivity of the micro-organisms to antibiotics, and the length of hospital stay of patients with diabetes compared with patients without diabetes. They concluded that the spread of the bacterial infection to the submandibular space was more common in patients and controls and that the second commonest area was the buccal space. Streptococcus species was more commonly isolated in both groups. Patients with diabetes were found to stay longer in hospital due to more severe infection and required more time to control their blood glucose levels (Rao *et al*.,2010).

1.7.5. Poor Oral Wound Healing

Poor soft tissue regeneration and delayed osseous healing in patients with diabetes are known complications during oral surgery. Therefore, the management and treatment of patients with diabetes undergoing oral surgery is more complex. It was reported that delayed vascularisation, reduced blood flow, a decline in innate immunity, decreased growth factor production, and psychological stress may be involved in the protracted wound healing of the oral cavity mucosa in patients with diabetes (Abiko and Selimovic,2010).

1.7.6. Other Oral Soft Tissue Lesions

Oral lesions that are not caused by candida infection have been reported to occur in patients with diabetes such as fissured tongue, irritation fibroma and traumatic ulcer. These lesions were more prevalent in diabetes compared to the controls (Saini *et al*.,2010).

1.7.7. Neuro-Sensory Oral Disorder

Oral dysesthesia or burning mouth syndrome (BMS) is a painful condition affecting the oral cavity (palate, tongue, throat and gingiva) (Scala *et al*., 2003; ADA, 2005).Other abnormal oral sensations may co-exist with the burning mouth sensation such as tingling, numbness, dryness or sore mouth at the same time. The exact cause of BMS is unknown, but it has been attributed to several conditions such as dry mouth, menopause, candidal infection, diabetes mellitus, cancer therapy, psychological problems and acid reflux. BMS is classified into two types: primary idiopathic, and secondary as a result of a systemic process; secondary BMS has been reported to occur with diabetes mellitus. It could adversely affect the ability to maintain good oral hygiene in patients with diabetes. Diabetic neuropathy could be the underlying cause of BMS in patients with diabetes. The nerve damage in diabetic neuropathy has been reported to show an increase in the langerhans cells that are associated with immune disturbance (Moore *et al*.,2007; Tavakoli *et al*.,2010). Therefore, it is crucial to screen patients who have symptoms of BMS for diabetes mellitus.

1.7.8. Dental Caries and Tooth Loss

It is well known that patients with diabetes are susceptible to oral infections that lead to tooth decay and loss. Salivary secretion dysfunction, periodontal and sensory disorders could increase the likelihood of developing new and recurrent dental caries and tooth loss. The relationship between

diabetes and development of dental caries is still unclear. It is well-known that the cleansing and buffering capacity of the saliva is diminished in patients with diabetes mellitus resulting in increased incidence of dental caries, especially in those patients who suffer from xerostomia (Collin *et al*.,1998).

1.8. Oral Exfolative Cytology

Exfoliative cytology is the study of cells which have been exfoliated or removed from the epithelial surface of several organs (Mehrotra,2012a).

This technique has opened several ways for working in health area, with favorable results and has been enhanced with some of the currently available technology programs, also allowing tabulating and analyzing information quickly and easily (Mehrotra ,2012b) **.**Using exfoliative cytology has been previously studied; morphological changes suffered by oral epithelial cells in diabetics, results are higher and significant when are compared to healthy patients. These changes are represented by variations in the nucleus, cytoplasm and the nuclear cytoplasmic ratio (Alberti *et al*.,2003; Prasad *et al*.,2010).

Accordinary, exfolative cytology, which is straight-forward and noninvasive diagnostic method (Sugerman and Savage,1996) , can be considered as more practical technique to evaluate the oral mucosa in diabetes. This method has been discussed over the last 30 years in the diagnosis of precancer in the cervical and vaginal mucosa and similar lesions of the oral mucosa. Most authors have come to the conclusion that exfolative cytology is of some value in the diagnosis of precancerous lesions, and particularly in determining their prognosis. (Sugerman and Savage, 1996 ; Alberti *et al*., 2003). However, use of this method to evaluate quantitative and qualitative changes in oral epithelial cells in diabetes is debatable. A few studies have used exfolative cytology to evaluate changes in the oral mucosa in diabetes mellitus and have

shown that this disease can produce alterations in oral epithelial cells that are detectable by cytomorphometric analysis.

Oral exfoliative cytology accounts as a more appropriate tool because it is easy, simple, and noninvasive as compared to conventional intervention techniques. Moreover, exfoliative cytology can be performed from multiple sites in the same patient in single/multiple visits (Alberti *et al*., 2003).

Exfoliative cytology is a diagnostic technique based on a microscopic evaluation of epithelial cells after a procedure of their fixation and staining.. There are 2 methods in use: the indirect cell--collecting method, such as aspiration subjects with self-exfoliated cells, and the direct method, rub cells of the mucosal surface. The exfoliated cells are put into a preservative fluid and the samples are processed according to the manufacturer directions, after staining using the papanicolaou method (Williams *et al*.,1999; Rajput and Tupkari , 2010).

The papanicolaou technique is a multichromatic staining histological technique developed by George papanikolaou and used to differentiate cells from smear preparations of various bodily secretions, This is a polychrome staining method which comprises a nuclear stain (hematoxylin) and two counterstains(Orange G and Eosin Azure dyes) (Renwik ,2009).

Papanicolaou (PAP) is still a big hit in the cytology staining procedures. The advantages of PAP staining lies in the fact that the dehydration and clearing solutions help in causing cellular transparency. This detects the overlapped cells and their individual morphology better, which otherwise would be confused for a giant cell, or a bi or multinucleated cell. The second significant advantage is the differential staining for different degrees of differentiation, green-blue cytoplasm for basal cells and yellow orange for a spinous or granular cell. Also a stability of stains over long periods, stability of colour and of course the better reproducibility of results make it popular (Sugerman and Savage,1996; Sundharam and Kalasagar, 2004).

1.8.1. Advantages and Disadvantages of Exfoliative Cytology

Exfoliative cytology is advantageous, as it is a painless, bloodless noninvasive, quick and simple procedure. It is suitable in patients with systemic disease who are contraindicated for biopsy. It guards against false negative biopsy and post-biopsy complications can be eliminated. This procedure can be repeated a number of times for diagnosis, follow up and research purposes (Sundharam and Kalasagar, 2004).

This technique is useful for preliminary diagnosis of many oral mucosal diseases but it is not a substitute for the routinely-used biopsy to obtain a definitive diagnosis. Lesions caused by reactive changes and inflammatory reactions are non-specific and non-diagnostic cytological findings. Exfoliative cytology is not appropriate as a diagnostic tool for patients with clinical symptoms of desquamative gingivitis. It adds to the cost and delays the definite diagnosis (Endo *et al*., 2008).

1.8.2. Areas for Collecting Smear Samples and Instruments for Exfoliative Cytology

The most common areas for collecting smears within the oral cavity are the buccal mucosa, hard and soft palate line, dorsum of the tongue, bottom of the mouth and the lower labial region. The cells may be detached naturally (mouthwash sampling) or artificially (tool sampling). Tools for collecting smears should be easy to use in any location, not irritating and provide an adequate number of epithelial cells. The types used and compared were wooden, plastic and metal spatulas, dermatological curette, Fisherbrand sterilized polyester swab and different types of brushes (interproximal brush, cytobrush, oral CDx brush and Cytobrush Plus GT) (Ogden *et al*., 1992; Reboiras-López *et al*., 2012).

Cytobrush sampling is used more frequently; it maximizes the number of cells obtained, and facilitates their uniform distribution on the microscope slide. The brush is recommended as an adequate instrument due to its easy use in sampling and due to the quality of the oral cytology sample. The oral cytobrsh used for exfoliative cytology is a simple, harmless and non-invasive (Scheifele *et al*., 2004 ; Kosicki *et al*., 2007).

All authors agreed that brushes are best for oral cytology, being much more effective than other instruments. The cytobrush is recommended for collecting cells from the lateral side of the tongue because it has been proven to be acceptable brush in this location (Kujan *et al*.,2006).

The cytological samples can be collected successfully from the oral tissues and characterized by its simplicity, quickness and a non invasive nature which make it favored by both patients and specialist over the surgical biopsy (Yadav and Jaggi, 2015).

1.8.3 Indications for Oral Cytology

It helps to differentiate an unidentified lesion or a benign lesion, also it can be performed when a suspicious lesion in a patient refuses the biopsy. It is of a value in the monitoring after an oral cancer treatment or even in the periodic examination of the treated and adjacent areas that possibly will show doubtful or positive malignant cells earlier to the reappearance of the clinical features.

Sometimes a non specific lesion may appear in an area that is previously irradiated or treated for cancer, and if the malignancy is excluded the lesion is considered as inflammatory (Kumaresan and Jagannathan, 2014).

1.9. Cytomorphometry and Its Role in Diagnosis of Oral and Systemic Disease

Cytomorphometry was used by Johnston in 1952 to measure the nuclear and cytoplasmic and nuclear to cytoplasmic ratios of 1000 normal and malignant epithelial cells. In a more extensive study, 20,000 malignant cells and 11,000 normal cells were analysed and a significant difference was observed between normal and malignant cells (Ogden *et al*.,1997a).

It was suggested that nuclear diameter (ND), cytoplasm diameter (CyD), nucleo-cytoplasmic ratio (N:CR) were important factors to consider in assessing normal exfoliated cells from the oral cavity. Since then, few researchers have quantitatively assessed oral mucosal smears (Ogden *et al*., 1997b). It was suggested that quantitative techniques, based on evaluation of parameters such as nuclear diameter, cytoplasm diameter and nucleo-cytoplasmic ratio N:CR, may increase the sensitivity of exfoliative cytology for early diagnosis since these are precise, objective and reproducible (Ogden *et al*.,1997a).

In a study on smears of buccal mucosa, dorsal surface of the tongue and floor of mouth were taken from 10 patients with histologically confirmed oral lichen planus and 12 healthy age and sex-matched controls. In buccal smears, no significant differences in cytoplasmic and nuclear areas were observed between lesional and control tissues. However, the cytoplasmic area in smears from lichen planus lesions on the dorsum of the tongue and adjacent clinically normal mucosa was reduced compared with healthy controls. The cytoplasmic: nuclear ratio in smears from clinically normal floor of mouth in oral lichen planus was similarly reduced. Papanicolaou-stained smears from buccal lichen planus showed increased keratinization compared with normal buccal mucosa. These findings demonstrate that quantitative cytology can detect both cytoplasmic and nuclear changes in oral lichen planus (Sugerman *et al*.,1996). A study was conducted to study cytomorphometric changes in exfoliated cells

of buccal mucosa of patients suffering from iron deficiency anemia. Results of the study showed that there was an increase in the cellular diameter, nuclear diameter and nucleocytoplasmic ratio of the iron deficiency anemia patients when compared with normal values (Gururaj *et al*,.2004).

Chapter Two

Subjects, Materials & Methods

Chapter Two

Subjects, Materials and Methods

2.1 The sample:

The present study comprised of 75 subjects, with an age range of (30-60) years of both gender. (50 patients with type 2 diabetes mellitus divided into controlled and uncontrolled on the bases of HbA1c levels and 25 non-diabetic healthy persons as the control group). The diabetic patients were all with confirmed diagnosis of non insulin dependent diabetes mellitus (type 2).The patients were examined at the Diabetic Clinic in Mawani General Hospital (Diabetic –Endocrinology Center) in Basra city during the period from (February-2017 to June 2017).

Analysis was performed in three groups:

Group1 (Control group): Consisting of 25 healthy subjects without any history of diabetes and did not suffer from any systemic disease)

Group2 (Controlled D.M): (25 patients with controlled type 2 diabetes mellitus (HbA1c ≤ 7.0%) (Hanas and John, 2010).

Group3 (Uncontrolled D.M): (25 patients with uncontrolled type 2 diabetes mellitus (HbA1c > 7.0%) (Hanas and John, 2010).

2.1.1 Inclusion criteria:

1. Patients aged (30-60) years.
2. Medical history of type 2 DM with no other systemic disease.
3. Diagnostic criteria for type 2 DM based on HBA1c

   a.controlled D.M (HbA1c ≤ 7%).

   b.uncontrolled D.M (HbA1c >7%).

4. Control group of volunteers with following criteria:
    a. Clinically healthy oral mucosa
    b. Negative for clinical signs of systemic diseases
    c. Negative for presence of diabetes and anemia
5. The smears were collected from a buccal mucosa and lateral border of tongue.

2.1.2 Exclusion criteria:

Patients/individuals with the following were excluded from the study:

1. Subjects suffering from any other systemic disease eg. Anemia and endocrine disease – malignancy - nutritional deficiency - reduced immune competency Cardiovascular complications and renal failure.

2. physiological states such as pregnancy and lactation.

3. habits such as smoking, alcohol, and tobacco chewing.

4. Subjects less than 30 years of age.

5. Patients who have undergone radiation therapy and chemotherapy

2.2 Materials, Instruments and Equipment's

2.2.1 Instruments and Equipment's:

1-Diagnostic tools such as: disposable mirrors, rubber gloves (Latex –free) and wooden tongue depressors.

2-Disposable cytobrushes (Disposable kit of pap.smear) (Figure 2-1).

3- Micro-scopical slides 25.4 × 76.2 mm (Haiman, China).

4- Glass slides covers 24×50 mm (AFCO brand/ China).

5- Glass coplin jars.

6- Plastic jars.

7- Plastic slides container.

8- Light microscope (Olympus/Japan) and Camera (Leica/Hungary) (Figure 2-2).

9. An eyepiece micro-oculometer.

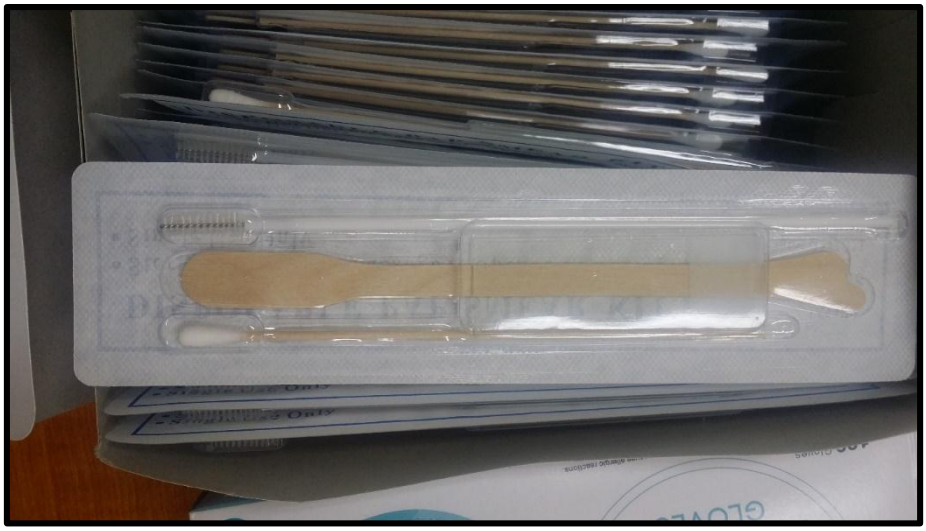

Figure (2-1) Disposable kit of pap smear.

Figure (2-2) Light microscope and Camera.

2.2.2 Chemicals:

1- Papanicolaou stain.

2- Xylene (Alphachemica, India).

3- D.P.X. mountant (A mixture of styrene, Plasticizer dissolved in Toluene-Xylene. (Qualikems, India).

4- Ethanol 96% (Scharlau, Spain).

5- Distal water.

6- Antiseptic solution.

2.3 Methods

2.3.1 Clinical examination

All the subjects were informed about the nature of the clinical experience, they had the right to participate or to refuse the participation, after that, a written consent form (Appendix I) was given and signed by the patient or one of his/her companion, after that, a baseline information including name, age, gender, address and occupation, dental and medical histories were taken from each subject and recorded in a case sheet (Appendix II).The diabetic patients were attending at the diabetic center for in Mawani General Hospital (Diabetic – Endocrinology Center) in Basra city for checking the glucose level using HbA1c which was done in the laboratory.

2.3.1.1. Oral examination:

A thorough oral examination was carried by using a disposable dental mirror including the lips, cheeks, tongue and the palate looking for oral findings in each subject.

2.3.2 Sample collection:

Smear was taken from two oral sites, the buccal mucosa and lateral border of tongue. Before samples were taken, patients rinsing their mouths with tap water to remove any debris, then cells from right and left buccal mucosa and right and left lateral border of tongue were collected by using a cytobrush of pap

smear (disposable kit of pap smear) (Figure 2-1). Ten rotations of the brush were applied against the mucosa starting from the center and increasing the circumference to increase the sampling area and prevent damage to one region . Scrapings were placed on the middle of glass slide and spread over a large area to avoid clumping of cells (Thomas *et al.*, 2009).

2.3.3 Fixation:

In order to prevent the cells from drying out and shrinking, as well as to maintain the specimen's structural features and to permit clear staining and differentiation, specimens were fixed immediately after being taken and while still moist. The classic method of fixing is done by immersing the glass slides in 96% ethanol for 30 minutes (Zare-Mirzaie *et al*., 2007).

2.3.4 Staining:

The staining was done in the same day of the smear collection to avoid over fixation (usually happens after 48 hours), the method was as follows:

• The slides were inserted in descending concentrations 90%, 80%, 70%, 50% of ethanol alcohol 10 seconds (10 dips) in each concentration.

• Distal water for 10 seconds or 10 dips.

• The slides were inserted in Hematoxylin Harris stain for 1 minute only.

• The slides rinsed under tap water for 1 minute (Note that the slides are put in a container where the water falls on the distant side of it and not directly on the slides to avoid distortion of the smear).

• Slides inserted into the acidic alcohol (0.5% hydrochloric acid added to 70% ethanol alcohol) for 5 seconds.

• The slides rinsed under the tap water for 1 minute.

• Slides then inserted into the Lithium carbonate solution (0.1%) for 1 minute.

• The slides rinsed under the tap water for 1 minute.

• The slides then inserted into the distal water for 10 seconds or 10 dips.

• Dehydration was done by inserting the slides in ascending concentrations of ethanol alcohol (50%, 70%, 80%, 90%) 10 dips in each one.
• The slides inserted in Orange -G6 stain for 1 minute.
• Insertion in 95% ethanol alcohol for 10 seconds.
• The slides then Stained with Eosin Azure solution for 2 minutes.
• Insertion in 95% ethanol alcohol then in 100% 10 seconds for each.
• The slides were then inserted in a mixture of 50% Xylene and 50% ethanol for 10 seconds.
• Clearing the slides with Xylen for 10-20 minutes.
• Mounting in DPX mountant (DPX is a mixture of distyrene, a plasticizer, dissolved in toluene- xylene, DPX Mountant rapidly dries and protects the stain).
• The slides were covering with a glass cover slip.

2.3.5 Examination of the slides:

The properly stained slides were examined with the assistance and calibration of specialist in oral pathology by using a light microscope, the smear was first screened at 10X lens for testing the excellence of staining and for screening, followed by examination at 40X lens for the scoring process.

2.3.6 Cytomorphometric evaluation:

Cytomorphometric evaluation will be prformed for cytological reading of the samples. The study parameters examined included nuclear diameter, cytoplasmic diameter, ratio of nuclear to cytoplasmic (N:CR), it performing using digital photographs obtained from the slides using microscope. An eyepiece micr-oculometer was used to obtain measurements of the nuclear diameter (ND), Cytoplasmic diameter (CyD) and nucleus to cytoplasm ratio (N:CR).

Two smears were taken from both sites (buccal mucosa and tongue) and from both sides (right and left), and were stained with Papanicolaou stain to visualize under compound light microscope for cytomorphometric analysis of cells for (ND, CyD and (N:C) ratio. The morphometric parameters of 40 cells in each patient (20 cells for the BM, and 20 cells for lateral side of T) were measured using an eyepiece micr-oculometer.

2.3.7 Statistical analysis:

The data were analyzed using SPSS software V.22 ((Statistical package for social science-version 22). Age and gender groups presented as frequency and percentage, therefore Chi square test was used to analyze the difference in distribution among groups. The parameters were represented as mean ± standard deviation (SD) and One-way ANOVA was used for their analysis. Also Independent Student's t-test was applied to compare between two groups. The level of significant was set as $P \leq 0.05$.

Chapter Three

Results

Chapter Three

Results

3.1. Demographic Distribution of study sample:

The demoghraphic data of 75 subjects entrolled in this study included 50 known type 2 diabetic adults divided into two groups based on their HbA1c levels (25controlled and 25uncontrolled DM),where as control group comprised of 25 healthy individual, with an age range of (30-60) years of both gender .

3.1.1. Distribution of samples according to the age

The result of age distribution between groups are illustrated in table (3-1) which shown that the mean age of control group was (36.2±0.81) and the mean age of controlled D.M group was (44.8±0.65) and the mean age of uncontrolled DM group was (53.6±0.55). There was non-significant difference in control healthy group p-value (0.28) and controlled DM p-value (0.32) regarding age. While in uncontrolled DM group there was significant increase with advance age p-value (0.04).

Table (3-1): Age distribution among study groups

Groups	Control healthy		Controlled DM		Uncontrolled DM	
Age	**No.**	**%**	**No.**	**%**	**No.**	**%**
30 - 40	**12**	**48**	**9**	**36**	**3**	**12**
41 - 50	**7**	**28**	**11**	**44**	**9**	**36**
51 - 60	**6**	**24**	**5**	**20**	**13**	**52**
Total	**25**	**100**	**25**	**100**	**25**	**100**
p-value	**0.28**		**0.32**		**0.04**	
Mean	**36.2**		**44.8**		**53.6**	
SD	**0.81**		**0.65**		**0.55**	

No= number of subject * A significant if p-value less than 0.05.

P-value depended on Chi-square test.

3.1.2. Distribution of samples according to the gender

The results illustrated in (Table 3-2) shown that the male: female number of control group was (10:15) and for controlled DM group was (12:13) and for uncontrolled DM was (11:14) with slight female predominance among patients 42 (56%), while male were 33 (44%). There was non- significant differences between study groups regarding gender.

Table (3-2): Gender distribution among study groups

Groups	Control Healthy		Controlled DM		Uncontrolled DM		Total	
Gender	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Male	**10**	**40**	**12**	**48**	**11**	**44**	**33**	**44**
Female	**15**	**60**	**13**	**52**	**14**	**56**	**42**	**56**
Total	**25**	**100**	**25**	**100**	**25**	**100**	**75**	**100**
p-value	**0.317**		**0.841**		**0.549**			

No=number of subject * A significant if p-value less than 0.05.

P-value depended on Chi-square test

3.2 Oral findings:

All participants in this study were examined to see if there are any oral manifestation. Table 3-3 shows different oral findings in patients with type2 DM. Oral manifestation in uncontrolled DM group showed highest frequency was for periodontal disease (76%) figure 3-1(a) ,followed by dry mouth (52%) while the lowest frequency was for altered taste, oral candidiasis and periodontal abscess figure 3-1(b), (8%,8%, 4%) respectively; While in controlled DM group showed highest frequency (28%) for periodontal disease and (4%) for dry mouth and the other oral findings were not seen such as altered taste, oral candidiasis, periodontal abscess in 0 patients (0%).

Table (3-3): Prevalence of oral manifestation in controlled and uncontrolled type 2 diabetes patients

Groups	Controlled DM		Uncontrolled DM	
O.M	No.	%.	No.	%.
Periodontal diseases	7	28	19	76
Periodontal abscess	0	0	1	4
Dry mouth	1	4	13	52
Altered taste	0	0	2	8
Oral candidiasis	0	0	2	8

O.M=oral manifestation.

No. =number.

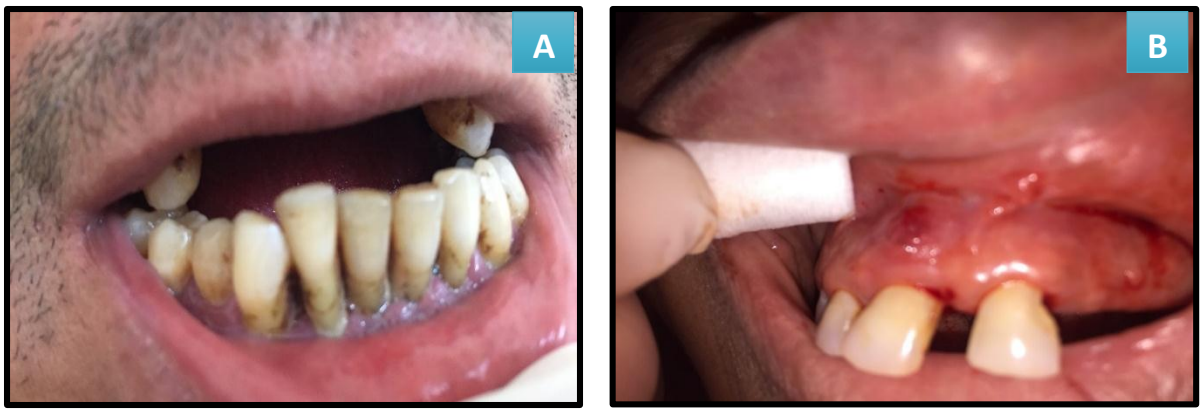

Figure (3-1): Oral Findings in type2 DM patients, a: Periodontal disease in uncontrolled DM, b: Periodontal abscess in uncontrolled DM

3.3 Cytomorphometric Analysis:

Statistical analysis of cytomorphometric data showed that the mean cytoplasm diameter, nuclear diameter and NCR ratio of study and control group of both smear sites (buccal mucosa and lateral border of tongue) are presented in Table (3-4) (3-5) (3-6) and Figures (3-2) (3-3) (3-4).

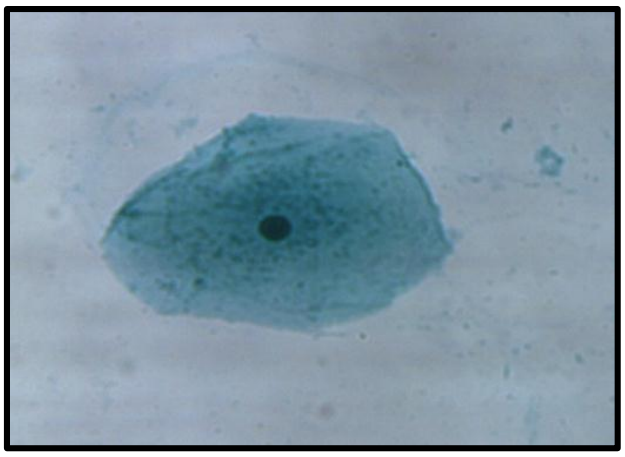

Figure (3- 2) : Pap stained cytological smear of normal healthy group (×40).

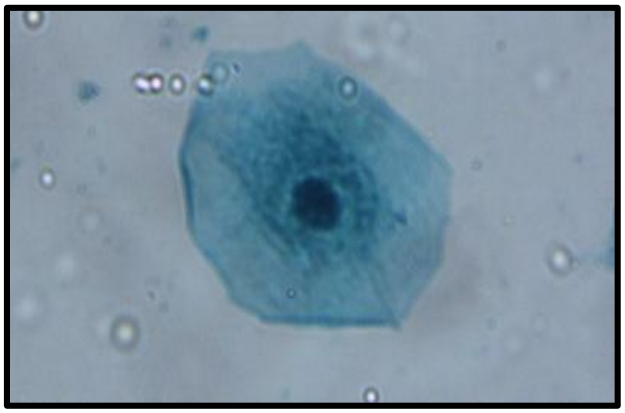

Figure (3-3): Pap stained cytological smear of controlled diabetic group (×40).

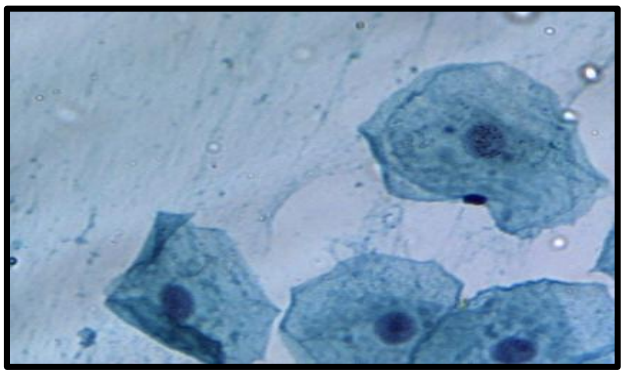

Figure (3-4) : Pap stained cytological smear of uncontrolled diabetic group (×40)

3.3.1 Nuclear Diameter:

The value of ND rises steadily from the control group to the uncontrolled diabetic group. ND in the UCD group is raised significantly when it is compared with the other groups. Table (3-4) Figures (3-5, 3-7, 3-8, 3-9).

On comparison, it was noted that the mean nuclear diameter was statistically different among all three group of study for uncontrolled, controlled and normal cases .The results showed significant increase in uncontrolled DM when compared with control (healthy) group for both (buccal mucosa *P=0.003* and lateral border of tongue *P=0.001)* and when compared with controlled DM *(P=0.007* for buccal mucosa, *P=0.003* for tongue*)*, also there's significant increase in controlled DM compared with control healthy group for both smear sites P-value *(0.001* for both tongue and buccal mucosa).

Table (3-4): Cytomorphometric Comparison of ND between control and two groups of diabetes of oral exfoliative cytology smears in both site (Mean± SD)

Cell parameter	Nuclear Diameter (µm)					
Groups	**Buccal mucosa**	**multiple comparison**	**p-value**	**Tongue lateral border**	**multiple comparison**	**p-value**
Control healthy	**7.72* ±0.58**	**Control healthy vs Controlled D.M.**	**0.001**	**8.41 ±0.00**	**Control healthy vs Controlled D.M.**	**0.001**
Controlled DM	**8.88 ±0.36**	**Control healthy vs Uncontrolled D.M**	**0.003**	**9.33 ±0.02**	**Control healthy vs Uncontrolled D.M**	**0.001**
Uncontrolled DM	**9.89 ±0.52**	**Controlled D.M. vs Uncontrolled D.M**	**0.007**	**10.02 ±0.01**	**Controlled D.M. vs Uncontrolled D.M**	**0.003**

* A significant if p-value less than 0.05 ($p<0.05$)

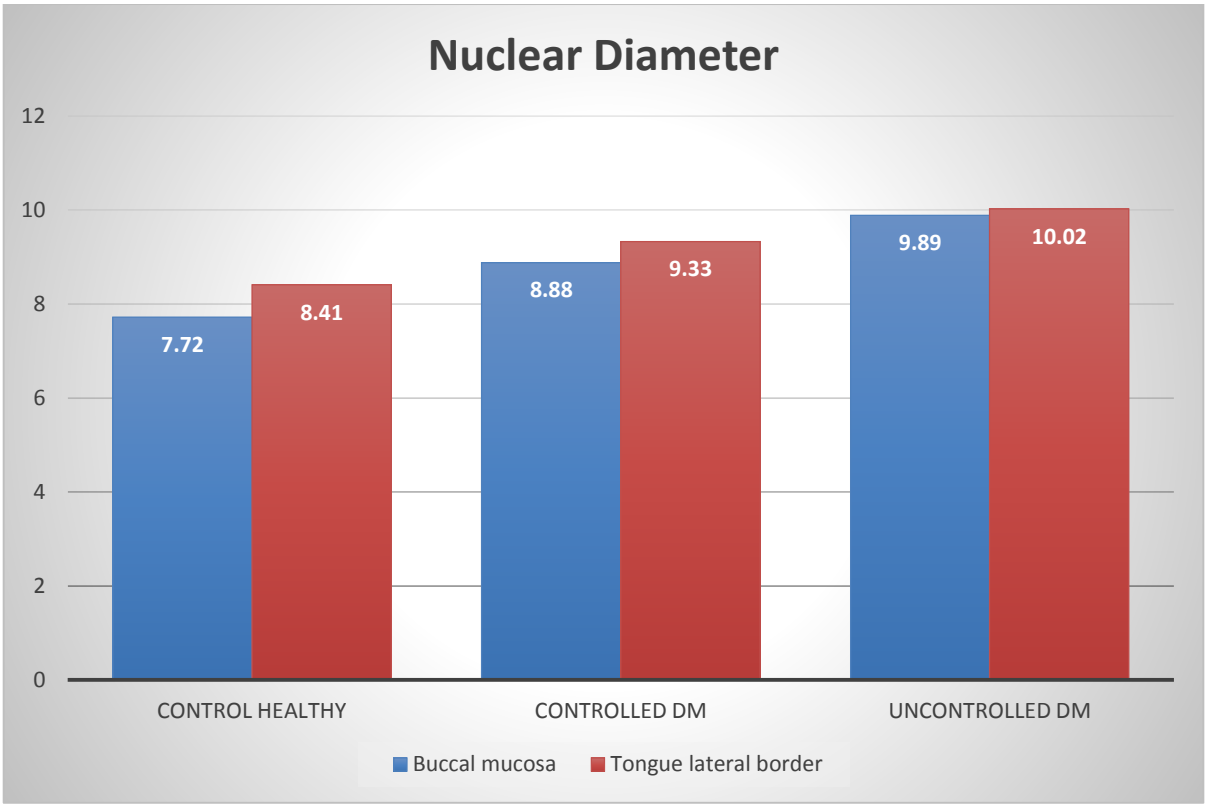

Figure (3-5): Mean nuclear diameter (ND) value in control and two groups of diabetes.

3.3.2 Cytoplasm Diameter:

The results of CyD showed significant decrease in buccal mucosa and lateral border of tongue for both uncontrolled and controlled diabetes groups when compared with control (healthy) group. In addition; there was significant decrease in uncontrolled DM when compare with controlled DM for both buccal mucosa and tongue as seen in table (3-5) .Figures (3-6, 3-7, 3-8, 3-9).

Table (3-5): Cytomorphometric Comparison of CyD between control and two groups of diabetes of oral exfoliative cytology smears in both sites (Mean± SD)

Cell parameter	Cytoplasm Diameter (µm)					
Groups	**Buccal mucosa**	**multiple comparison**	**p-value**	**Tongue lateral border**	**multiple comparison**	**p-value**
Control healthy	50.93 ±0.06	Control healthy vs Controlled D.M.	0.001	51.48 ± 0.05	Control healthy vs Controlled D.M.	0.001
Controlled DM	49.20 ±0.02	Control healthy vs Uncontrolled D.M	0.001	50.39 ±0.11	Control healthy vs Uncontrolled D.M	0.001
Uncontrolled DM	45.33 ±0.02	Control D.M. vs Uncontrolled D.M	0.004	46.24 ±0.11	Controlled D.M. vs Uncontrolled D.M	0.002

* A significant if p-value less than 0.05 (p<0.05)

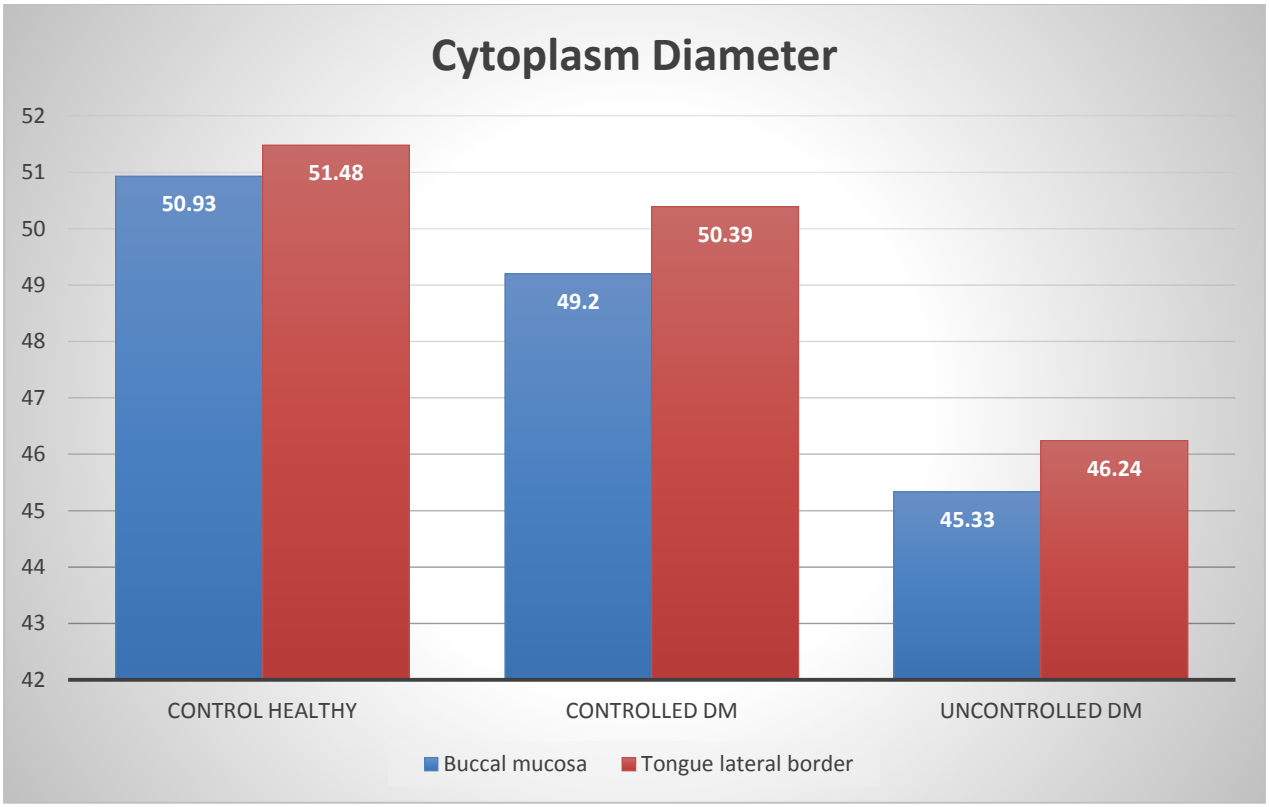

Figure (3-6): Mean cytoplasmic diameter (CyD) value in control and two diabetes groups

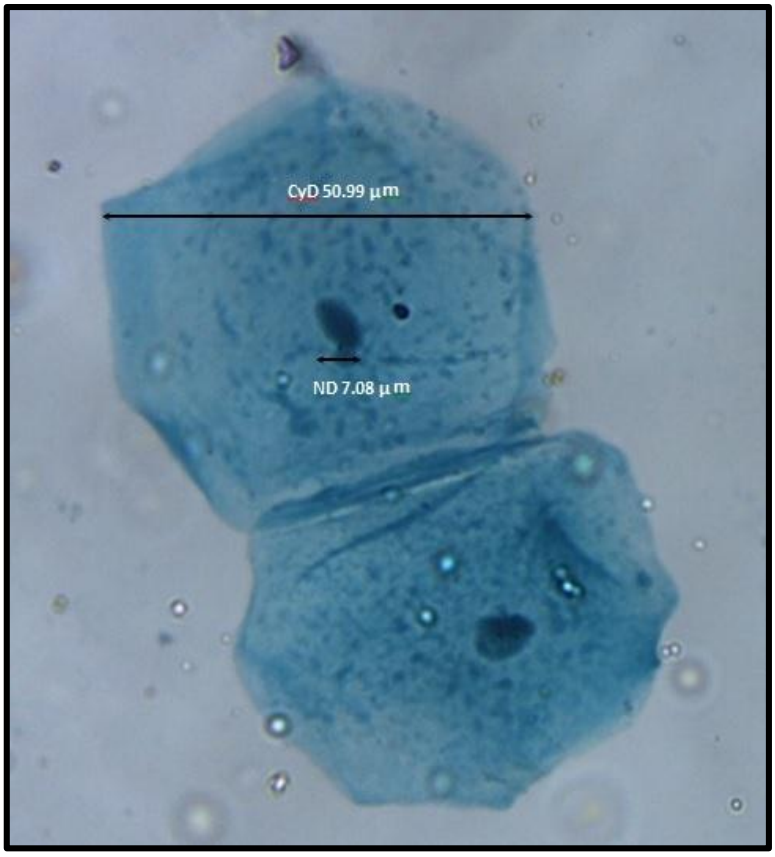

Figure (3-7) : Pap stained cytological smear of normal healthy group (×40) showing nuclear and cytoplasmic diameters

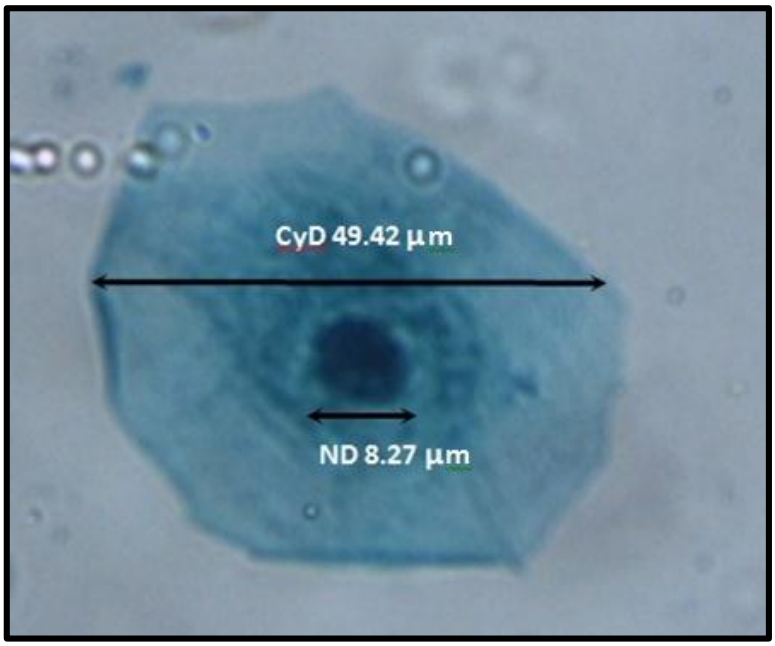

Figure (3-8) : Pap stained cytological smear of controlled diabetic group (×40) showing nuclear and cytoplasmic diameters

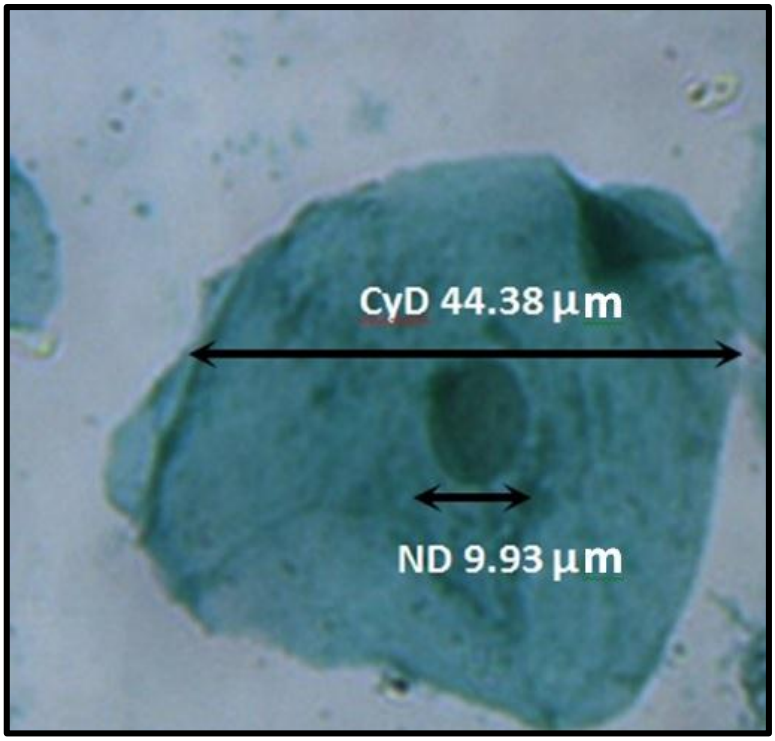

Figure (3-9) : Pap stained cytological smear of uncontrolled diabetic group (×40) showing nuclear and cytoplasmic diameters

3.3.3 Nuclear: Cytoplasmic Diameter ratio (N:CR)

The present study showed statistical significant increase in results of N: C ratio in buccal mucosa and lateral border of tongue for uncontrolled DM and controlled DM groups when compared with control healthy group. Also there's significant increase in both sites for uncontrolled DM when compare with controlled DM, as shown in table (3-6).Figure (3-10).The N: C ratio again showed similar trends as the ND. It is raised significantly in the UCD group when it is compared with the other groups.

Table (3-6): Cytomorphometric Comparison of Nuclear/Cytoplasmic ratio between control and two groups of diabetes of oral exfoliative cytology smears in both sites (Mean± SD)

Cell parameter	Nuclear/Cytoplasmic Diameter ratio (N:CR)					
groups	**Buccal mucosa**	**multiple comparison**	**P-value**	**Tongue lateral border**	**multiple comparison**	**p-value**
Control healthy	0.151 ±0.01	Control healthy vs Controlled D.M.	0.005	0.163 ±0.00	Control healthy vs Controlled D.M.	0.003
Controlled DM	0.180 ±0.01	Control healthy vs Uncontrolled D.M	0.001	0.185 ±0.00	Control healthy vs Uncontrolled D.M	0.013
Uncontrolled DM	0.210 ±0.02	Controlled D.M. vs Uncontrolled D.M	0.001	0.216 ±0.00	Controlled D.M. vs Uncontrolled D.M	0.001

***** A significant if p-value less than 0.05 ($p<0.05$)

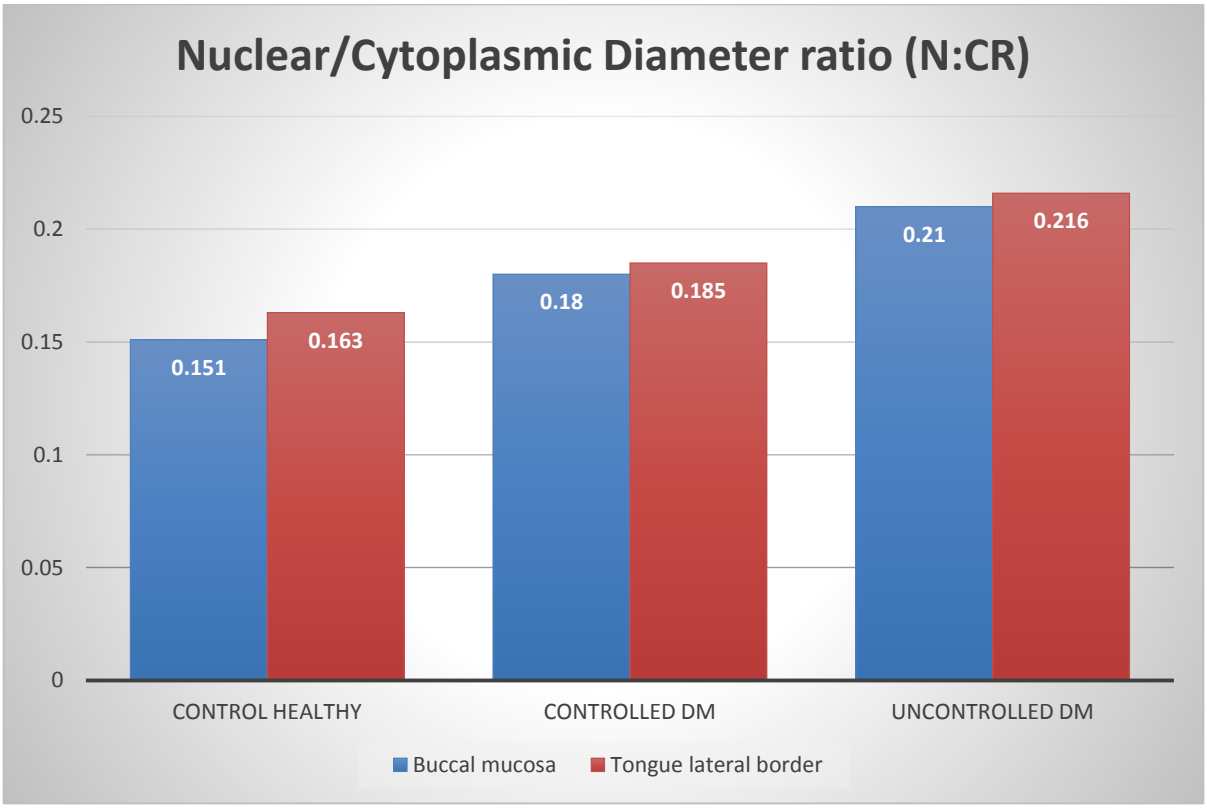

Figure (3-10): Mean Nuclear/Cytoplasmic ratio (N:CR) value in control and two diabetes groups.

3.4 Intergroup comparison of cytomorphometric parameters according to site:

In the current study cytomorphometric parameters (ND, CyD and N:C ratio) showed non-significant differences between tongue smear and buccal smear for all groups. As seen in table (3-7)

Table (3-7): comparison of cytomorphometric parameters according to site (Mean± SD)

Site			
Cell Parameter	**Buccal mucosa**	**Tongue lateral border**	**p-value**
Nuclear Diameter	8.833 ±1.041	9.283 ±0.793	0.09
Cytoplasmic Diameter	48.490 ±2.448	49.375 ±2.398	0.124
Ratio Nuclear/Cytoplasmic	0.183 ±0.029	0.188 ±0.024	0.379

***** A significant if p-value less than 0.05 ($p<0.05$)

3.5 Intergroup comparison of cytomorphometrical parameters according to site in relation to gender:

In the present study, the intergroup gender comparison for all the parameters illustrated in table (3-8). On intergroup gender comparison of control males with females, the results overall was statistically non-significant for two sites BM and T in all cytomorphometrical parameters.

In intragroup gender comparison, for (controlled and uncontrolled diabetic) males with females, as like control group also the result was statistically non-significant for both smear sites BM and T of all parameters.

However on intergroup gender comparison of diabetic males and females (controlled DM and uncontrolled DM) with control healthy males and females a higher mean ND with lower mean CyD was noted.

Tables (3-8): Intergroup gender comparison between control and diabetes groups (Mean± SD)

Parameter		Cytoplasm diameter		Nuclear diameter		Nuclear / Cytoplasmic ratio	
Groups	**sex**	**Buccal mucosa**	**Tongue lateral border**	**Buccal mucosa**	**Tongue lateral border**	**Buccal mucosa**	**Tongue lateral border**
Control Healthy	**F**	51.237 ±0.328	51.930 ±0.144	8.173 ±0.356	8.743 ±0.196	0.161 ±0.009	0.170 ±0.006
	M	50.590 ±0.266	50.590 ±0.266	7.266 ±0.261	8.078 ±0.241	0.141 ±0.004	0.156 ±0.004
P-value		**0.112**	**0.08**	**0.061**	**0.174**	**0.091**	**0.065**
Controlled DM	**F**	49.786 ±0.440	50.950 ±0.578	9.316 ±0.248	9.605 ±0.255	0.190 ±0.006	0.190 ±0.008
	M	48.628 ±0.321	49.845 ±0.380	8.461 ±0.341	9.060 ±0.115	0.173 ±0.005	0.180 ±0.005
P value		**0.084**	**0.097**	**0.102**	**0.078**	**0.085**	**0.081**
Uncontrolled DM	**F**	45.818 ±0.422	46.951 ±0.509	10.276 ±0.439	10.415 ±0.212	0.225 ±0.008	0.223 ±0.005
	M	44.843 ±0.417	45.531 ±0.249	9.505 ±0.233	10.170 ±0.367	0.208 ±0.007	0.211 ±0.007
P value		**0.062**	**0.089**	**0.144**	**0.188**	**0.075**	**0.080**

F=Female, M=male

***** A significant if p-value less than 0.05 ($p<0.05$)

3.6 Intergroup comparison of cytomorphometrical parameters according to site in relation to age:

The current study found statistically significant maximum ND values for age group of 50–60 years followed by 40–50, and 30-40 years age group with significant maximum CyD values for age group of 30–40 years followed by 40–50, and 50-60 years age group seen in Table (3-9)

The statistical result of cytomorphometrical parameters according to site in relation to age showed that the difference in ND, CyD, and N:C ratio in control healthy group of different age groups >30 and <60 was statistically significant for both sites BM and T. As shown in (Table 3-9)

On stratification it was noted in diabetic groups (controlled and uncontrolled) the results overall was statistical significant differences for both smears sites BM and T with respect to ND, CyD , N:C ratio in different age groups > 30 and < 60 years of age except in N:C ratio for T. Seen in (Table 3-9).

However on intergroup age comparison of diabetic (controlled DM and uncontrolled DM) and control healthy was noted that the ND increase with age and CyD decrease with age for both BM and T

Table (3-9): Intergroup Age comparison between control and diabetes groups (Mean± SD)

Parameters		Cytoplasm diameter		Nuclear diameter		Nuclear/ Cytoplasmic ratio	
Groups	**Age**	**Buccal mucosa**	**Tongue lateral border**	**Buccal mucosa**	**Tongue lateral border**	**Buccal mucosa**	**Tongue lateral border**
Control Healthy	**30-40**	51.425* ±0.296	52.002 ±0.877	7.130 ±0.289	7.952 ±0.154	0.140 ±0.005	0.155 ±0.007
	41-50	50.922 ±0.072	51.667 ±0.180	7.680 ±0.231	8.442 ±0.176	0.147 ±0.005	0.162 ±0.004
	51-60	50.447 ±0.188	50.795 ±0.415	8.350 ±0.123	8.837 ±0.154	0.167 ±0.004	0.172 ±0.005
p-value		**0.03**	**0.04**	**0.002**	**0.021**	**0.001**	**0.009**
Controlled DM	**30-40**	50.030 ±0.257	51.200 ±0.533	8.257 ±0.203	9.005 ±0.128	0.170 ±0.013	0.180 ±0.005
	41-50	49.140 ±0.246	50.305 ±0.228	8.492 ±0.107	9.237 ±0.091	0.182 ±0.006	0.180 ±0.009
	51-60	48.447 ±0.147	49.687 ±0.376	9.467 ±0.162	9.755 ±0.099	0.192 ±0.006	0.195 ±0.008
p-value		**0.003**	**0.005**	**0.04**	**0.02**	**0.01**	**NS**
Uncontrolled DM	**30-40**	44.640 ±0.012	45.407 ±0.012	9.407 ±0.017	10.070 ±0.428	0.205 ±0.007	0.116 ±0.004
	41-50	45.302 ±0.032	46.160 ±0.123	9.802 ±0.012	10.270 ±0.121	0.217 ±0.006	0.129 ±0.004
	51-60	46.050 ±0.017	47.157 ±0.018	10.462 ±0.012	10.537 ±0.105	0.227 ±0.030	0.118 ±0.005
p-value		**0.006**	**0.004**	**0.02**	**0.01**	**0.009**	**NS**

***** A significant if p-value less than 0.05 ($p<0.05$)

NS= not Significant

Chapter Four

Discussion

Chapter Four

Discussion

Diabetes mellitus (DM) is a syndrome characterized by abnormal carbohydrate, fat and protein metabolism that results in acute or chronic complications due to absolute or relative lack of insulin (Ship, 2003). Dentists come across various oral manifestations of this disease. If they have proper understanding of these manifestations, disease can be diagnosed. Early diagnosis is helpful in control of blood sugar level at an early stage to prevent various complications. Several studies have examined the deleterious effects of DM on oral mucosa. It was reported that DM adversely affects the morphology of oral mucosa, which may compromise tissue functions to favor the occurrence of oral infections & oral neoplasia.These effects can be studied at cellular level by using oral exfoliative cytology (Eduardo *et al*., 2004; Auluck, 2007).

The current study was opted for Hb1Ac to measure the blood glucose level as it not affected by factors like diet or medication intake, and it gives an accurate and objective measure of glycemic control over the past 3 months, thus acts as a more reliable parameter (Kilpatrick, 2004).

In the Iraq population, cytomorphometrical study done to detect the oral sign and symptoms and the state of hyperglycemia of diabetic patient type 2 (Baban and Garib ,2013). On our knowledge, first attempt has been made by this study to study morphometric and cytologic changes in the exfoliated cells of normal buccal mucosa and lateral border of tongue in type 2 diabetic patients.

4.1. Demographic Features

4.1.1. Distribution of samples according to the age and gender

On statistical analysis no significant difference between the mean ages of groups except in uncontrolled DM group there was significant increase with advance age. Also no significant difference between gender of the all groups was found with slight female predilection. In a study on Indian population by Hong *et al*. (2004), showing slight female predilection. In a similar study in United States population it was reported that the prevalence of diabetes in adults was slightly higher in women and increased significantly with age (Paul *et al*.,2003), and this in accordance with present study.

4.2 Oral findings:

The role of oral medicine specialist is established firmly in recognition of oral manifestation of diabetes mellitus. Diabetic patients suffer various oral change among which gingival and periodontal diseases are main. Some authors believe that inadequate metabolic control of diabetes is related to certain oral manifestations. (Skamagas *et al*.,2008).Others believe that it might be due to altered immunological response in diabetes such as lower chemotaxis and phagocytosis, and due to the involvement of microcirculation because of reduction in blood supply. This makes diabetic patients more prone to oral infections and alterations (Beikler and Flemmig, 2003).

The results of present study exhibited higher percentage of periodontitis in uncontrolled D.M. group (76%) than the controlled D.M was (28%),There were some studies (Javed *et al*.,2007 and Bridges *et al*.,1996) agree with this current finding reported that severity of gingival bleeding and periodontal disease was more in individuals with poorly controlled type 2 diabetes ,but contradict with study of Nichols *et al*. (1978). that seem glycemic control is not correlated with periodontal status in diabetic patients. Chandna *et al*. (2010***)*** was

also showed periodontitis to be a recognized complication of diabetes and it was more common in individuals with elevated glucose levels. Maike *et al*. (2011) suggested that the incidence and severity of periodontitis were influenced by the presence or absence of DM, as well as the severity of hyperglycemia. A number of surveys have suggested that numerous contributing factors are responsible for increased susceptibility of diabetes mellitus to periodontal diseases with increases the risk threefold because of compromised polymorph nuclear leukocyte function resulting from impaired neutrophil adherence, chemotaxis, and phagocytosis prevent destruction of bacteria in the periodontal pocket and markedly enhance periodontal destruction, angiopathy, altered microbial flora, abnormal collagen metabolism, alterations in salivary flow and composition (Mealey and Oates,2006). Abnormalities of collagen metabolism, impaired proliferation of osteoblasts and weakened mechanical properties of newly formed bone have been documented in hyperglycemic patients. (Lalla and D'Ambrosio,2001; Ship,2003 and Azodo,2009).

Also the result of present study showed that xerostomia was more frequent in the uncontrolled diabetics group (52%) when compared to controlled diabetics (4%), various studies have reported that xerostomia is one of prominent manifestation in diabetic patient; in this study the data revealed that xerostomia is a common oral manifestation among uncontrolled diabetes. This result is in agreement with study (Barbara *et al*., 2014)**.** Also the result agree with another study by Maria et al. (1995) which found xerostomia more frequent in the uncontrolled diabetics when compared to controlled diabetics.

Studies of Chavez *et al*. (2001) have suggested that older adults with poorly controlled diabetes may have impaired salivary flow in comparison with subjects with better controlled diabetes.

Xerostomia is very common symptom of the DM and has been linked with dysfunction of the parenchyma of the major salivary glands and with polyuria, and the substitution of the functioning tissue by adipose tissue has

been suggested to quantitatively and qualitatively modify saliva production, facilitating hyposalivation and burning mouth symptoms.

In addition; the result of current study showed other oral findings were less frequently such as altered taste, oral candidiasis, periodontal abscess.

Taste dysfunction has been reported in study to occur more frequently in patients with poorly controlled diabetes compared to healthy controls (Lalla and Ambrossio , 2001).Candidiasis may result from xerostomia, hyperglycemia or deficient leukocyte function and is more prevalent in diabetics with poor blood glucose control (Guggenheimer *et al*.2000).For periodontal abscess it has been statistically proven that diabetes is one of the predisposing factors for the development of periodontal abscess and more prevalent in the uncontrolled and controlled diabetics than in the non-diabetic (Ogunbodede *et al*.,2005).

Shrimali *et al*. (2011) observed hyposalivation as the most common oral manifestation, seen in 68%, followed by halitosis in 52%, periodontitis in 32%, burning mouth sensation in 32%, candidiasis, and taste alteration in 28% of cases with controlled DM. In the same study, subjects with uncontrolled DM also presented with these manifestations, with hyposalivation seen in 84%, followed by halitosis in 76%, periodontitis in 48%, taste alteration in 44%, candidiasis in 36%, and burning mouth sensation in 24%. Hence these manifestations were more in uncontrolled diabetics.

4.3 Cytomorphometrical Analysis of Parameters:

4.3.1 Nuclear Diameter:

The first parameter assessed was nuclear diameter, which was increased in diabetics. The present study evaluated the morphometric and cytologic changes in the exfoliated cells of normal buccal and tongue mucosa in type 2

diabetic patients. There was a significant increase in ND obtained from both sites. Statistical Analysis of the ND parameter among study groups was found a consistent and uniform increase from control healthy to uncontrolled diabetic group. This increase in ND is highly significant in uncontrolled diabetic patients when compared to controlled DM2 patients and to control healthy group. This finding concurs with the study by Prasad *et al*. **(**2010) where he found that diabetes severity (or in other words, the amount of glycemic control), measured with Hb1Ac, definitely influenced ND. Also agree with other study by Sadia *et al*.(2017) that gave the close results when compared to this study for the variable of ND and found the nuclear diameter showed gradual increase in size from control to uncontrolled diabetic group. This finding are also in consistent with several studies (Alberti *et al*., 2003; Jajarm *et al*., 2008; Shareef *et al*., 2008; Tozoglu and Bilge 2010; Hallikerimath *et al*.,2011; Suvarna *et al*.,2013).Where found the nuclear change was significantly higher in diabetic group.

First reason for its increase among the study group might related to sustained hyperglycemia which could be explained by delay in keratinization of oral epithelium, effects of ageing, dehydration/atrophy, and inflammatory process.

Delay in the keratinization is attributed to glycation changes. Sustained hyperglycemia causes greater accumulation of advanced glycation end products by abnormal glycation of proteins, lipids, and nucleic acids in the walls of large blood vessels as well as in the basement membrane of the microvasculature.

The progressive narrowing of the vessel lumen leads to decreased perfusion of the affected tissue and consequently decreases cell turnover, thereby explaining the delay in the keratinization process of the epithelium. This delay in the process of epithelial differentiation leads to an increase in the number of mature cells, which present a large nucleus as a primary

characteristic. (Alberti *et al*., 2003; Martin and Michael, 2003; Kumar *et al*., 2003; Jajarm *et al*.,2008).

Second factor could be xerostomia due to reduced salivary flow. Xerostomia ultimately causes increased oral mucosa trauma which leads to cell loss. Therefore, the activity of the basal cells is enhanced to replenish the lost cells by increasing the proportion of actively dividing cell compartment, which constitutes cell with prominent and large nucleus. (Suvarna *et al*., 2013).

4.3.2 Cytoplasm Diameter:

Another parameter assessed was cytoplasm diameter, the results of the present study showed the difference in mean of CyD between study groups was statistically significant decrease in cells from patients with uncontrolled and controlled diabetes when compared to control patients in both lateral border of tongue and buccal mucosa. This is similar to the findings of Prasad *et al*. (2010) noted a clear and definite decrease in cytoplasmic diameter in uncontrolled diabetes.

Also agree with Shareef *et al*. (2008) in a similar study found a statistically significant decrease in cytoplasm area, which could be due to the cell shrinkage caused by dehydration. This theory is supported by the findings of Ogden *et al*. (1999) have reported a decrease in CyD in patients with alcoholism, and they have proposed that this might be due to the dehydration seen in them. A similar condition of dehydration is also found in diabetic patients, and this might explain the decrease in CyD. Also this finding is indicated to the findings of Frost *et al*. (1997) according to him, in an actively growing cell the cytoplasmic size decreases whereas nuclear contents increases because it is undergoing replication. In another study by Sadia *et al*. (2017) who found decrease in cytoplasm diameter CyD, but no significant difference was noted among all the study groups. Alberti *et al*. (2003) in his study also found

decrease in cytoplasm area among diabetic group but he concluded that cytoplasmic area did not show any significant difference in diabetic individuals. This finding is contradictory to the findings of Jajarm *et al*. (2008) who reported a significant increase in cytoplasmic area the in diabetic patients.

4.3.3 Nuclear : Cytoplasmic Diameter (N:C) ratio

In the present study, an increase in N:C ratio was evident in patients with type2 DM. On statistical analysis significant increase was found in the mean values of N:C ratio of the exfoliated cells from the buccal mucosa and tongue smears with uncontrolled and controlled diabetes when compared to control patients.

A gradual increase in N:C ratio was noticed as progress from control healthy group to uncontrolled diabetics which is highly significant. The relatively greater increase in nuclear diameter compared to cytoplasmic diameter may explain this phenomenon. The N:CR values were coincident with the study realized by Prasad *et al*.(2010) where he found that diabetes severity, measured with Hb1Ac, had a relation with the increase of the ND and N:CR. Also this finding similar to other studies (Alberti *et al*., 2003; Shareef *et al*., 2008). But inconsistent with the findings by Jajarm *et al*. (2008) according to him the mean N:CR was significantly lower in diabetic group as compared to controls.

4.4 Inter-group comparison of cytomorphometric parameters according to site

In this study, the smears was taken from different oral sites (the buccal mucosa and lateral border of tongue). On intergroup comparison according to site, the present study showed no significant variation in ND, CyD and N:CR. This finding Agree with study by Saritha *et al.*(2017) that reported no statistical

significant difference was found in the cytoplasmic area, nuclear area and nuclear cytoplasmic ratio between the two sites. This finding is disagree with the result by Cowpe *et al*. (1985) in that they display a significant variation in nuclear and cytoplasmic size between different sites.

4.5 Intergroup gender comparison of cytomorphometrical parameters according to site in relation to gender in study and control groups :

The result of the present study on intergroup comparison according to gender in BM and T smears sites showed that there was no significant variation in cytoplasm diameter, nuclear diameter, and N:CR in control group. This agree with a sex‑related survey by Cowpe *et al.* (1985) on normal oral squames showed that there was no significant variation in cytomorphometrical parameters according to gender. Also concure with Sahay *et al.* (2017) who found the majority of the parameters did not show a significant result on intragroup gender comparison in control group.

On intergroup comparison between males and females in diabetic group (controlled and uncontrolled DM) the result of present study showed that there was no significant variation in cytoplasm diameter, nuclear diameter, and N:CR for both sites. This finding agree with a study by Sahay *et al.* (2017) who reported that statistically nonsignificant result was found in intragroup gender comparison in diabetics.This reflected that, in diabetics, gender has no effect on morphometric changes in the cell. Contradictory to the present study with Patel *et al.* (2011) observed some morphometric changes on gender comparison, which could be due to sexual dimorphism and hormonal differences.

4.6 Intergroup age comparison of cytomorphometrical parameters according to age in study and control groups :

There was a significant variation of ND, CyD and N:C ratio with respect to age. The result showed that ND was significantly increase with age from 30-40 to 50-60 years and CyD was significantly decrease of all age groups in diabetic groups and control healthy group. The result found statistically significant maximum ND values for age group of 50–60 years followed by 40–50, and 30-40 years age group with significant maximum CyD values for age group of 30–40 years followed by 40–50, and 50-60 years age group.

In comparison with a study carried by Nayar and Sundharam (2003) who reported that the ND was increased with age and CyD was decreased with age, and this finding was in accordance with present study.

Another study stated that age related variations were present in the buccal mucosal cells in female subjects (Preethy *et al*., 2013). Similarly Cowpe *et al.* (1985) also showed a significant variation with age. And these finding were agreement with present study. Sadia *et al.* (2017) was noted that there was significant difference with respect to nuclear diameter according to age group. But no significant difference was noted with respect to cytoplasm diameters among the study groups of diabetes. However, present study is not in agreement with the results of Lee *et al.* (1973) who reported no significant variations in nuclear diameter and cytoplasmic diameter with age.

In an attempt to justify the increasing the size of the nucleus in patients who are diabetic, the evidence is practiced by epithelial cell aging. Aging is linked to the age of patients, because the type 2 diabetes is a disease also associated with more advanced decades of life. Another pattern that is directly related to aging, is the rate of cell turnover, which undergoes a decrease related adverse effect of ischemia due to atherosclerosis patients suffering from diabetes (Morris *et al*., 2000). There is also an accumulation of the end products

of advanced glycation, which involved not only in the pathogenesis of diabetes, but also in cellular aging (Kumar *et al*., 2012). Previous studies have described the altering cell turnover as one possible explanation for the observed changes in diabetic patients, Finally, it is possible to say that cellular alterations occurred in patients with DM2, are related to blood glucose levels.

The present study showed significantly increase in nuclear diameter and significantly decrease in cytoplasmic diameter in the diabetes groups as compared to controls. The cytomorphometric alterations demonstrated in the present study suggests that even though the oral mucosa of diabetic patient appears clinically normal, cytologic changes are present. The general understanding of the alterations in the cellular pattern of oral mucosal cells in diabetic patients provide health professionals with a non-invasive tool for verification of clinical diabetes. In the background of the association of diabetes mellitus with various oral neoplastic and inflammatory diseases the early changes in oral cavity can be ascertained through cytology, more so through cytomorphometry.

Thus, cytomorphometry may be an efficient tool to understand the extent of cellular changes that occur in oral epithelial cells in diabetics that are secondary to microscopic changes like vascular occlusion and molecular changes such as oxidative stress. Therefore, cytomorphometric analysis of oral mucosal cells could be implicated as a noninvasive technique for screening and monitoring of the disease status in diabetic patients.

Chapter Five

Conclusions & Suggestions

Chapter Five

Conclusions and Suggestions

5.1. Conclusions

1. Most frequent oral manifestation in type 2 DM patient were periodontal disease and dry mouth

2. There was a clear and definite statistical significant increase in ND as we progress from normal individuals to patients with uncontrolled diabetes.

3. In contrast to the increase in ND, there was a statistical significant decrease in CyD in uncontrolled diabetes.

4. The N:C ratio gradually and steadily significant increased from normal individuals to uncontrolled diabetics.

5. In addition to these morphometric changes, no statistical significant difference was found in the ND, CyD, and nuclear cytoplasmic ratio between the two sites

6. On intergroup comparison according to gender in BM and T smears sites showed that there was no significant variation in Cytoplasm diameter, Nuclear diameter, and N:CR in all study group.

7. On stratification it was noted that there was a significant variation of ND, CyD and N:C ratio with respect to age.

5.2. Suggestions for further studies

1. Assessment of the cytomorphometrical changes in patients with other types of diabetes mellitus like type 1 DM .
2. Further study are needed regarding the duration of the disease or the effect of different type of treatment.
3. Using other cytomorphometrical parameters such as nuclear roundness factor (NRF) , nuclear perimeter (NP), nuclear width (NW), and nuclear lengeth (NL).
4. Carrying same study on other nutritional diseases like Iron Deficiency Anemia, autoimmune diseases like Rheumatoid Arthritis and Systemic Lupus Erythmetosus.

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Appendices

Appendices

Appendix I: Consent Form

موافقة المشترك

لقد قرأت استمارة القبول هذه وفهمت مضمونها وتمت الاجابة على اسئلتي جميعها وبناء عليه فأنني حر مختار اجيز اجراء هذا البحث واوفق على الاشتراك فيه وانني اعلم ان الباحثة الدكتورة وزملاءها ومعاونيها او مساعديها سيكونون مستعدين للاجابة على اسئلتي وانه باستطاعتي الاتصال بهم على الهاتف واذا شعرت لاحقاً ان الاجوبة تحتاج الى مزيد من الايضاح فسوف اتصل باحد اعضاء لجنة الاخلاقيات (350000-01) المقسم (5445). كما اعرف تمام المعرفة بانني حر في الانسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقة دون ان يؤثر ذلك على العناية الطبية المقدمة لي كما اعلم اني سوف احصل على نسخة طبق الاصل من هذه الموافقة .

_____ اسم المشترك

_____ توقيع المشترك او ممثله القانوني او وليه الجبري او وصيه

_____ اسم الممثل القانوني او الولي الجبري او الوصي

_____ التاريخ والساعة (بيد المشترك او ممثله القانوني او وليه الجبري او وصيه)

_____ اسم الشاهد (اذا كان المشترك او الوصي امياً)

_____ توقيع الشاهد

التاريخ والساعة _____

Appendix II: Case Sheet

Pt. Name: Age: Gender:

Date: Adress: Occupation:

HbA1c:

Medical history:

Oral manifestations: yes no

Periodontal disease:

Dry mouth:

Altered taste:

Candidiasis:

Others:

الخلاصة

المقدمة:

داء السكري هو اضطراب الغدد الصماء الأيضي الشائع للغاية الذي يؤدي إلى ارتفاع مزمن لمستوى السكر في الدم. له تأثيرات على أنسجة الجسم المختلفة. بسبب زيادة مستويات الجلوكوز في الدم ، تحدث تغيرات خلوية كبيرة في تجويف الفم أيضًا. التقشير الفموي الخلوي يعتبر تقنية سريرية بسيطة غير مدمرةيمكن استخدامها لتحديد التغيرات الشكلية والتغيرات الكمية الخلوية في الخلايا المقشرة.

اهداف الدراسة:

كان الهدف من الدراسة هو تحليل القياس الكمي للخلايا الظهارية الفموية في مرضى السكري من النوع 2 والاصحاء بواسطة مسحات للخلايا التقشرية ، لمقارنة القطر السيتوبلازمي ، القطر النووي ، ونسبة النواة الى السيتوبلازم في مرضى السكري من النوع الثاني والأصحاء . بالإضافة إلى تقييم المظاهر الفموية في مرضى السكري من النوع 2.

المواد و طرئق العمل:

العينة الكلية مكونة من 75 بالغ ، تتراوح أعمارهم بين 30-60 سنة. (50 مريضا يعانون من داء السكري من النوع 2 ينقسم إلى المسيطر وغير المسيطر على أسس مستويات HbA1c و 25 شخص غير مصاب بالسكر كمجموعة سيطرة). تم الحصول على المسحات من (الخلايا المخاطية المبطنة للخد والحدود الجانبية للسان) . وضعت العينات على شريحة زجاجيّة نظيفّة جافّة وثبتت على الفور في 96٪ إيثيل كحول بعد ذلك صبغت بصبغة بابانيكولاو لتحليل القياسات الكمية الخلوية . استعملت العدسة العينية الميكروميتر وأخذت لكل عينة قيم متوسّطة من القطر النووي ، القطر السايتوبلازمي ، و نسبة النواة الى السايتوبلازم .

النتائج:

أظهرت النتائج أن زيادة معنوية ذات دلالة إحصائية في (P = 0.001) في القطر النووي للغشاء المخاطي المبطن للخد والحدود الجانبية للسان ، مع انخفاض ذو دلالة إحصائية في القطر السايتوبلازمي (P = 0.001) لكل من اللسان ومخاطية الخد تم العثور عليها في مرضى السكري مقارنة مع المجاميع الاخرى. درجة السيطرة على نسبة السكر في الدم أثرت بشكل ملحوظ على القطر النووي لمخاطية الخد (P = 0.007) وللحدود الجانبية للسان (P = 0.003) وعلى نسبة النواة الى السايتوبلازم (P = 0.001 لكل من اللسان ومخاطية الخد . بشكل عام ، كلما زادت شدة مرض السكري ، ارتفعت نسبة القطر النووي ونسبة النواة الى السايتوبلازم تدريجياً. أظهرت النتائج عدم وجود فروق ذات دلالة إحصائية في قطر سايتوبلازمي ، القطر النووي والنسبة السيتوبلازمية النووية بين الموقعين. اختلفت

قطر النووية وقطر السايتوبلازمي بشكل كبير مع التقدم في السن. لم يكن هناك اختلاف كبير في أي معيار بين الذكور والإناث.

الاستنتاجات :

تشير النتائج إلى أن النوع 2 من داء السكري يمكن أن يؤدي إلى تغيرات محددة في الخلايا الظهارية الفموية ، يمكن اكتشافها عن طريق التحليل المجهري والقياس الكمي باستخدام علم الخلايا التقشري الذي يمكن استخدامه في تشخيص المرض. بالإضافة إلى المظاهر الفموية الرئيسية التي وجدت في مرضى السكري النوع الثاني كان الاكثر شيوعا مرض اللثة والجفاف الفموي.

وزارة التعليم العالي والبحث العلمي

جامعة بغداد

كلية طب الاسنان

تحليل القياسات الكمية الخلوية للخلايا الظهارية المخاطية الفموية في مرضى السكري من النوع 2

رسالة مقدمة

الى مجلس كلية طب الاسنان/ جامعة بغداد

كجزء من متطلبات درجة الماجستير في طب الفم

من قبل

رؤى شاكر فرحان

بكالوريوس طب وجراحة الفم والاسنان

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ماجستير امراض الفم

العراق- بغداد

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