Ministry of Higher Education

and Scientific Research

University of Misan

College of Science

Department of Biology

Comparative Cytological Study on Oral Mucosa in Diabetic and Healthy People in Misan Governorate.

A Thesis

Submitted to the Council of the College of Science/University of Misan as Partial Fulfillment of the Requirements for the Master's Degree in Biology

By

Noor Saeed Anead

B.Sc. Biology (2019 – 2020)

Supervised

Assist. Prof. Dr. Ali Khalaf Ali

Assist. Prof. Dr. Maytham Abdul Kadhim Dragh

2024 A.D. **1446 A.H.**

بسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قَالُوا سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ

أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العلي العظيم

سورة البقرة (الآية32)

Supervisor Certificate

We certify that this thesis entitled "(Comparative Cytological Study on Oral Mucosa in Diabetic and Healthy People in Misan Governorate) "

Submitted by (Noor Saeed Anead)has been prepared under my supervision at the College of Science,
University of Misan; as partial fulfillment of the requirements for the Degree of Master in Biology.

Signature
Assist. Prof. Dr. Ali Khalaf Ali

Assist. Prof. Dr. Maytham Abdul Kadhim Dragh

Department of Biology
College of Science/ University of Misan
Date: / /2024

Recommendation of Head of Biology Department
Because of the available recommendations; I forward this thesis to debate by the examining committee.

Signature
Assist. prof. Dr.Maytham Abdul Kadhim Dragh
Head of Department of Biology
College of Science/ University of Misan
Date: / /2024

Dedication

To our absent sun…

To our long-awaited hope...

To our savior from the darkness of falsehood to the light of truth

To our refuge when life strikes us…

To our prayers in every prayer that Allah makes us from his supporters …

To the rest of Allah is in his land…imam (AL-Mahdi)

To my strong self who endured all the pitfalls and continued despite the difficulties

To the one who filled me with love, always provided me with strength, and was my husband to lean on in all my stumbles

To the one who supported me without limits and gave me freely, my father

To the one whose prayers were the secret of my success, my mother

To those who were my source of support, strength, and pride, my aunt and uncle

To those whom God has blessed me with so that I may know from their permissible taste the taste of life, my children (John - Aba Al-Fadl)

Noor Saeed

Acknowledgments

Praise be to God Almighty first and last, who has blessed me with the gift of patience, ability and perseverance to complete the work, and praise be to God Almighty, the Almighty, the Blessed, the Exalted, filling the heavens and the earth, for the honor He has bestowed upon me in completing this study. I hope that it will gain your approval, and thanks to the Messenger of Mercy and Humanity, Muhammad al-Hadi al-Amin, and his good and pure family... and his loyal companions who taught us to perform the trust and seek knowledge. And many thanks to Dr. Saadi Yahya Ayed, who helped me in the statistical work of the results. I am pleased to put the final marks on my thesis to express my sincere thanks and gratitude to my dear Assist. Professor Dr. Ali Khalaf Ali, and Professor Dr. Maytham Abdul Kadhim Dragh, who kindly supervised my thesis and suggested the topic of the thesis, and their continuous encouragement to me in all stages of the research work. I wish him every success and success in health and wellness, and may God reward them on my behalf with the best reward. I also sincerely thank my distinguished professors and the Deanship of the College of Science, especially the distinguished Dean.

I would like to extend my sincere thanks and gratitude to my family, especially my father, mother, brothers, and sisters, and to the donors who deserve this message. May God reward them on our behalf with the best reward. I ask God to grant them health, wellness, and a speedy recovery.

Noor Saeed

Summary

The study aims to identify the phenotypic changes in oral epithelial cells using exfoliative cytology from type 1 and type 2 diabetic patients and compare them with healthy people, and to know the relationship between the number of cells calculated from the oral mucosa and different areas of the oral cavity and the incidence of diabetes; In this experiment, the ages of males and females used in the study ranged from 1-70 years. After obtaining the patient's consent and the approval of the Scientific Research Ethics Committee at the University of Maysan, samples were collected from the Diabetes and Endocrinology Center in Maysan Governorate, and the period took from October 10\24 to June 6\1, and samples were taken from four areas of the oral mucosa (cheek, gums, tongue and palate) to collect these samples. This study included 100 people, who were divided into two groups, the first group (healthy control group) consisted of 50 people, while there were 50 people in the second group.

The diagnosis of diabetes was based on doctors who performed the diagnostic examination at the Diabetes Center, the cumulative blood sugar analysis, and the rapid analysis, where blood samples of about 5 ml were taken from each person for testing. Samples were taken in the morning from people who had their blood sugar levels measured. After taking the samples, they were stained with several stains: Papas, hematoxylin, eosin, MitoTracker, DCFH-DA, and MitoSox.

The results of the current study indicate that there was a significant difference at the level (0.05) in the number of squamous cells falling from

the oral cavity between patients with type 1 and type 2 diabetes compared to healthy individuals.

The number of cells increased in the cheek area, while a decrease was observed in the gum area. The results showed significant differences in the number of cells observed in all areas of the oral cavity, and the number of cells in the oral mucosa in the cheek, gum, tongue, and palate areas was diagnosed according to age groups and based on the level of sugar and cumulative sugar. From the results of this table, it is clear that there is a significant statistical relationship at the level of P≥0.05 between the number of cells counted from the oral cavity and the sugar level according to age groups.

The current results showed a significant difference of 0.05 between the diameters of the nuclei of people according to age between diabetics and non-diabetics, as the ND rate in the non-diabetic group increased significantly compared to the other group. The age ratio level equals (0.417), while the Moral level ratio for diabetic patients is (0.174). The results also showed that the age groups 0-15 and 46-70 did not have statistically significant differences, meaning that A = B, while the age groups 16-30 and 31-45 have statistically significant differences, as A ≥ B. The current results showed statistically significant differences in the diameters of the cytoplasm in all age groups, as A ≥ B when comparing the control group with the diabetic group, according to age groups. The present results showed that there was a statistically significant increase in the nuclear-to-cytoplasmic ratio results in diabetic and non-diabetic subjects in the age groups 0-15, as was greater than B, showing significant different 0.05, while the rest of the age groups from 16 to 70 years were not significantly affected.

Summary

In non-diabetic subjects, careful examination of the microstructure of mitochondria under the sarcoma and between myofibrils revealed well-defined and uniform peaks after staining with lead citrate and uranyl acetate detector the results showed that mitochondria in non-diabetic subjects function normally. In diabetic subjects, microscopic features of cheek cells revealed several abnormalities: loss of mitochondrial peaks, rupture of mitochondrial outer membranes, and endothelial hyperplasia. Clear disarray and a decrease in the number of mitochondrial peaks were observed. The results showed that diabetic subjects have degenerated mitochondria that do not function normally.

The present results of fluorescent microscopic cell sections showed increased release of mtROS in diabetic cells, and fluorescent microscopic results also showed increased release of ROS.

Other fluorescent microscopic cell sections showed increased release of mitoTreaker in diabetic cells, and fluorescent microscopic results also showed increased release of ROS. (mitoTreaker). The cells show enlarged mitochondria with broken cristae there are many enlarged mitochondria of different sizes and varying degrees of cristae disorganization.

List of contents

List of contents

List of contents

List of Tables

List of Figures

List of Figures

List of Diagram

List of Graphs

List of Abbreviations

Periodic acid Schiff	PAS
Haematoxylin & Eosin	H&E
Diabetes Insipidus	DI
Cytoplasmic diameter	CyD
Nuclear to Cytoplasmic ratio	N: CR
Nuclear diameter	ND
Cytoplasmic diameter	CD
beta cell	ß-cell
Glycosylated Hemoglobin	HbA1c
Social Package of Social Sciences	SPSS
Percentage	%
American Diabetes Association	ADA
Antidiuretic hormone	ADH
Fasting plasma glucose	FPG
Oral glucose tolerance test	OGTT
Degree of freedom	d.f
Grams	G
Dichlorodihydrofluorescein diacetate	DCFH-DA
Figure	Fig.
Number	N
Milligram	Mg
blood glucose level	BGL

List of Graphs

Mitochondrial ROS	mtROS
World Health Organization	*WHO*
standard deviation	SD
Unit per litre	(*U*/*L*)
Id est	i.e
Reactive oxygen species	ROS
p-value	P
Papanicolaou	PAP
Phosphate Buffer saline	PBS
Neutrophils	PMN
Diabetes mellitus	DM
Cytoplasmic area	CYA

Chapter One

Introduction

Introduction

Diabetes is a group of metabolic diseases characterized by high blood sugar levels. The disease is caused by a lack of insulin in the body's cells, making it difficult for the body to use the insulin secreted by the pancreatic beta cells. The only hormone that lowers blood glucose levels is insulin; other hormones that raise blood glucose levels include thyroid, glucagon, growth hormone, catecholamines (adrenaline and noradrenaline),and glucocorticoids (World Health Organization, 2009).

No matter what type of diabetes a person has, their mouth tissues react and show distinctive symptoms. to destroy the oral and dental structures, these manifestations can be used as a diagnostic tool for diabetes during normal oral examinations and screening programs to identify oral disorders. (Braunwald *et al*., 2003; Shoback *et al*., 2011).

The blood glucose level (RBS) and glycosylated hemoglobin level (HbA1c) are important markers of metabolic regulation, which is a major predictor of this frequent disorder and the potential consequences of diabetes. Glycosylated hemoglobin (HbA1c) is a single measurement that provides the average plasma glucose over the last two to three months. It may be taken at any time of day and doesn't require any particular preparation, such as fasting. The (HbAlc) test is presently regarded as the gold standard and best indicator of glycemic management. HbA1c is a measurement of total hemoglobin that falls between 4% and 6% in healthy people, although it can be higher in hyperglycemic patients. Given that the HbA1c test, which gauges the quantity of glucose bound to hemoglobin A1C

molecules, controls the severity of diabetes mellitus and the effectiveness of metabolic.

therapy (Delong & Burkhart, 2008). Glycated hemoglobin (HbA1c), a measure of average plasma glucose over many weeks, was endorsed by an international committee for the diagnosis of diabetes in 2009 as a marker for the illness (American Diabetes Association, 2010). The American Diabetes Association (ADA) also recommended in 2010 that an HbA1c of > 6.5% (48 mmol/mol) be used as a diagnostic indicator for diabetes (Selvin et al., 2010). When it comes to predict diabetes, HbA1c is seen as being on par with fasting plasma glucose (FPG) (D'Emden *et al*., 2012).

The detrimental effects of diabetes on the oral mucosa have been the subject of several investigations. Diabetes has been shown to hurt the morphology of the cheek mucosa, which may impair tissue function and increase the risk of oral infections and neoplasia (Eduardo *et al*., 2004; Auluck, 2007).

Exfoliative cytology is the microscopic analysis of shed or desquamated cells from the mucous membrane or other epithelial surface. The study of cells obtained by scraping the surface of the tissue or from bodily fluids like saliva, sputum, etc. is also included in this (Das Bijoy Kurnar, Mallik.,2000)

With the advancements in quantitative oral exfoliative cytology, oral cytology once again emerged as a diagnostic aid in oral lesions. The accuracy of cytologic techniques in diagnosing oral lesions can be enhanced by morphometry. (Cowpe JG, &Longmore, 1981)

Computer-assisted morphometric analysis of cells improved the ability to accurately measure the various cell parameters such as nuclear diameter (ND), cell diameter (CD), nuclear area (NA), cytoplasmic area (CyA), and nuclear-to-cytoplasmic ratio(Sumanthi J *et al*.; 2012).

As the science of quantitative exfoliative cytology has advanced, oral exfoliative cytology has emerged as a potent diagnostic instrument. It is a

non-aggressive method that the patients readily embrace.

For this reason, it can be a very effective alternative for squamous cell carcinoma, mouth cancer, and epithelial atypia early detection. image analysis techniques due to recent improvements, which yield faster, more accurate, more repeatable findings. precision and in a much shorter amount of time(Ogden *et al*.;1997).

Thus far, only one study (Hoseinpour *et al*., 2010) has reported on the comparison of cytomorphometric changes of the oral mucosa of people with diabetes types I and II. A few studies have examined changes in the oral mucosa of diabetic patients and reported the replacement change in epithelial cells of the oral mucosa by cytology method (Hoseinpour *et al*.,2010; Shareef.,2008). There are several ways to diagnose diabetes, by measuring FPG. Still, this method has the disadvantage that the person must fast for no less than 8 hours, because the diagnosis cannot be made in most patients who come in the afternoon appointments or if they eat before the morning appointment. In addition to this, is done by obtaining venous blood, and there is another way to diagnose diabetes, which is measuring the HbA1c level, as it only requires venous blood, as this method is considered to be easier than the HbA1c method (Gavin *et al* .,1997). A new method has been discovered to diagnose diabetes based on the number of cells taken from the oral cavity. This is considered an easy, simple, harmless, quick method that does not require venous blood.

Active and vital organelles known as mitochondria play a key role in many crucial cellular functions many diseases, including cancer, cardiovascular disease, and neurological disorders, have been linked to their

dysfunction, which results in their inability to perform their function properly.(Mizushima *et al*.,2008; Choi *et al*.,2013).

The imaging of mitochondria in live cells may be done non-invasively using conventional fluorescence microscopy. Electron microscopy has been used to identify sites of physical contact between organelles.(Soubannier *et al*.,2012)

Aims of the study:

Due to the lack of studies on this topic, this study was conducted, which aims to do the following:

1- Identifying cellular changes in the oral mucosa in person with diabetes compared with healthy..

2- Find out the difference between stains and the distinction of each one in terms of speed, cheapness, and method of staining

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

4- Investigate the relationship between the number of cells calculated from the oral mucosa and different areas of the oral cavity and the incidence of diabetes. Furthermore, the study indicates the possibility of adopting this relationship as a novel method for diagnosing diabetes

5- Knowing the extent of activity, and cell gene expression by studying

Chapter Two
Literatures Review

2-1 Human Oral Mucosa:

Three kinds of oral mucosa may be distinguished histologically: masticatory, lining, and specialized. The hard palate and gingiva are covered by the masticatory mucosa. The lamina propria securely attaches the masticatory mucosa to the underlying bone, and the epithelium covering it is keratinized to endure the continuous pounding of food during mastication. In contrast, the lining mucosa has to be as flexible as possible in order to carry out its protective role. The lamina propria is shaped for mobility and is loosely attached to surrounding tissues; the epithelium is not keratinized. A unique mucosa covering the tongue's dorsal surface is made up of a highly extensible masticatory mucosa with taste buds and papilla(Glim *et al*.,2013)

The oral mucosa is entirely coated in squamous stratified epithelium. The thickness and level of keratinization of this highly structured, avascular, semipermeable tissue vary depending on where it is located in the mouth cavity and the mechanical and functional requirements of the region. The lamina propria and the epithelium are connected by an interdigitated contact. The underlying papillary projections of the lamina propria are attached to the rete pegs, which are the undulating projections of the deeper layer of the epithelium. The epithelium is firmly attached to the non-cellular basement membrane that separates these two tissues. The basement membrane links the epithelium to the connective tissue and supports it. It appears as a boundary between the lamina propria's connective tissue and epithelium under light microscopy. Electron microscopy, on the other hand, provides a clearer image of the basal lamina, which is further separated into lamina lucida and lamina densa.

Three distinct forms of oral mucosa may be distinguished based on their histology, clinical characteristics, and functional makeup. Lining, or movable mucosa, refers to the mucosa that lines the mouth's mobile tissues. This mucosa is located on the floor of the mouth, vestibular fornix, cheeks, lips, and soft palate. An epithelium known as non-keratinized stratified squamous epithelium covers the lining mucosa (Groeger &Meyle, 2019). Masticatory mucosa refers to the hard palate and associated gingiva's stiff mucosa that is firmly linked to the underlying bone. The keratinized or para-keratinized stratified squamous epithelium that covers these surfaces gives the masticatory mucosa its ability to withstand the stress that it experiences during mastication. The tongue's dorsum also has a specific mucosa that displays a squamous stratified epithelium that may or may not be keratinized. Its distinct characteristic of possessing several lingual papillae and taste buds that enable taste perception earns it this moniker. The tongue's dorsum actively engages in mastication, so this mucosa is occasionally referred to as masticatory mucosa. In keratinized oral mucosa, or the masticatory mucosa, the oral epithelium is composed of four layers. We discovered the stratum spinosum, stratum granulosum, and stratum corneum starting with the deeper layer. There are two layers above the stratum basale where the epithelium is nonkeratinized, like in the lining mucosa: the stratum filamentosum and the stratum distendum.(Groeger &Meyle, 2019). Additionally, the granular layer is absent from the non-keratinized epithelium that lines the mucosa, and the spinous layer is typically considered to be thinner.(Otsuka-Tanaka etal., 2013).

2-1-1 Non-keratinocytes in The oral Epithelium:

Studies clearly show that these cells represent a range of distinct cell types, including pigment-producing cells (melanocytes), Langerhans' cells, Merkel cells, and inflammatory cells like lymphocytes, which collectively can make up as much as 10% of the cell population in the oral epithelium.(Squier& Finkelstein,1998)These cells can be found in many histologic sections of the oral cavity. Except for Merkel cells, none of these cells have desmosomal linkages to neighboring cells. As a result, the cytoplasm shrinks around the nucleus to form the transparent halo during histologic processing. Since none of these cells engage in the maturation process observed in oral epithelia or have the abundance of desmosomes and tonofilaments found in epithelial keratinocytes, they are often collectively called non-keratinocytes. (Squier& Finkelstein,1998)

2-1-2 Keratinocytes in The Oral Epithelium

Keratinocytes cover both the skin and some oral mucosa, but the morphology of each tissue and the behavior of the keratinocytes from these two sites are different. One significant dissimilarity between the two sites is the response to injury (Bogdan Calenic.,2015). Oral mucosal wounds heal faster and with less inflammation than equivalent cutaneous wounds.

keratinocytes might have intrinsic differences at baseline as well as in the response to injury, and such differences would be reflected in gene expression profiles.

Oral keratinocyte stem cells reside in the basal layers of the oral epithelium,representing a minor population of cells with a great potential to self-renew and proliferate for their lifetime. As a result of the potential uses

of oral keratinocyte stem cells in regenerative medicine and the key roles they play in tissue homeostasis, inflammatory conditions, wound healing, and tumor initiation and progression, intense scientific efforts are currently being undertaken to identify, separate, and reprogram these cells. Although currently there is no specific marker that can characterize and isolate oral keratinocyte stem cells, several suggestions have been made (Calenic *et al*.,2010).

2-1-3 The Function of Oral Mucosa

1-Protective Function

The mechanical, chemical, and biological stressors of daily living present a continual challenge to the mouth cavity. The oral mucosa is crucial in shielding the underlying tissues from harmful chemicals from the food, external antigens, and mechanical forces involved in regular mastication, such as stretching, compression, and abrasion from a hard diet. Moreover, alcohol, tobacco, and betel nut use in some areas expose the oral mucosa to carcinogenic chemicals. Acting as a barrier against these pathogenic and physiological stressors is the oral epithelium. It serves as an immune system and physical barrier against foreign aggressions and stops pathogens from penetrating the mouth cavity's natural bacterial flora, which might lead to illness. The oral epithelium does this by preserving immunological responses to antigens through the presence of dendritic cells, which are made of multilayers of epithelial cells and cell-cell junctions.(Wang *et al*., 2019).

2-Secretion

Saliva, which is expelled by the ducts of the major and minor salivary glands, is the primary material secreted by oral mucosa. The submucosa contains the small salivary gland that are extensively dispersed throughout the oral cavity. However, the main source of saliva, the large salivary glands, is situated outside the boundaries of the mouth mucosa. Nonetheless their excretory ducts open into the mouth cavity, which helps to keep the tissue moist. Less sebaceous glands than those seen in the majority of adult populations on the lips, labial, and buccal mucosa, and occasionally in the alveolar mucosa are present in the oral mucosa. The purpose of the fatty material secreted by these glands, called sebum, is yet unknown. Nonetheless, new research on skin sebum indicates that it could be related to immunity**. (**Wertz, 2018**).**

3-Sensory Function

The trigeminal nerve's three branches provide sensory innervation to the mouth cavity. The oral mucosa is primarily made up of three types of sensory ends: Merkel's disks, Meissner's corpuscles, and free nerve endings. These endings enable the oral mucosa to sense and react to stimuli related to temperature, touch, and pain. In addition, it detects the flavors of salty, sweet, sour, bitter, and umami; nevertheless, it has recently been proposed that fat may also be tasted. (Laugerette *et al*., 2007; AlJulaih &Lasrado , 2023).

The oral dorsum of the tongue and the soft palate contain taste receptor cells. These receptors are also present in the mucosae of the larynx, pharynx, and upper esophagus. The ability to identify items, control

mastication activities, and trigger the swallowing reflex are all dependent on the oral cavity's sensory function. The ability to feel and touch allows the tongue, lips, and soft palate to work together in unison to produce sounds when speaking. (Bearelly& Cheung,2017).

2-2 Molecular and Cellular Organization of The Oral Epithelium

Along with the skin's epithelium, the stratified oral mucosa epithelium is one of the most resilient and protective epithelia. It is made up of two layers: the lamina propria, an underlying connective tissue, and the first layer, which consists of epithelial cells with a basement membrane.(Squier&Kremer,2001)

The gingiva is made up of connective and epithelial tissues that come together to form a masticatory mucosa collar that is affixed to the alveolar bone and teeth. The oral sulcular epithelium seems to be stratified and non-keratinized, in contrast to the stratified squamous keratinized epithelium that makes up the gingival epithelium.

2-3 Epithelial Proliferation and Turnover:

Dynamic processes that are a component of kinetic events throughout the growth, maturation, and function of tissues and organs are understood by epithelial cell proliferation and differentiation. (Rivard *et al*.,1999).

cell turnover in a tissue is the process whereby cell formation is balanced by cell loss. Close examination of the turnover systems in epithelia, such as the epidermis and intestinal epithelium, reveals that the mechanism of the process is, in fact, very complex and may differ widely between

epithelia of different morphology and function. The existence of cell turnover in an epithe1ium may be shown morphologically. Cell formation is indicated by the presence of mitotic activity.

This may be restricted to one region of the epithelium, as in the deeper layers of the epidermis and the crypts of the intestinal epithelium. Regions of cell loss are indicated in the epidermis by cornification and death of the surface cells, and in the intestinal epithelium, by the presence of extrusion zones at the tips of the villi(Von Herbay &Rudi.,2000).

Cell turnover in an epithelium is probably an inherent property rather than a repair process induced by trauma. However, the rate of cell turnover may be altered by a number of internal influences (e.g., hormones) as well as by factors in the external environment (e.g., temperature, feeding). Alteration of the turnover rate may be shown by changes in mitotic activity or the size of the cell population of the epithelium. Thus, in contrast with non-renewing tissues, whose architecture is always constant, the histologic picture of an epithelium undergoing renewal may vary with the interplay of factors controlling its cell turnover (Ijssennagger *et al* .,2012). Epithelial organs undergo steady-state turnover throughout adult life, with old cells being continually replaced by the progeny of stem cell divisions. To avoid hyperplasia or atrophy, organ turnover demands strict equilibration of cell production and loss. However, the mechanistic basis of this equilibrium is unknown (Liang *et al*., 2017).

The oral epithelium, like other covering and lining epithelia, maintains its structural integrity by a process of continuous cell renewal in which cells produced by mitotic divisions in the deepest layers migrate to the surface to

replace those that are shed (Nakamura *et al*.,2007). Thus, the epithelial cell may be thought of as consisting of two functional populations: a mature population and a progenitor population, whose role is to divide and produce new cells.(Squier & Kremer 2001). Epithelial cells in different tissues divide at greatly different rates. Apart form measuring the number of cell in division, it is also possible to estimate the time necessary to over time of the epithelium and is derived from aknowledge of the time it take for acell to divide and pass ghough the entire epithelium . The rate of cell proliferation in the different epithelia has been estimated using a variety of approaches, but overall, the skin's epithelial turnover time is slower than that of the oral mucosa, which is slower than that of the gut. Different turnover rates appear to be related to regional differences in epithelial maturation patterns; non-keratinized buccal epithelium, for instance, turnovers more quickly than keratinized palate epithelium.(Squier & Kremer 2001).

2-4 Oral Exfoliative Cytology:

The study of cells that have been removed or exfoliated from the epithelial surface of various organs is known as exfoliative cytology (Mehrotra, 2012a).

With positive outcomes, this approach has opened up several avenues for working in the health sector. It has also been improved by some of the technological programs presently in use, making it possible to swiftly and conveniently tabulate and analyze information (Mehrotra, 2012b).

Exfoliative cytology has been used in the past to examine the morphological alterations in diabetes patients' oral epithelial cells. The findings are more substantial when compared to healthy individuals. Variations in the

cytoplasm, nuclear-cytoplasmic ratio, and nucleus are indicative of these modifications (Alberti *et al*.,2003; Prasad *et al*.,2010)

A more practical way to assess the oral mucosa in diabetes is extraordinary, exfoliative cytology, a simple, noninvasive diagnostic procedure (Sugerman & Savage, 1996).

Over the last 30 years, this technique has been explored for the detection of precancer in the oral mucosa, cervical mucosa, and vaginal mucosa. Exfoliative cytology has been shown by most writers to be somewhat useful in the identification of precancerous lesions, especially when establishing the prognosis of these lesions. (Sugerman & Savage, 1996 ; Alberti *et al*., 2003). It is controversial to employ this technique to assess both quantitative and qualitative alterations in oral epithelial cells in diabetics. Exfoliative cytology has been employed in a few studies to assess changes in the oral mucosa associated with diabetes mellitus, and the results have demonstrated that the illness can modify oral epithelial cells in a way that can be identified by cytomorphometric analysis. Comparing oral exfoliative cytology to traditional intervention procedures, it is easier, simpler, and noninvasive, making it a more suitable tool. Furthermore, in a single or numerous visits, exfoliative cytology can be done on the same patient at multiple places (Alberti *et al*., 2003).

Exfoliative cytology is a diagnostic method that involves labeling and fixing epithelial cells, and then evaluating them under a microscope. There are two techniques used: the direct technique, which involves rubbing mucosal surface cells, and the indirect technique, which involves aspirating individuals with self-exfoliated cells. After staining with the Papanicolaou

technique, the exfoliated cells are placed in a preservation solution and the samples are treated by the manufacturer's instructions (Williams *et al*., 1999; Rajput & Tupkari, 2010)

The papanicolaou technique is a multichromatic staining histology method that was created by George Papanikolaou. It is used to distinguish cells from smear preparations of different body secretions. The polychrome staining method consists of two counterstains (Orange G and Eosin Azure dyes) and a nuclear stain (hematoxylin) (Renwick,2009). Papanicolaou (PAP) remains a popular staining method in cytology.

The benefits of PAP staining come from the way the cleaning and dehydration solutions contribute to the transparency of the cells. This improves the detection of overlapping cells and their distinct appearance, which would otherwise be mistaken for a gigantic, bi- or multinucleated cell. The differential staining for varying degrees of differentiation yellow orange for spinous or granular cells, and green-blue cytoplasm for basal cells is the second important benefit. It is also well-liked because of the long-term stain stability, color stability, and, of course, improved repeatability of findings (Sugerman & Savage, 1996; Sundharam & Kalasagar, 2004).

Exfoliative cytology has the benefit of being a rapid, easy, noninvasive, painless, and bloodless process. Patients with systemic illness for whom a biopsy is contraindicated can benefit from it. It prevents misleading negative biopsy results and can remove difficulties following the biopsy. For research, diagnostic, and follow-up reasons, this process can be done several times (Sundharam & Kalasagar, 2004). Although this method can be helpful in the early detection of many oral mucosal illnesses, it cannot replace the

regular biopsy that is required to get a conclusive diagnosis. Cytological findings that result from reactive alterations and inflammatory responses are non-specific and non-diagnostic lesions. Exfoliative cytology should not be used as a diagnostic method on individuals who have desquamative gingivitis clinical signs. It increases expenses and postpones the conclusive diagnosis (Endo *et al*., 2008).

2-5 -Smear and Exfoliative Cytology Sample Collection Areas

Within the oral cavity, the buccal mucosa, the line between the hard and soft palates, the dorsum of the tongue, the lower labial region, and the bottom of the mouth are the most often sampled locations. The cells can be separated intentionally (tool sampling) or spontaneously (mouthwash sample). Smear collection instruments should be simple to use in any setting, non-irritating, and equipped with a sufficient quantity of epithelial cells. Wooden, plastic, and metal spatulas; dermatological curettes; Fisherbrand sterilized polyester swabs; and several brush types (interproximal brush, cytobrush, oral CDx brush, and Cytobrush Plus GT) were among the varieties that were used and compared (Ogden *et al*., 1992; Reboiras-López *et al*., 2012). More often than not, cytobrush sampling is employed because it increases the number of cells collected and makes it easier for them to be distributed uniformly on the microscope slide.

Because it is simple to use for sampling and the oral cytology sample is of high quality, the brush is suggested as a suitable tool. Exfoliative cytology uses an oral cytobrush, which is a straightforward, safe, and non-invasive procedure (Scheifele *et al*., 2004; Kosicki *et al*., 2007). Because brushes are so much more efficient than other tools, all writers agreed that they are the best for oral cytology. Since the cytobrush is an appropriate

brush in this region, it is advised for the purpose of gathering cells from the lateral side of the tongue (Kujan *et al*.,2006)

Cytological samples may be effectively obtained from oral tissues and are preferred by patients and specialists over surgical biopsy due to their ease of use, speed, and non-invasiveness (Yadav & Jaggi, 2015).

2-6 Indications for Oral Cytology

It is useful in distinguishing between a benign lesion and an unknown lesion. It can also be carried out when a patient declines a biopsy for a worrisome lesion. It is useful for monitoring following oral cancer therapy, as well as for routinely examining the treated and surrounding regions, which may reveal positive or suspicious malignant cells before the clinical characteristics return.

A non-specific lesion may occasionally develop in a region that has had radiation therapy or cancer treatment; if the malignancy is ruled out, the lesion is classified as inflammatory (Kumaresan &Jagannathan, 2014).

2-7 Cytomorphometry and Its Use in Oral and Systemic Disease Diagnosis

In 1952, Johnston employed cytomorphometry to determine the nuclear-to-cytoplasmic of one thousand normal and malignant epithelial cells. In a larger investigation, 11,000 normal and 20,000 malignant cells were analyzed; a substantial difference was found between the normal and malignant cells (Ogden.,1997a).

When evaluating normal exfoliated cells from the oral cavity, it was indicated that nuclear diameter (ND), cytoplasm diameter (CyD), and

nucleo-cytoplasmic ratio (N: CR) were crucial variables to take into account. Oral mucosal smears have not been quantitatively evaluated by many studies since (Ogden *et al*., 1997b). Given that quantitative techniques are exact, objective, and repeatable, it was proposed that they could boost the sensitivity of exfoliative cytology for early diagnosis. These techniques would involve evaluating parameters like nuclear diameter, cytoplasm diameter, and nucleo-cytoplasmic ratio N: CR (Ogden et al., 1997a).

In research using buccal mucosa smears, 12 healthy age- and sex-matched controls and 10 patients with histologically proven oral lichen planus had their dorsal surfaces of the tongue and mouth collected. There were no discernible variations in the cytoplasmic and nuclear regions of the control and lesional tissues in buccal smears. In contrast to healthy controls, the cytoplasmic region in smears from lichen planus lesions on the tongue's dorsum and the nearby clinically normal mucosa was smaller. Similar reductions were seen in the cytoplasmic: nuclear ratio in smears from clinically normal floor-of-mouth samples in oral lichen planus. When compared to normal buccal mucosa, Papanicolaou-stained smears from buccal lichen planus revealed more keratinization.

According to Sugerman &Savage,(1996), these results show that quantitative cytology may identify nuclear and cytoplasmic alterations in oral lichen planus. To investigate cytomorphometric alterations in exfoliated cells, a search was carried out of the buccal mucosa in individuals with iron-deficient anemia. The 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).

2-8 Diabetes Mellitus Oral Manifestations

Diabetes mellitus has been linked to some soft tissue pathologies and inflammatory illnesses in the oral cavity. (Baldwin 2009; Vernillo 2003).

The primary causes of oral manifestations of diabetes are decreased polymorphonuclear (PMN) leukocyte function and abnormal collagen metabolism. PMN dysfunction also leads to impaired resistance to infections, which may facilitate bacterial persistence in the tissue and increase the risk of disease in diabetic patients. In the connective tissues, altered protein metabolism brought on by reduced glucose consumption may be a factor in the increased collagen degradation. Furthermore, people with diabetes may also have reduced wound-healing responses due to altered neutrophil chemotaxis and macrophage activity (Straka, 2011). These symptoms include dysphagia, which results in altered salivary flow and composition, periodontal disorders (periodontitis and gingivitis), and taste impairment. Patients with diabetes have also been observed to suffer oral fungal and bacterial infections. Moreover, cases of stomatitis, geographic tongue, benign migrating glossitis, fissured tongue, traumatic ulcer, lichen planus, lichenoid response, and angular cheilitis have been reported as oral mucosa diseases. (Chomkhakhai *et al*., 2009; Collin *et al*., 2000; Sandberg *et al*., 2000) Diabetes patients have also been linked to delayed mucosal wound healing, mucosal neuro-sensory abnormalities, dental caries, and tooth loss. (Lamster *et al*., 2008; Saini *et al*., 2010).

2-9 Diabetes Mellitus (DM)

A class of metabolic disorders known as diabetes mellitus is defined by hyperglycemia brought on by deficiencies in either insulin action or secretion or both. (Guyton and Hall, 2012; American Diabetes Association, 2007). The primary characteristic of diabetes is hyperglycemia, which increases the risk of microvascular damage, retinopathy, nephropathy, and neuropathy. It is linked to a lower life expectancy, considerable morbidity from certain microvascular diseases associated with diabetes, a higher risk of macrovascular consequences (such as peripheral vascular disease, ischemic heart disease, and stroke), and a worse quality of life (World Health Organization, 2009).

2-9-1 Symptoms:

The symptoms of both forms of diabetes are identical, and they appear more quickly in a matter of days or weeks. These consist of excessive drinking and eating, unexplained weight loss, atrophy of muscle, exhaustion, cramps, constipation, hazy vision, and skin infections. Diabetes is frequently identified by looking for aftereffects of untreated individuals, such as eye issues and foot ulcers. The three main categories of long-term problems associated with diabetes mellitus are nephropathic, microvascular, and macrovascular. These conditions result in distinct dysfunctions and are the primary causes of morbidity and death among diabetics. (Kumar & Clark, 2007; Culling *et al*,1985; Ahmed, 2010)

2-9-2 Diagnosis

When a patient has a random blood glucose value of 200 mg/dL (11.1 mmol/L) or greater and exhibits the characteristic signs of hyperglycemia, the diagnosis of diabetes mellitus can be made with ease and confirmed at a later time. The basic diagnosis is made using the tests listed below:
When a person hasn't eaten for at least eight hours, their blood glucose is measured using the fasting plasma glucose (FPG) test. Diabetes and prediabetes are identified with this test. An oral glucose tolerance test (OGTT) checks blood glucose two hours after a person consumes a glucose-containing beverage and at least eight hours after the individual fasts. Because it is inexpensive and convenient, the FPG test is the one that is recommended for the diagnosis of diabetes. It could, however, overlook some cases of diabetes or prediabetes that the OGTT can detect. Early in the morning is when the FPG test is most dependable. Studies have demonstrated that the OGTT is less practical to use but more sensitive than the FPG test in the diagnosis of prediabetes. A casual plasma glucose test, also known as a random plasma glucose test, tests blood glucose without taking into account the subject's most recent meal. This test is used to diagnose diabetes, not prediabetes, in conjunction with a symptom evaluation.A second test on a separate day should be performed to confirm test findings that indicate a person has diabetes (Twillman, 2002).

According to the Report of a WHO Consultation (1999), the current WHO diagnostic criteria for diabetes should be kept at either fasting plasma glucose of 7.0 mmol/l (126 mg/dl) or 2-hour plasma glucose of 11.1 mmol/l (200 mg/dl).

2-10 Epidemiology of Diabetes Mellitus:

over retirement age (International Diabetes An estimated 366 million individuals worldwide are expected to have diabetes, of which half are ignorant that they have the illness. Six of the ten nations with the highest rates of diabetes prevalence are in the Middle East: Saudi Arabia, Qatar, Kuwait, Bahrain, Lebanon, and the United Arab Emirates. Approximately 20.5 million persons in the 20 Arab nations for which data are available have diabetes, while an additional 13.7 million have poor glucose tolerance and are in the prediabetes stage. Almost three-quarters (73.4%) of diabetics in Arab countries are under 60 therefore in their most productive years, which increases the burden of disability due to diabetes.

2-10-1- Diabetes Type I (Insulin-Dependent Diabetes Mellitus1-IDDM)

diabetes Type 1 can be diagnosed at any age; however, most individuals are diagnosed before the age of twenty, meaning that the disease often first appears in childhood, adolescence, or early adulthood. who are 54genetically predisposed have normal beta cell mass at birth, but for months or years, they begin to lose beta cells due to autoimmune destruction. of all cases of DM are of this kind (Beers *et al*., 2006).

Insulin secretion is compromised in type 1 diabetes mellitus due to the autoimmune destruction of the pancreatic beta cells in the islets of Langerhans. Type 1 diabetes is mostly immune-mediated, and the loss of beta cells is an autoimmune response driven by T cells (Rother, 2007).

2-10-2-Diabetes Type II (Non-Insulin Dependent-NIDDM):

Insulin resimmune-mediated resistance, which may be linked to comparatively lower insulin production, is a hallmark of type 2 diabetes mellitus (Shoback *et al*., 2011). Even if the first defect is debatable, the majority of research lends credence to the theory that insulin resistance develops before an insulin secretory defect, with diabetes developing only after a deficiency in insulin secretion. The illness is complex and polygenic because of the environment in addition to genetic vulnerability, variables (such as obesity, physical activity, and diet) also influence the phenotypic. The most prevalent kind is known as adult-onset diabetes. Type 2 diabetes may strike anyone at any age, including in childhood, but it often strikes those over 40. (Shoback *et al*., 2011).

Type 2 diabetes, on the other hand, is more common in teenagers and is characterized by peripheral tissue resistance to insulin action without loss of beta islet cell function a state in which the liver muscle and fat cells use the insulin incorrectly) (Shoback *et al*., 2011) . Even when insulin resistance is evident, glucose tolerance is relatively normal in the early stages of the disease because the pancreatic beta cells adjust by producing more insulin. Because of this, the body needs more insulin to let glucose enter cells so that it can be used as fuel. Initially, the pancreas produces more insulin to counterbalance the increased demand. By the time the pancreas is unable to produce enough insulin in response to food. Although not predisposed to ketosis, these patients may experience it in stressful situations (Defronzo *et al*., 2004). In the early stages of type 2 diabetes, decreased insulin sensitivity is the main problem. This stage of hyperglycemia regression is brought on by many therapies and drugs that either increase the sensitivity of insulin or

decrease the liver's synthesis of glucose (Horn *et al*., 2007). Adipocyte-derived circulating free fatty acid levels, which are elevated when levels of circulating free fatty acids and other fat cell products are elevated, inhibit glucose uptake, glycogen synthesis, and glycolysis. Obesity concomitant with type 2 diabetes also contributes to insulin resistance. Insulin resistance is often offset in obese people by increased insulin production. However, ß-cell apoptosis increases significantly in one-third of obese persons, reducing ß-cell bulk and inadequately producing insulin (Mealey & Oates, 2006).

Compared to type 1 diabetes, type 2 diabetes has a stronger hereditary foundation; nonetheless, the risk gene or genes are still unclear. People who have one type 2 diabetes parent are more likely to have the disease themselves; if both parents have the disease, the risk increases to about 40%.

Although the causes of type 2 diabetes vary, most patients are thought to have a combination of hyperinsulinemia/insulin resistance and ß-cell failure, which is an insulin shortage brought on by a compromised ability of cells, tissues, and peripheral organs to respond to insulin activity (Abrairaa *et al*., 2003).

2-10-3- Etiologic Classification of Diabetes Mellitus:

Diabetes occurs as a result of the body's loss of ability to use the hormone insulin, or decreased insulin secretion, which leads to the accumulation of glucose or sugar in the blood. Insulin is a hormone that facilitates the entry of glucose molecules into cells for storage or energy production. It also helps control blood sugar levels. One of the most important causes of diabetes is that the pancreatic cells that produce insulin

are attacked and destroyed by the immune system or by genetic components or a specific virus that targets the pancreatic cells and stimulates the immune system or weight gain and other reasons.(Nall.,2018).

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

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

III. Gestational diabetes mellitus.

IV. Other specific types.

A. Genetic defects of beta cell function

B. Genetic defects in insulin action

C. Diseases of the exocrine pancreas

D. Endocrinopathies

E. Drug- or chemical-induced

F. Infections

G. Uncommon forms of immune-mediated diabetes

H. Other genetic syndromes sometimes associated with diabetes

2-10-4- Diagnostic Criteria for Diabetes Mellitus: (James *et al*., 2003)

1. Traditional signs and symptoms of diabetes mellitus, such as polydipsia, polyuria, and inexplicable weight loss, which help diagnose the disease and a c1ausative blood glucose level of more than 200 mg/dl (11.1 mmol/L); or:3. Random plasma glucose test

2. Oral glucose tolerance test (OGTT)

Studies have indicated that when it comes to identifying pre-diabetic persons, the OGTT test is more sensitive than the FPG test, although its administration is not as well accepted. It is necessary to fast for at least eight hours before to the OGTT. The measurement of plasma glucose level acts 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 who 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 &Inzucchi, 2012).

3. Glycated Hemoglobin (HbA1c)

The patient's level of hemoglobin A1C offers a picture of long-term glycemic control and represents average glycemic control over the previous 2–3 months. Although the term "glycosylated" was previously included in the nomenclature, it has been clarified that this term only pertains to glycosides. As a result, according to the Joint Commission on Biochemical Nomenclature, any process that joins a sugar to a protein is referred to as "glycation," or, in the specific instance of a reaction involving hemoglobin, "glycated hemoglobin" (Abrairaa *et al*., 2003).

The American Diabetes Association (ADA) (2007) refers to HbA1c as A1C. It is the accepted technique for evaluating long-term glycemic control and is thought to be a reliable indication of average glycemic concentrations during the preceding 90 to 120 days (Sacks, 2005; Sultanpur *et al*., 2010). According to the American Diabetes Association

(ADA) (2007), patients who are fulfilling their treatment objectives and have stable glycaemic control should have their A1C tested at least twice a year. Patients whose medication has changed or who are not meeting their glycemic goals should have their A1C tested every quarter. When blood glucose levels are regularly high, Patients who are meeting their treatment goals and maintain stable glycaemic control have to have their A1C checked at least twice a year, according to the American Diabetes Association (ADA) (2007).

Every quarter, patients should have their A1C checked if their medication has changed or if they are not reaching their glycemic targets. When blood sugar levels are consistently elevated, of Chapter One Literature Review 15: Glycemic management throughout the long term (Hanas & John, 2010). According to Marshall (2010), an HbA1c value of 6% to 7% was thought to represent good diabetes control, a value of 7.1% to 8% suggested intermediate control and a value of >8% was considered poor diabetes management. It is advised to use (HbA1c) as a screening and diagnostic tool for diabetes mellitus because they are easy to use, accurately represent long-term hypoglycemia, and are standardized (Saudek *et al*., 2008).

Chapter Three

Materials and Methods

3- Materials and Methods

3-1- Chemicals, Apparatus and Instruments used

3-1-1-chemicals

Table (3-1): Shows the origin and names of the chemicals used in this study

Chemicals	Company	Country
Acid al cohol	Misan	**Iraq**
Canada Balsam	Roth	**Germany**
Chloroform	Sigma	**Switzerland**
Charcoal Activated	BDH	**England**
Ethanol (absolute 100%)	BDH	**England**
Formalin	BDH	**England**
Fuchsin Basic	Dakocytomation	**Denmark**
Glacial acetic acid	BDH	**England**
Haematoxylin & Eosin	BDH	**England**
Methanol	Merck	**Germany**
Periodic acid	Dakocytomation	**Denmark**
Sodium metabisulphite	BDH	**England**
Xylene	BDH	**England**
DCFH-DAStain	Abcam	**CHINE**
Mitotracker stain	Thermo Fischer	**USA**
PAP Stain	Thermo Fischer	**USA**
MtRose	Thermo Fischer	**USA**
PAS Stain	Thermo Fischer	**USA**

3-1-2- Instruments

Table (3-2): the table shows the origin and names of the instruments used in this study

Instrument	Company	Country
Cyto Brush	Janetzki	**Germany**
Scrap	Mindray	**China**
Slide	Roche	**Germany**
Swab	Tglassco	**India**
Cover Slide	Thermo Fischer	**USA**
rubber gloves	Tglassco	**India**
Glass Coplin jars	Binder	**USA**
Plastic jars	LG	**USA**
Plastic slides container.	Leitz	**Germany**
An eyepiece micro-coulometer	Binder	**Germany**
Ocular stage	LED	**USA**

3-1-3- Apparatus

Table (3-3): shows the origin and names of the apparatus used in this study

Name Apparatus	Manufacture Company	Country
Electron Microscope	Japan	Olympus
Hot plate	India	Tglassco
Light Microscope	Japan	Olympus
Refrigerator	Korea	LG
Fluorescent Microscope	Japan	Olympus
Frozen	China	AUCMA

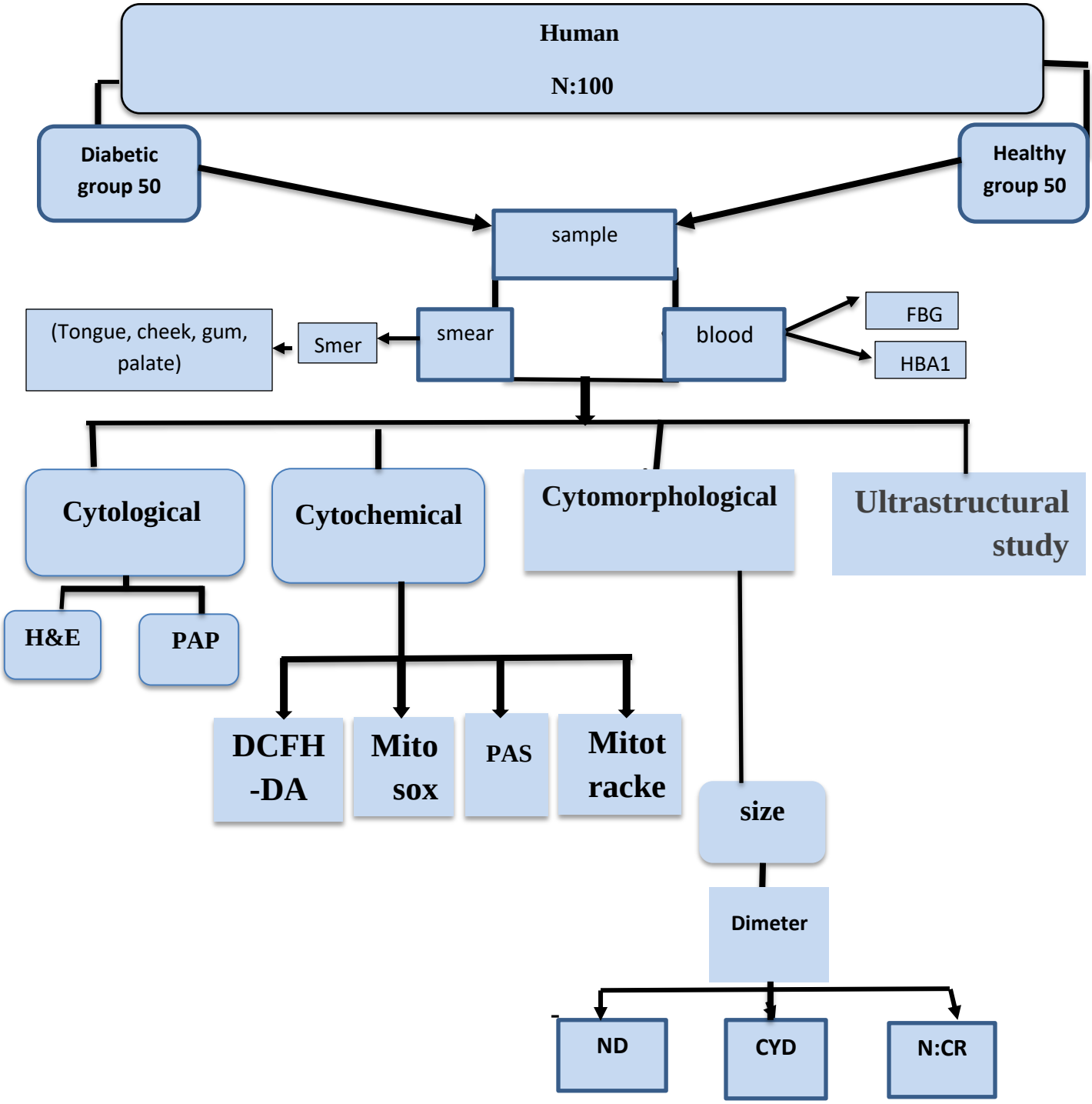
3-2- Experimental Design

The recent study was completed on diabetic or healthy individuals form both sexes used in the study ranging between 1-70 years. After obtaining the patient's consent and the approval of the Scientific Research Ethics Committee at the University of Maysan, samples were collected from the Diabetes and Endocrinology Center in Maysan Governorate, and samples were taken from four areas of the oral mucosa to collect these samples. This study included 100 people, who were divided into two groups.

The first group (the healthy group) consisted of 50 people, then,

The second group(the diabetes group) consisted of 50 people. The diagnosis of diabetes was based on the doctors who carried out the diagnostic examination at the diabetes center, The cumulative blood sugar analysis, and the rapid analysis, where blood samples of about 5 ml were taken from each person to conduct the tests. The samples were also taken in the morning, and after taking the samples, they were stained with several dyes: Pap, PAS, DCFH-DA, Mitotracker, mitosox and hemotoxylin &Eosin.

3.3. Experimental Design



`Diagram: Experimental Design

3-4- MethodSample Collection:

3-4-1 Smear

A brush was used to collect swabs from the oral cavity to four areas: the cheek, gums, tongue, and palate. The smears were spread on a clean glass slide and left to dry for a few seconds. Specimens were quickly fixed to avoid cell dehydration and shrinkage and maintain their structural integrity. For this, the glass slides were submerged in increasing amounts of ethanol: 100%, 95%, 80%, 70%, and 50%. The slides were stained using Papanicolaou stain (PAP‘ PAS ‘ H&E, DCFH-DA, Mitotracker, mitosox). Afterward, the slides were placed under a light microscope once they had dried. The Ocular Stage lens was operated to accomplish cell counting, with non-diabetic cell counts per square millimeter. However, since diabetics have a lower cell count overall, their cell counts were determined over a broader region than 1 square millimeter Finally, we placed the cover of the slide and attached the cover to it DPX Then we counted or measured cells in the laboratory using a light microscope.

3-5 Serological Test (Blood glucose)

The blood sugar level was measured for people with diabetes and healthy, where blood was drawn using the (TOSOH) device Sampling times were in the morning.

3-6 cytological Study :

3-6-1 Fixation:

Specimens were fixed as soon as possible to preserve the specimen's structural characteristics, keep the cells from drying out and shrinking, and allow for clear staining and differentiation. This is done by immersing the glass slides in a graded concentrations series: 100%, 95%, 80%, 70%, and 50%.

3-6-2Staining

3-6-2-1Staining :-H&E staining

Staining Procedure: Hematoxylin & Eosin staining

The general component of the cell has been demonstrated by using hematoxylin and eosin as a standard histological stain (Luna, 1968).

1. The slides were fixed by xylene for (5) min.
2. After being dried in xylene, slides were rehydrated for approximately three minutes in each concentration using a graded series concentration of alcohol (100%, 95%).
3. Rinsed with one minute of water.
4. Hematoxylin type Ehrlich stains were applied to the slide and left for twelve minutes.
5. After that, every slide was water-washed.
6. After that, wash with water and stain with eosin stain type Y for three minutes.

7. After that, the samples were dehydrated in a series of graded alcohol concentrations (95%, 100%) for five to six seconds at a time.

8. For five minutes, the slides were submerged in xylene.

9. After mounting the slides with D.P.X (Dextrin-Plastizer-Xylene), they were covered and then examined with a light microscope.

Hematoxylin dye stains the nucleus and eosin stains the cytoplasm because hematoxylin is considered a basic dye that reacts with the nucleus, which contains DNA, because it is acidic, while eosin is an acidic dye In contrast, hematoxylin stains the cytoplasm because it is basic. Thus, when diagnosing the cells of the mouth, when the molecules are purple in color, it means nuclei, while the cytoplasm is pink, and the two dyes differ. In terms of components.

3-6-2-2PAP staining

Staining Procedure: Papanicolaou(PAP) The PAP stain is used to compare it with other dyes for the purpose of evaluating its efficiency in diagnosis and to detect crusty cells in the oral cavity.

1- Fixation in the appropriate fixation solution. We take the slides and place them in descending ethanol 95%, 80%, 70%, and 50%.
2- Stain with Harris hematoxylin for aboute 3 minutes **.**
3- Wash the slide in tap water for 1-2 minutes
4- Differentiate in acid alcohol unite only the nuclei retain the stain(few seconds)
5- Then we put it again in 70% ethanol, and then we put it in 95% alcohol.
6- Stain in O.G.6 for approximately 2 minutes.

7- We wash the slide in 95% alcohol.
8- Stain in E.A.50 for about 2-4 minute until the desired intensity of colour has been obtained .
9- We wash the slide again with 95% ethanol for a few seconds each
10- Dehydrate in alcohol ‘clear in xylene

RESULTS:

Cytoplasm of cornified cell _reddish _pink.

Cytoplasm of non- cornified cell_green .

Nuclei _blue.

Pap stain stains both the nucleus and the cytoplasm.This dye is considered extremely fast, as it takes a short time compared to other dyes. This dye is considered the most famous.

3- 7Cytochemical:

3-7-1 Periodic acid Schiff stain (PAS).

Use the stains to explain the components of the cell and its contents, including the nucleus and cytoplasm. (Culling *et al.*,1985).

1. The slides were fix by xylene for (5)min.
2. Slides dried from xylene and rehydrated in graded series concentration of alcohol (100%, 95%) for about (3) min in each concentration .
3. Bring slides to disilled water.
4. Treat with used to explain the components of the cell and its contents, including the nucleus and cytoplasm used to explain the components of

the cell and its contents, including the nucleus and cytoplasm periodic acid for (5) min.

5. Washing slides well with distilled water.
6. Cover with Schiff's reagent for 5-10 min.
7. Wash in running tap water 5-10 min.
8. Counter stain with Herris hematoxylin for approximately 15 sec.
9. Wash in tap water.
10. Rinse in increasing concentration of alcohol (70%,80%,95%,100)
11. Clear in Xylene.

After staining, the sections were inspected under a microscope with magnifications of (40 x and 100x) times, and pictures were taken.

-PAS stain stains carbohydrates, PAS stain interacts with the carbohydrates present in the cell membrane, the cells are stained on this basis. The nature of the staining differs between a person with diabetes and a healthy person, because in a person with diabetes, the percentage of carbohydrates is low, so the staining is less than in healthy people. The nucleus is stained violet and the cytoplasm is pink because it is passed with hematoxylin and eosin stains, but the cell membranes are stained pinker than the cytoplasm.

2-Preparation of DCFH-DA stain

In order to create the DCFH-DA stain, one milliliter of the stock solution was added to fifty milliliters of PBS in a black container. This allowed for the detection of cytoplasmic ROS, which is a sign of changes in tissue.

-To detect hydrogen peroxide and release it to cells as a whole. This is to detect free radicals emanating from the mitochondria or from the reticulum.

We add rose stain, which when added produces fluorescent images that are clearly visible and easy to manage. (Dragh et al.,2017).

3-Preparation of Mitotracker stain

Using the stock solution, the Mitotracker stain was made and put in a black container by mixing one milliliter of Mitotracker stain stock solution with fifty milliliters of PBS, and using it for stained mitochondria and used as a measure of the morphology of the mitochondria. (Dragh et al.,2017).

4-Preparation of Mitosox stain :The Mito-Sox stain, which is used to identify mitochondrial ROS and is thought to be an indicator of mitochondrial defects, was made from the stock solution and placed in a black container by mixing 1 ml of the stock solution with 50 PBS. (Dragh et al.,2017).

3- 8 Cytomorphology Study:

Cytomorphometric evaluation will be performed 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 microscope. Aneyepiece 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 (buccal mucosa ,tongue , cheek and palate and jungiva) and were stained with Papanicolaou stain and PAS and H&E to visualize under compound light microscope for cytomorphometric analysis of cells for (ND, CyD and (N:C) ratio.

3-9Ultrastructural Study:

Samples were collected from Maysan Governorate and sent to an electron microscope for sectioning and preparing sections to learn information related to mitochondrial morphology.

3-10Examination of the slides:

The properly stained slides were examined with the assistance and calibration of special 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 It was also scanned using 100x lens.

3-11 Statistical Analysis

The data were statistically processed using SPSS (Statistical Package of Social Sciences) to determine the relationship between the number of counted cells and the incidence of diabetes, followed by ANOVA and Chi-square tests and the significance level was set at $P \leq 0.05$. (Al-Rawi and Khalaf Allah, 2000).

Chapter Four
Results

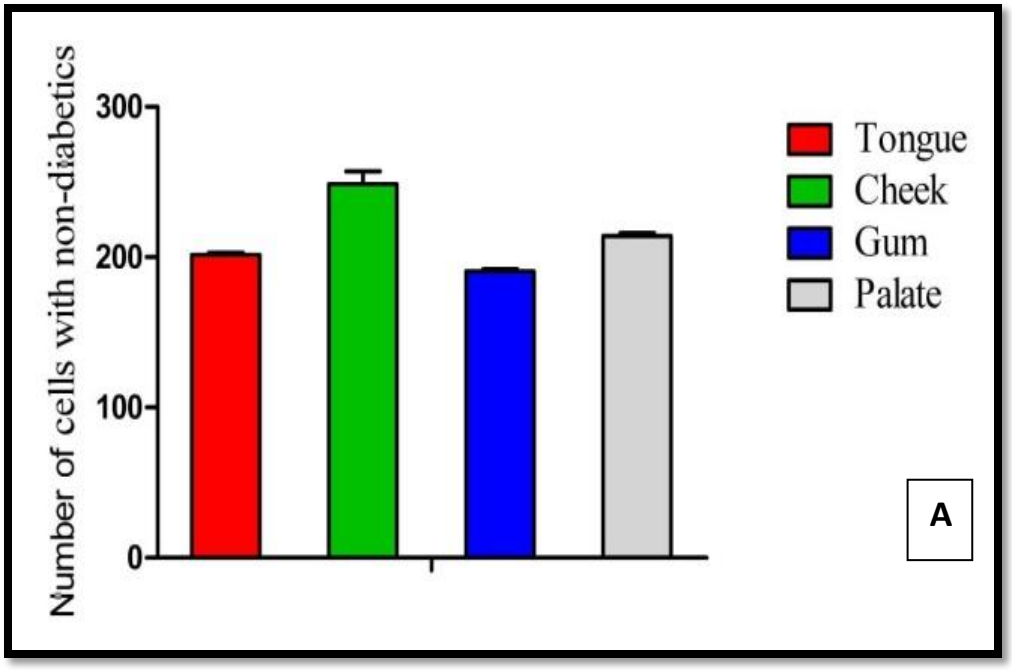
4- Results of the cytological study

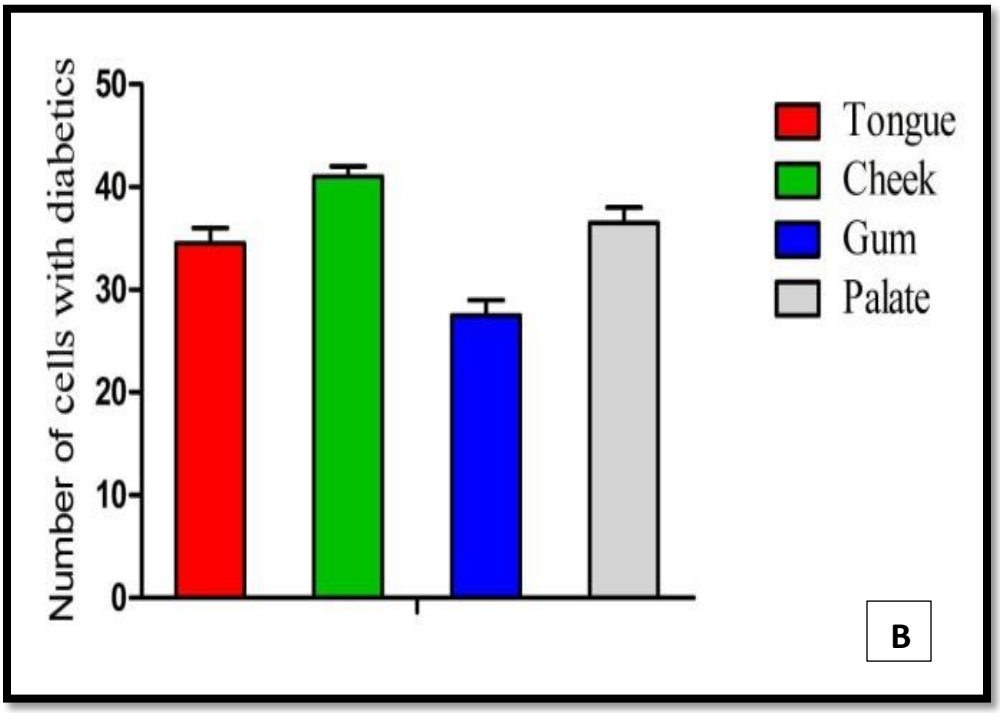
4-1 Distribution of samples according to the number of cells in the mouth areas.

The cell counts varied significantly between the oral sampling sites of 100 subjects (50 diabetic and 50 healthy) of both sexes and different age groups. These samples were taken with brushes and taken from four areas (tongue, cheek, gums, and palate). The study's results revealed a significant variation and statistically significant differences (at a significance $p \leq 0.05$ variation) in the number of squamous cells falling from the oral cavity between patients with type 1 and type 2 diabetes and those with healthy. Specifically, the slides stained with hematoxylin-eosin and PAP and PAS stains showed a large cell proliferation in all oral sites of healthy individuals, especially in the cheek area, as well as in diabetic individuals, accompanied by a decrease in cell numbers in the gingival area of diabetic and healthy individuals. The data from the mucous membrane were compared, where the percentage of cell numbers in the gum area (n = 192) was found for healthy individuals, while the cell numbers in the gum area of diabetic individuals (n = 29). These differences were observed in the cell numbers in the oral cavity (gum) areas and the percentage of cell numbers in the cheek area of non-diabetic individuals (n = 257) while the percentage of cell numbers in the cheek area of diabetic individuals (n = 42), as shown in Tables (4-1) and visually represented in Figures (4-1) and graph (4-1).

Table (4-1): The table includes the number of cells according to the areas of the mouth for people with healthy and diabetes.

Region	Count of healthy	%(non-diabetics)	Count of diabetic	%(diabetics)
Tongue	203	23%	36	25%
Cheek	257	30%	42	29%
Gum	192	22%	29	20%
Palate	216	25%	38	26%
Significance *P<0.05, NS = No Significance X2 Chi-square at 11.161, d.f 3, p-value .011, Total of non- diabetes,868 Total of diabetes ,145				





Graph (4-1): The number of cells by mouth area for non-diabetic people (A) and the number of cells by mouth area for diabetic people(B).

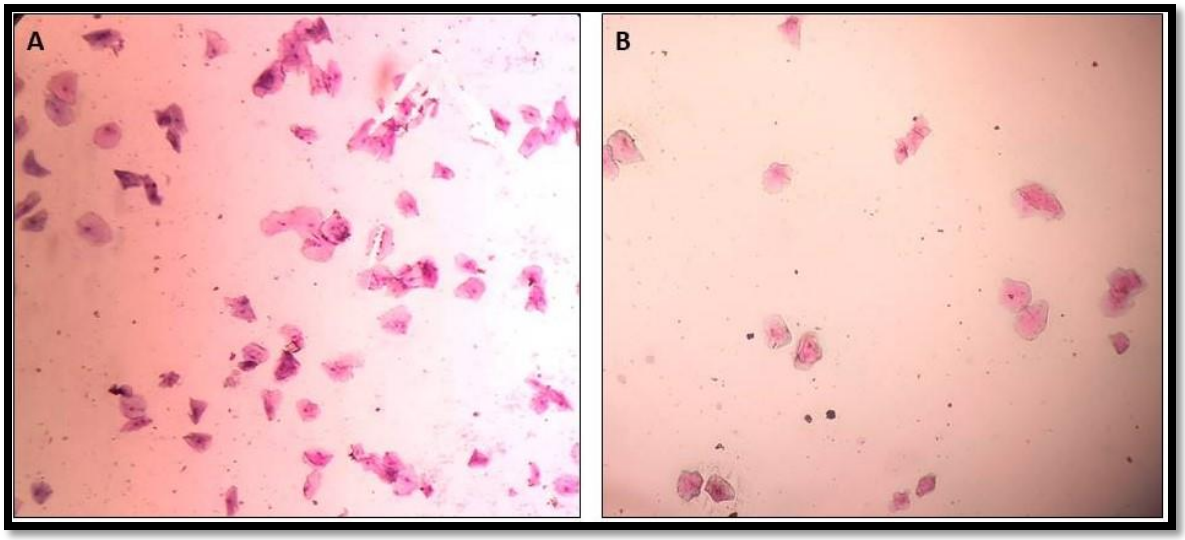


Figure 4-1 A: The number of decidual cells in a healthy individual is greater. B: The number of decidual cells in a diabetic patient reveals that the diabetic patient has fewer cells than the healthy.(PAP,10X)

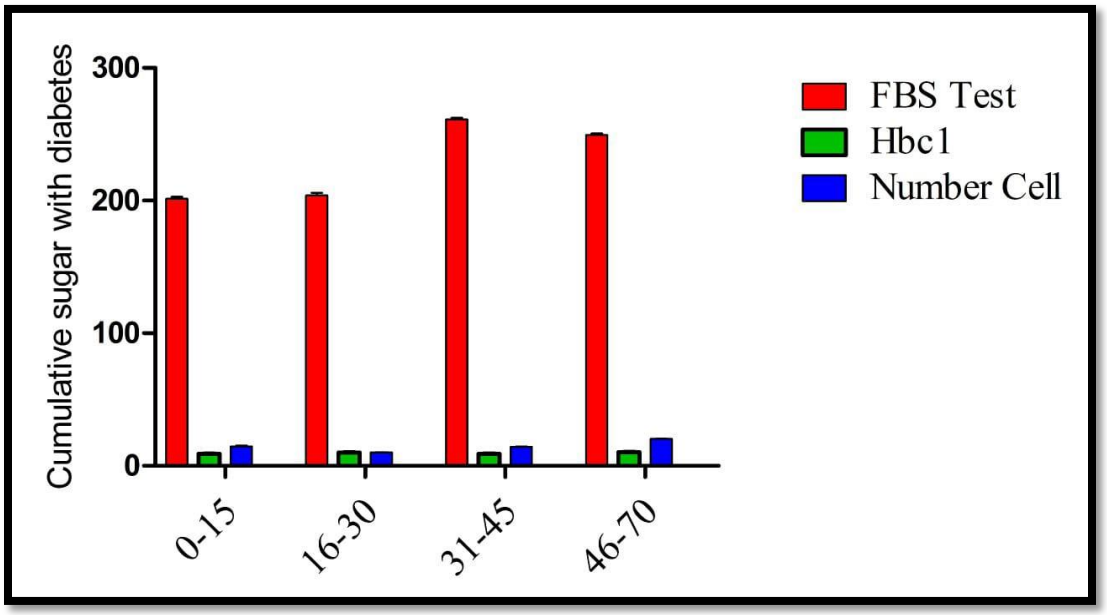
4.1.1 The relationship between the number of cells, the fasting sugar, and the accumulated sugar.

After confirming the examination of the accumulated sugar and the sugar level, samples were taken from diabetic and non-diabetic patients. According to the results, there were statistically significant differences between the sugar level and the number of cells in diabetic and non-diabetic patients. While there was no statistically significant relationship between the accumulated sugar and the number of cells in diabetic and healthy , according to the age groups. The results highlight a statistically significant association ($P \leq 0.05$) between the number of cells derived from the oral cavity and the sugar levels across different age groups. For example, individuals aged 1 to 15 years, with sugar levels of about 202.9 and accumulated sugar levels of 9.26, showed a number of cells ranging from

15.4. Participants aged 16 to 30 years, with sugar levels of about 205.79 and accumulated sugar levels of 10.34, showed a number of cells ranging from 10.4. Participants aged 31-45 have a blood sugar level of 262.29, a cumulative sugar level of 9.4, and a cell count of 14.6. As for people aged 46-70, their blood sugar level is 250.69, a cumulative sugar level of 10.56, and a cell count of 20.6. The number of cells is directly proportional to the increase in age, especially in the age groups 40-70, where the highest percentage of increase was recorded, as shown in Tables (4-2) .

Table (4-2) shows the cumulative sugar, diabetes level, and cell numbers for people with diabetes.

Age(years)	FBS Test	HbA1c	Number cell
1-15	±202.94	±9.26	15.4
16-30	±205.79	±10.34	10.4
31-45	±262.29	±9.43	14.6
46-70	±250.69	±10.56	20.6
FBS	Correlation	0.413	
	P- value	0.008	
HbA1c	Correlation	0.012	
	P- value	0.940	
Significance *P<0.05 Chi-Square 2.448 ,df 3 p- value. .485			



Graph (4-2): Shows the cumulative sugar, diabetes level, and cell numbers for people with diabetes.

4-2 Results of cytomorphometrical

4.2.1 Cytomorphometric Analysis:

The cytomorphological analysis of the study and control groups in the smear sites (tongue, gum, cheek, and Platae) included determining the average cytoplasmic diameter, nucleus diameter, and nucleus-to-cytoplasm ratio (NCRthe results showed that the cells of diabetic subjects were few in all age groups compared to non-diabetes subjects without diabetes. The figure shows that the number of cells in people with diabetes is less than in non-diabetics and all age groups. These results are summarized in Figure (4-2), (4-3).

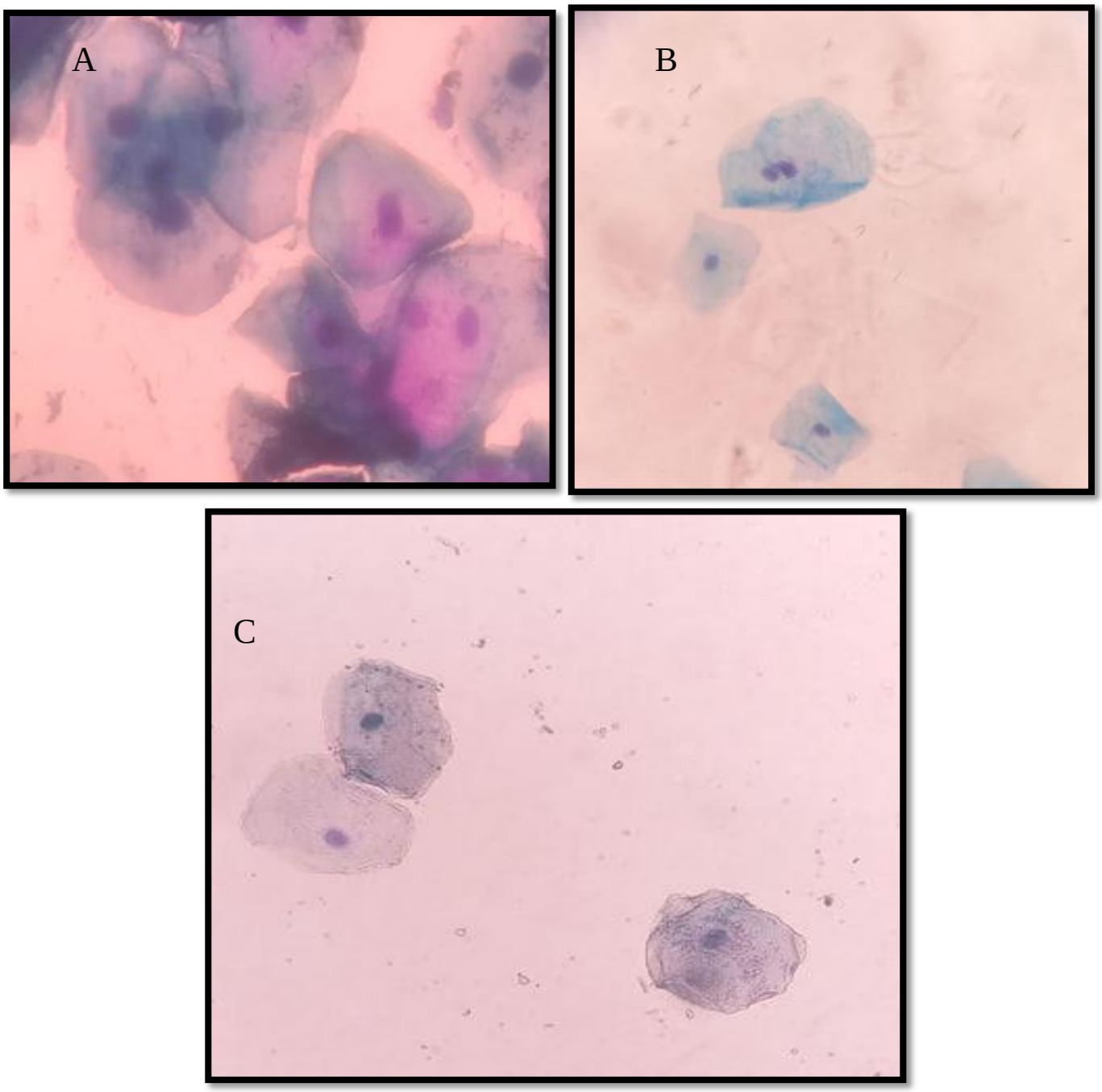


Figure 4-2: Cytological smears of non-diabetes taken from the oral mucosa of different age groups as (A) 10-15 years, (B)20-30 years, and (C)31-70 years (H&E,40x).* Note: A combination of two stains was used as hematoxylin-eosin stain and PAP stain.

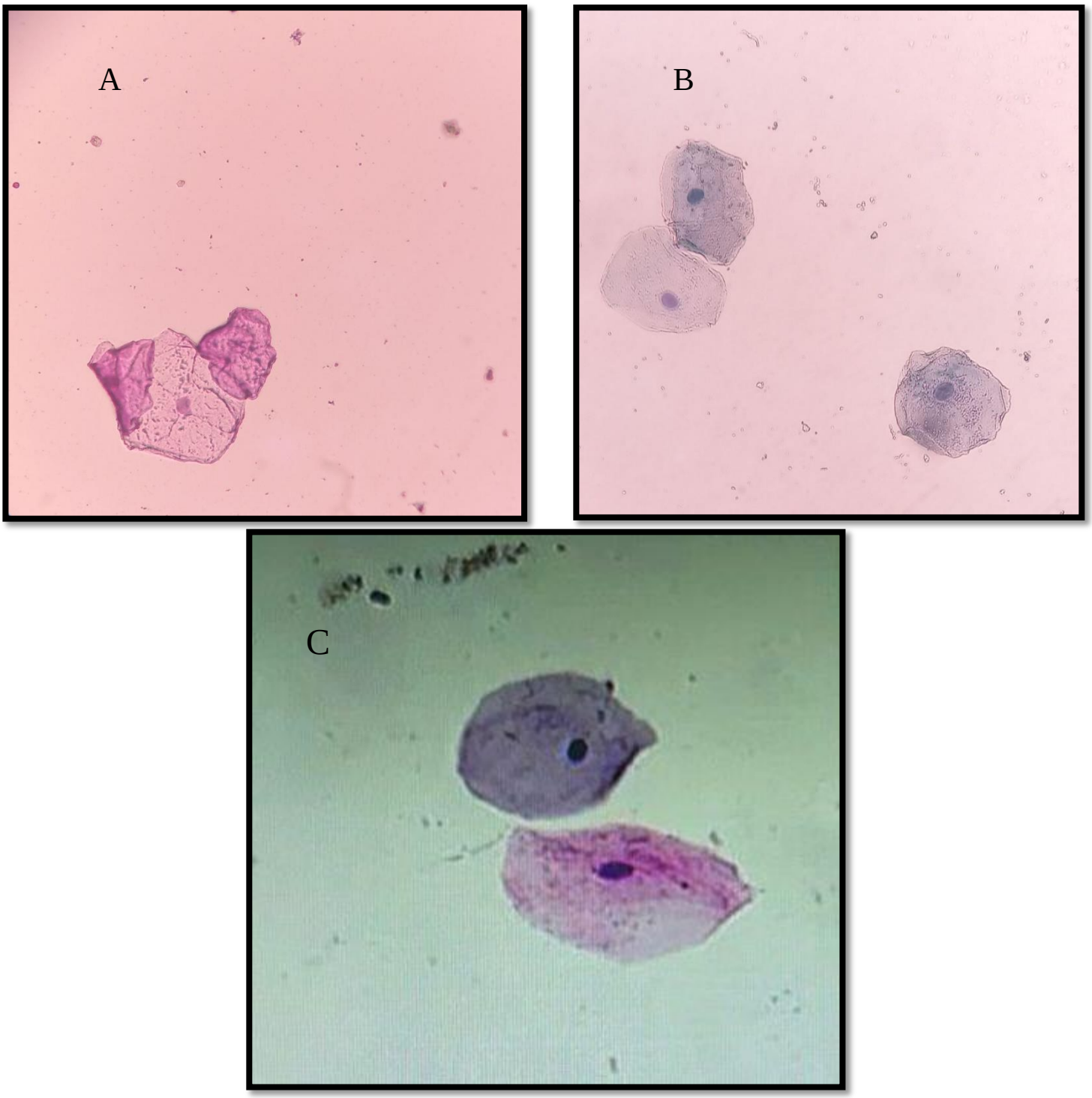


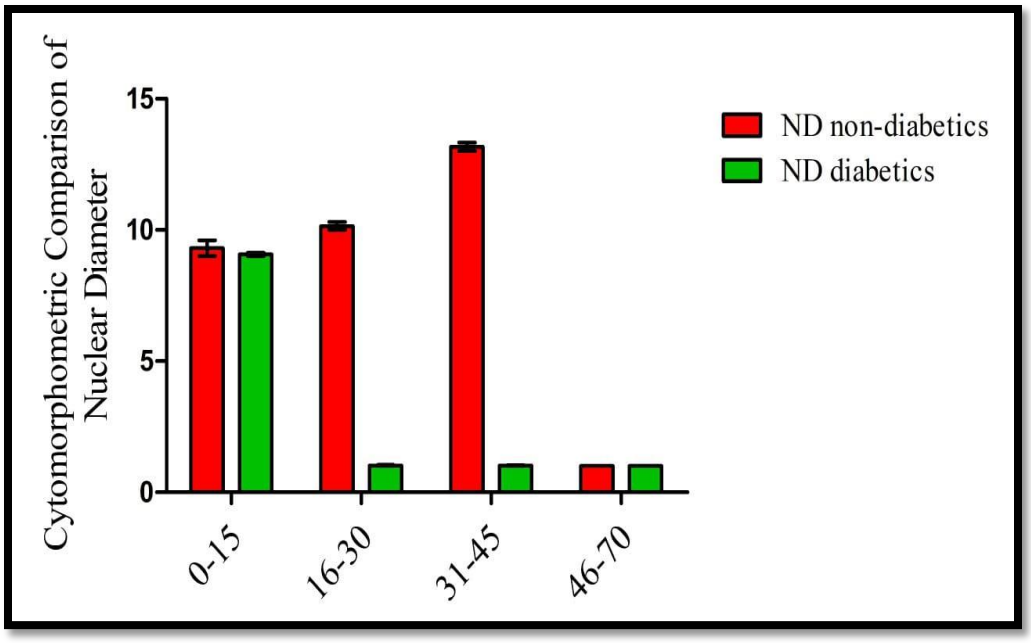
Figure 4-3: Cytological smears of diabetes taken from the oral mucosa of different age groups as (A) 10-15 years, (B)20-30 years, and (C)31-70 years (H&E,40x).* Note: Two stains were combined as hematoxylin-eosin stain and PAP stain.

4.2.1.1 Nuclear Diameter:

The present results indicate a significant ($p \leq 0.05$) variability in nucleus diameters between individuals, depending on age, between diabetic and non-diabetic subjects, as shown in Table 4-3. It is worth noting that the non-diabetic group showed a significant increase in nucleus diameter compared to their diabetic counterparts. The age F pr ratio is 0.417 for the non-diabetic group and 0.174 for the diabetic group the variability in nucleus diameter across age groups and diabetic status. Furthermore, the analysis reveals that the age groups 1-15 and 46-70 showed no significant differences, indicated by A closer to B, while the age groups 16-30 and 31-45 showed significant differences, with A outperforming B as shown in Figures. (4-4),(4-5) and graph (4-3).

Table 4- 3: Cytomorphometric Comparison of ND between control and diabetics.

Age(years)	Nuclear Diameter of healthy (µm) (A)	Nuclear Diameter of diabetic (µm) (B)	Standard errors of means
1-15	9.6	9.125	Table age
16-30	10.3	1.045	Diabetics
31-45	13.326	1.03	rep. 2 4
46-70	1	1	d.f. 3 3
d.f.	3		e.s.e. 3.11 2.20
Significance *P<0.05, d.f 3, F Pr for age 0.417, F Pr for diabetes0.174			



Graph 4-3: Cytomorphometric Comparison of ND between control and diabetic

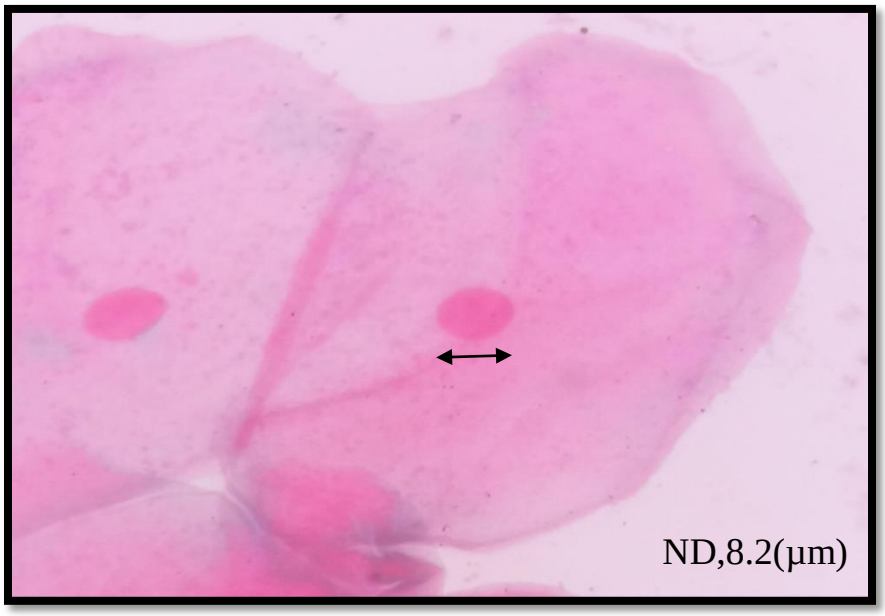


Figure 4-4: Cytological smear from the oral cavity of a healthy group showing nuclear diameter(H&E,100X).

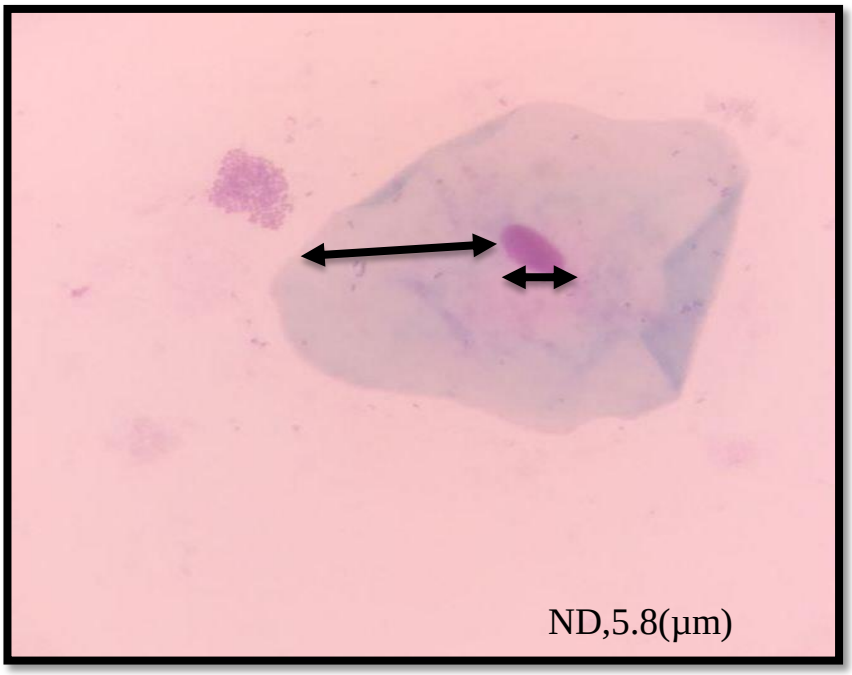


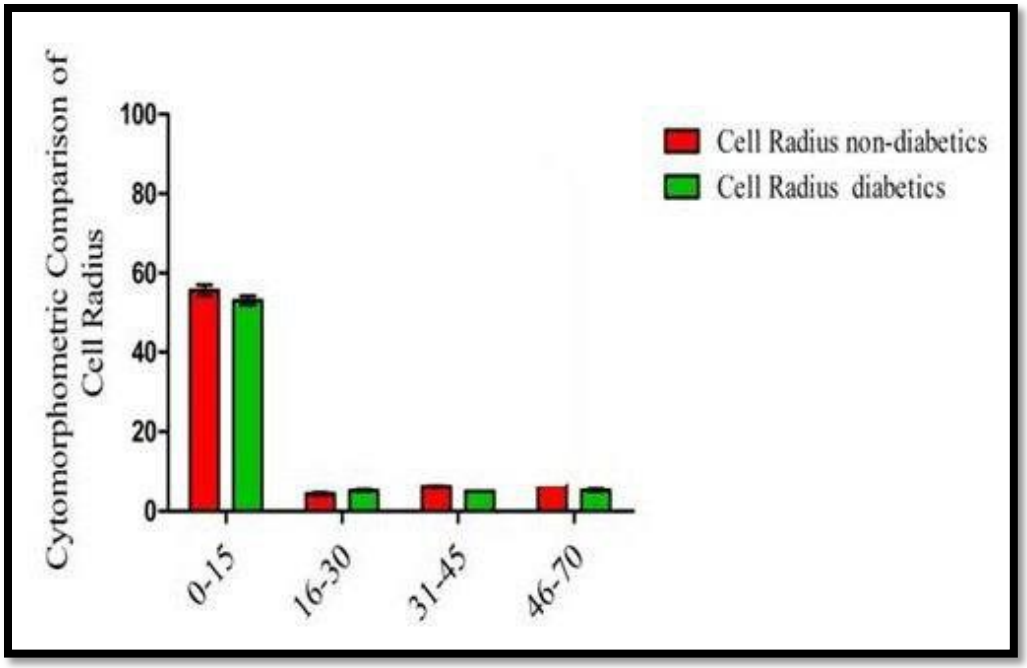
Figure 4-5: Cytology smear from a diabetic group showing nuclear diameter(H&E,100X).

4.2.1.2 Cytoplasm Diameter :(Cell Radius):

The current results showed significant differences in cell radii across all age groups, as the control group (A) for age groups 1-15 showed diameters equal to those of the diabetic group (B), and the age groups 16-30 of the control group showed cytoplasmic diameters equal to 4.727 smaller than those of diabetics equal to 5.454, and the age groups 31-45 of non-diabetics showed cytoplasmic diameters of 6.288 and those of diabetics 5.076, while the age groups 46-70 showed cytoplasmic diameters of non-diabetics 8.554 and those of diabetics 5.642, as detailed in Tables (4-5), (4-6), Figure (4-6), (4-7) and Graph (4-4).

Table 4-5: Cytomorphometric Comparison of CYD between control and diabetes of oral in terms of age

Age(years)	Cell Radius of healthy (µm) (A)	Cell Radius of diabetics (µm) (B)	Standard errors of means
0-15	±54.375	±54.275	rep. 2 4 d.f. 3 3 e.s.e. 107.5 76.0
16-30	±4.7272	±5.4545	
31-45	±6.2884	±5.076	
46-70	±8.5542	±5.6428	
Significance *P<0.05, F Pr for age 0.374, F Pr for diabetes0.305			



Graph 4-4: Cytomorphometric Comparison of CYD between control and diabetes of oral in terms of age

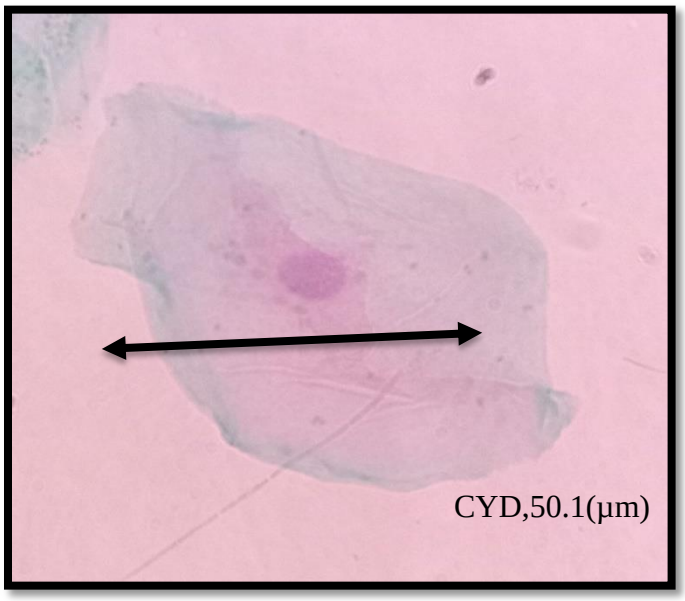


Figure 4-6: Cytological smear from the oral cavity of a healthy group showing cytoplasm diameter (H&E,100X).

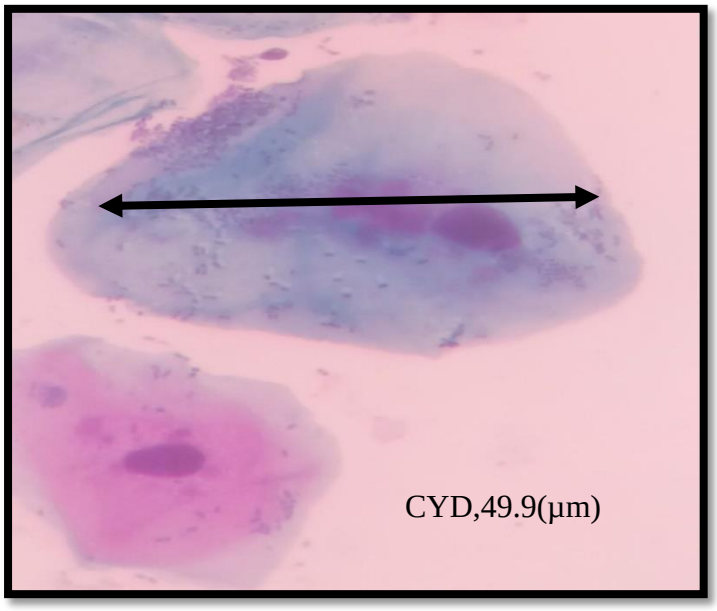


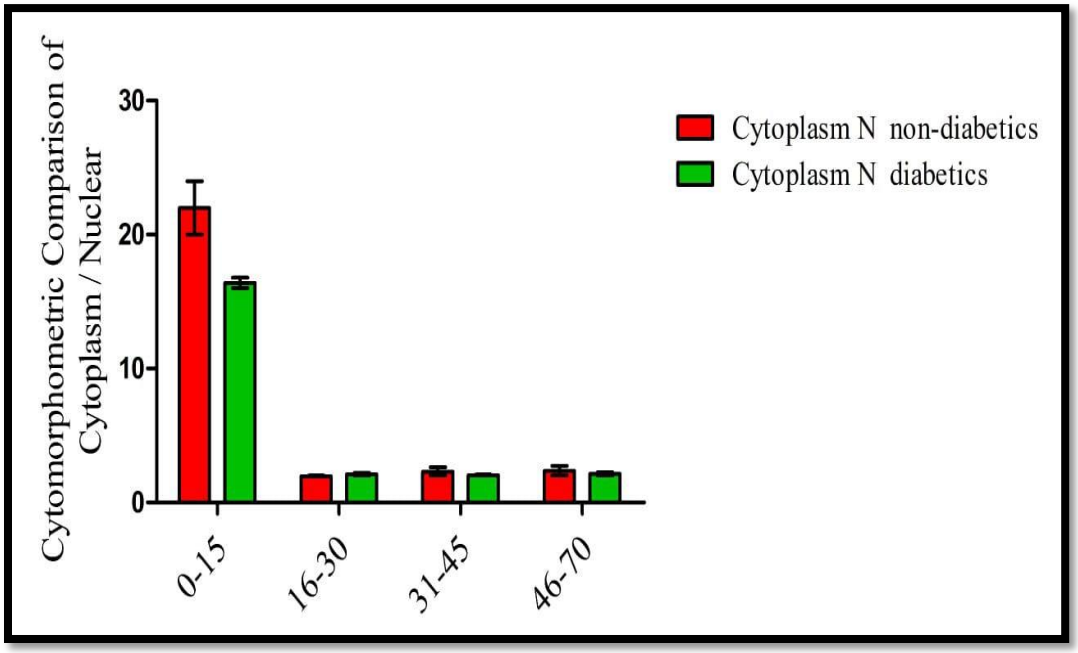
Figure 4-7: Cytology smear from a diabetic group showing cytoplasmic diameter(H&E,100X).

4.2.1.3Nuclear: Cytoplasmic Diameter ratio (N: CR)

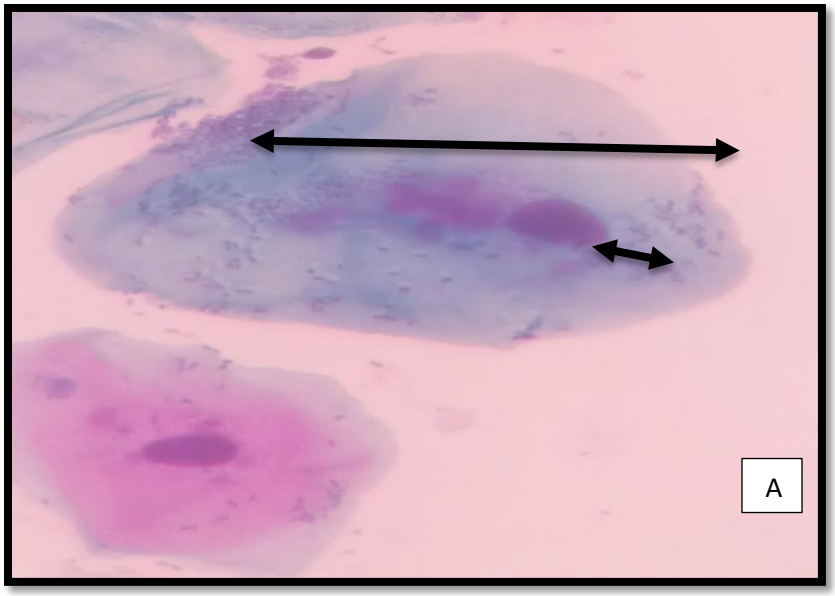
The results showed a statistically significant increase in the ratio of nucleus to cytoplasm between individuals with and without diabetes within the age group 1-15 years, where group (A) was greater than group (B) in all groups except the age groups 16-30, where group A was less than group B, indicating the presence of significant differences at the 0.05 level. In contrast, no significant differences were observed in the age groups 31-45,46-70 years, the results were equal, as shown in Table (4-6), Figure (4-8), and Graph (4-5).

Table 4-6: Cytomorphometric Comparison of Nuclear/Cytoplasmic ratio between control and oral diabetes.

Age(years)	**Cytoplasm \ Nuclear The ratio of healthy (µm) (A)**	**Cytoplasm \ Nuclear Ratio of diabetics (µm) (B)**	**Standard errors of means** **rep. 2 4** **d.f. 3** **e.s.e. 107.5 76.0**
0-15	±23.985	±16.797	
16-30	±1.9364	±2.218	
31-45	±2.6457	±2.096	
46-70	±2.7357	±2.271	
Significance *P<0.05, d.f 3, s-s 490.187, m-s,26.80, v-r,0.011			



graph 4-5: Cytomorphometric Comparison of Nuclear/Cytoplasmic ratio between control and two groups of diabetes of oral



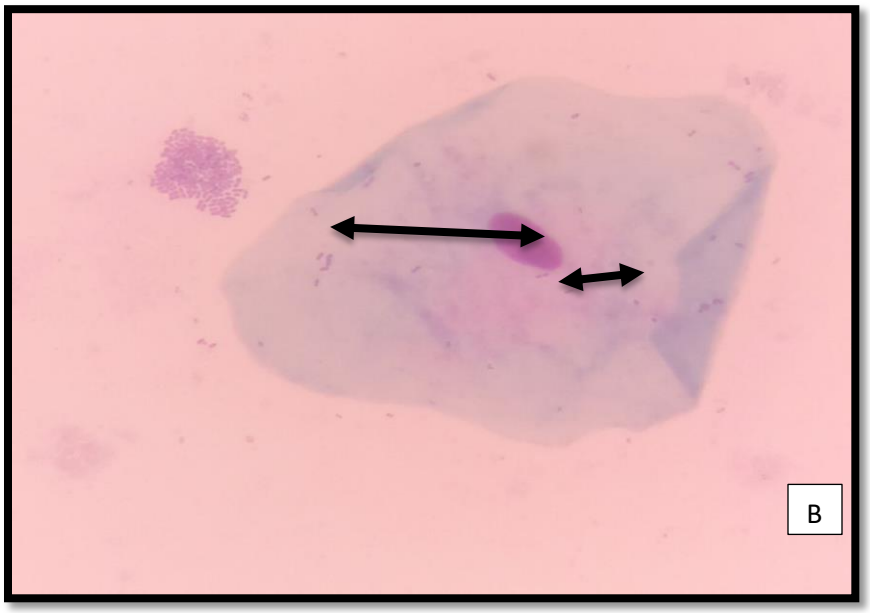


Figure 4-8: **(A)**Cytological smear from the oral cavity of the diabetic group showing nuclear: cytoplasmic diameters ratio(B) Cytological smear from the oral cavity of the healthy group showing nuclear: cytoplasmic diameters ratio (H&E,100X).

4.3-Results of cytochemical

Comparison of cell counts in non-diabetics and diabetics. Samples were taken from the four areas of the mouth: cheek, tongue, gums, and palate. They were then stained with several stains (PAP, and PAS). The samples were then examined under a light microscope. The smears of diabetics and non-diabetics were examined. The results showed that the number of cells in diabetics was less than the number of cells in healthy, as shown in the figures (4-9),(4-10),(4-11),(4-12)

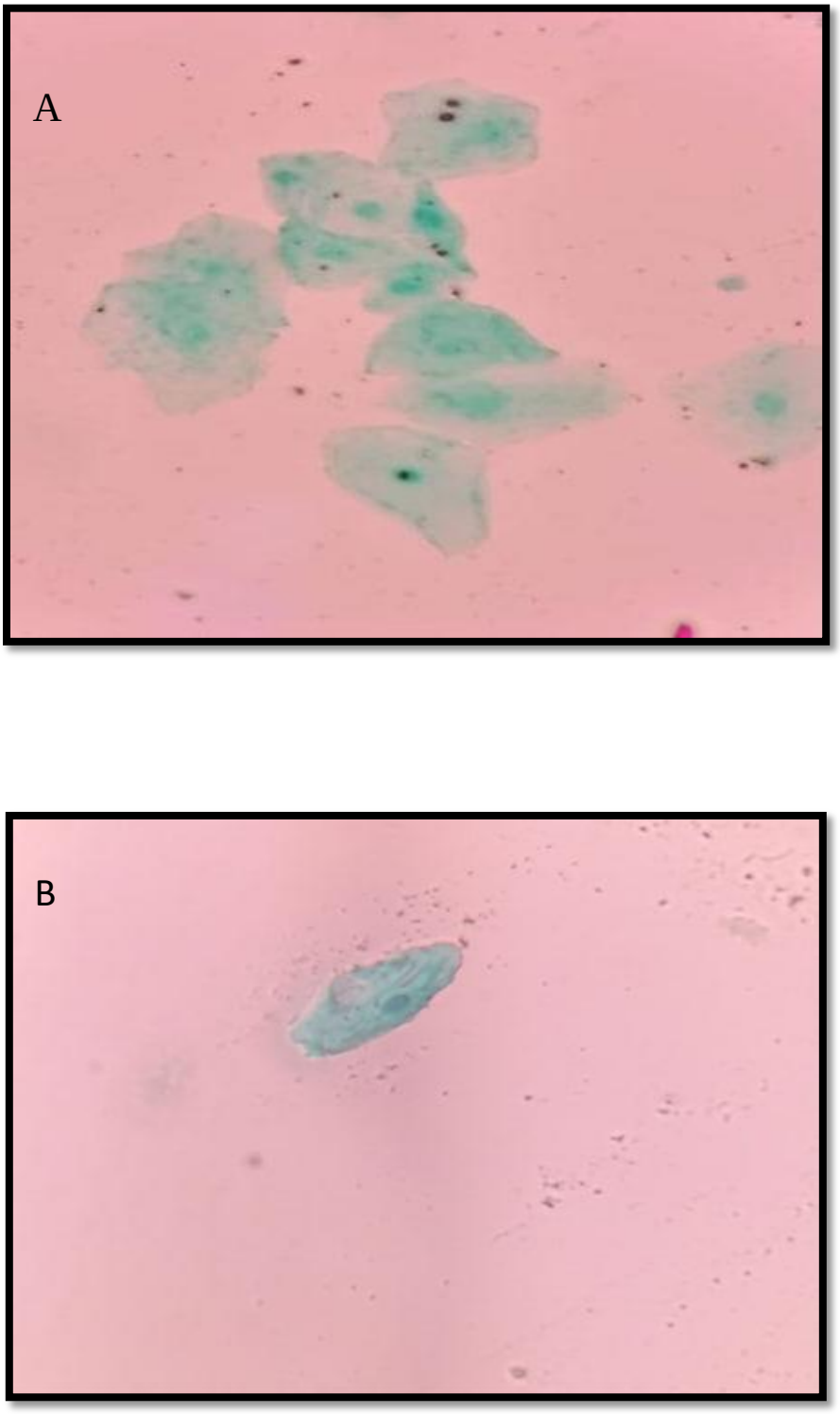


Figure 4-9: Cytological section of the cheek area (A) healthy people(B) diabetic people (PAS,40x)

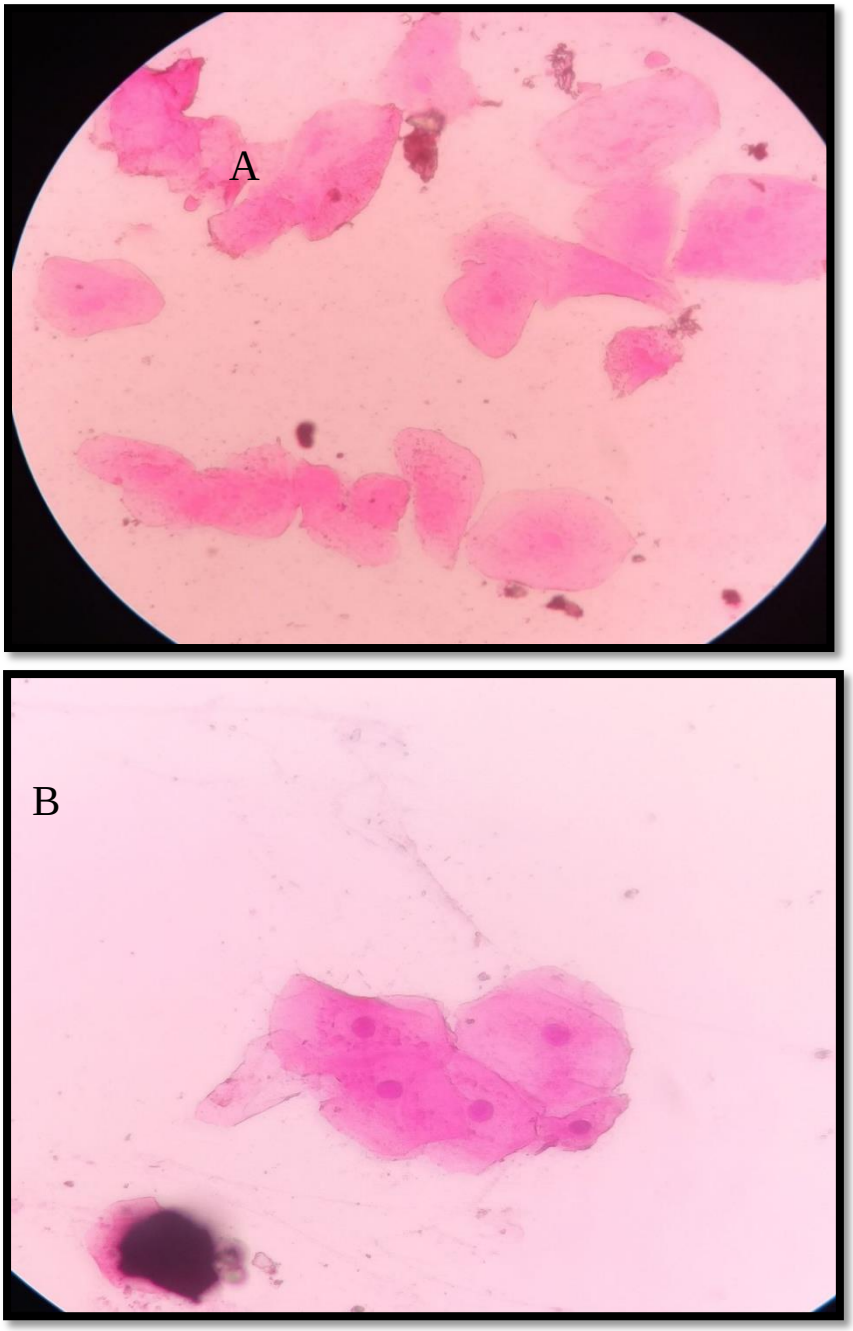
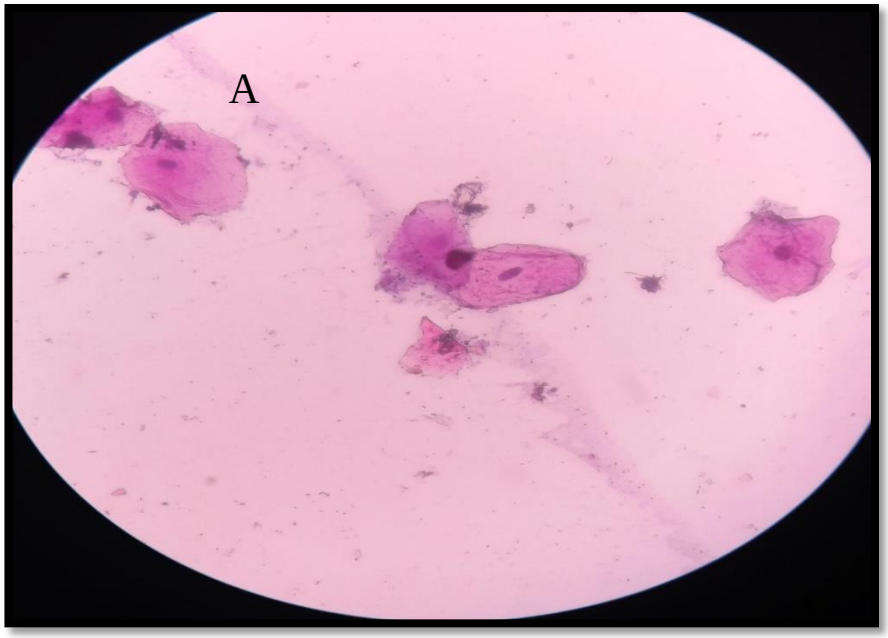


Figure4-10: A cytological section of the palate area (A) healthy people (B) people with diabetes (PAP, 40 x)



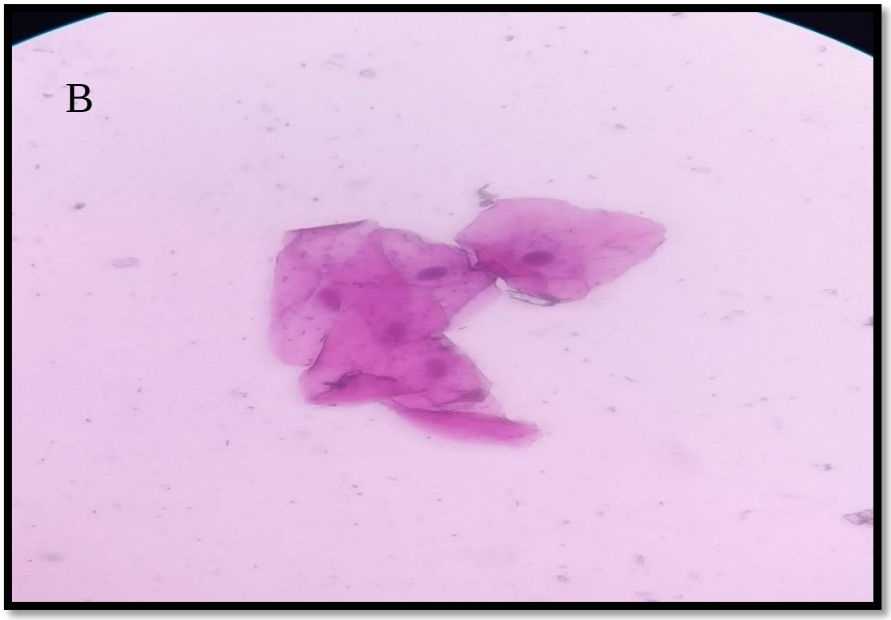


Figure 4-11: A cytological section of the tongue area (B) People with diabetes (A) Healthy people (PAP,40x)

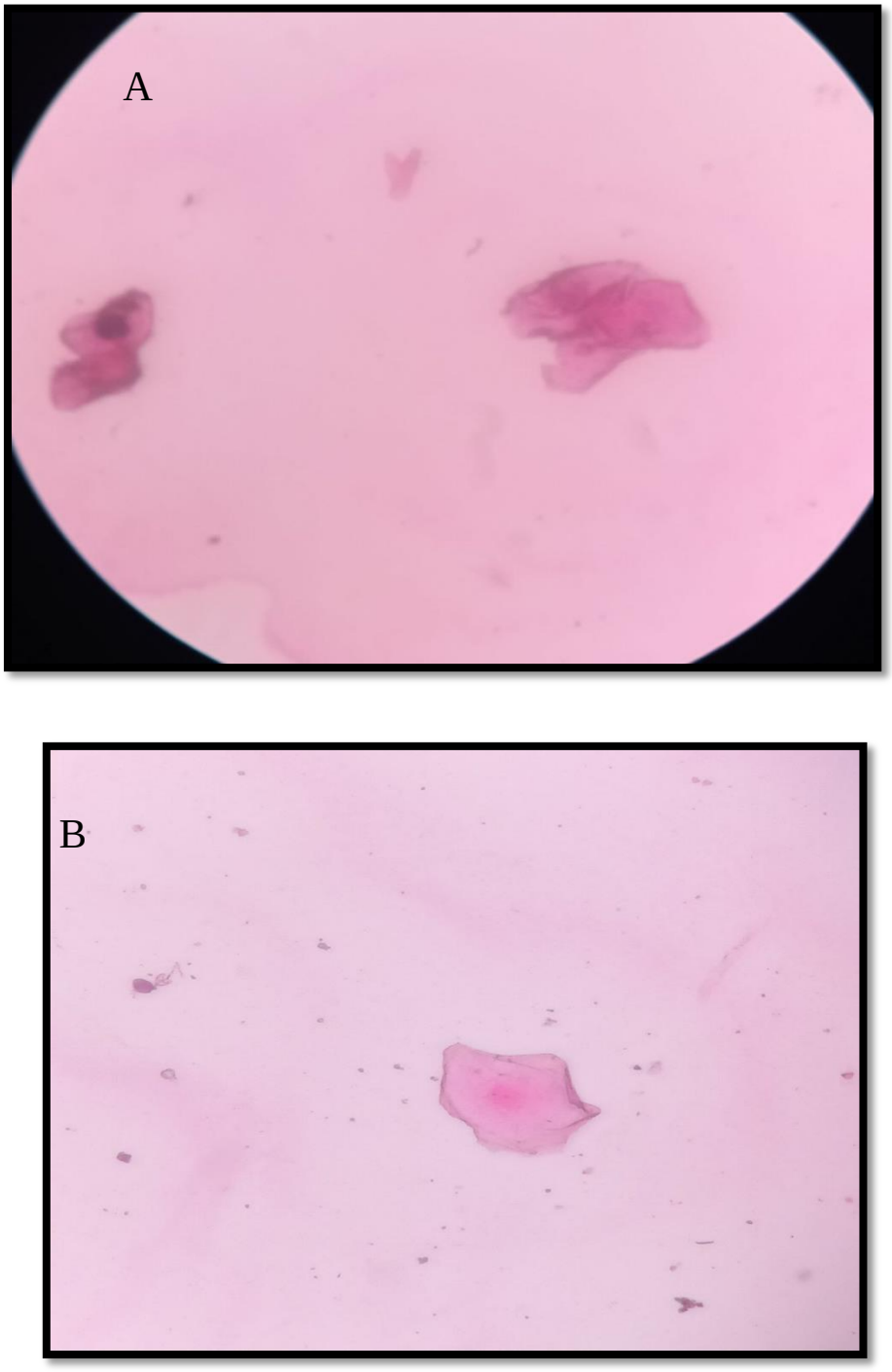
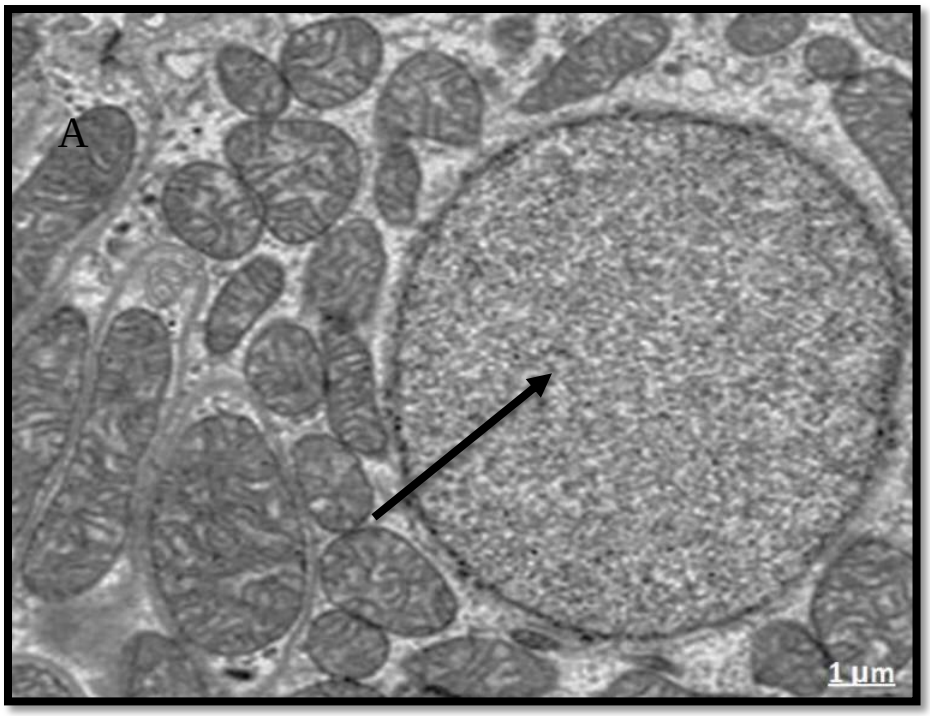


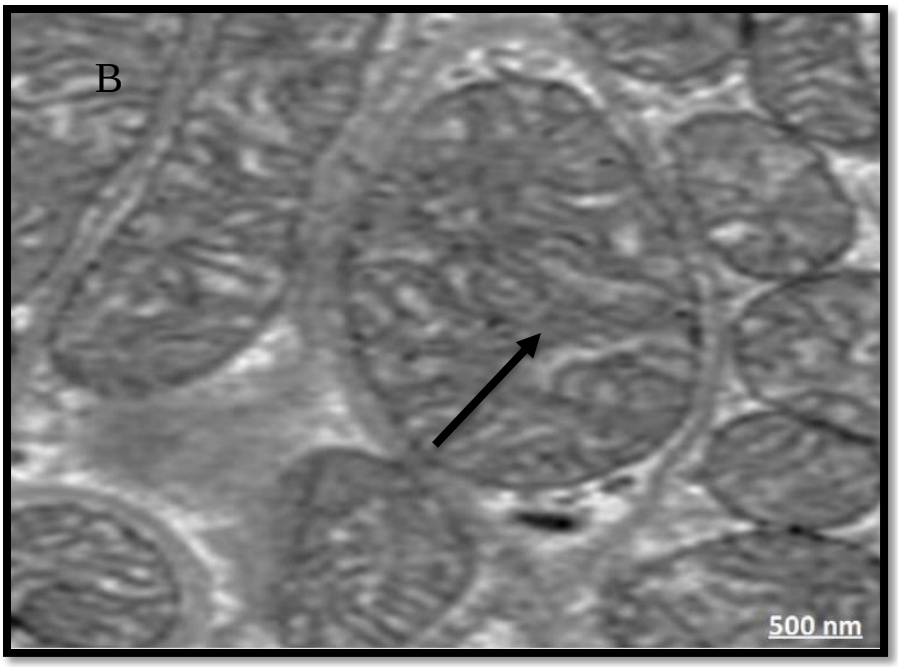
Figure 4-12: Cytological section of the Gum swab (B) for people with diabetes (A) healthy people (PAP, 40x)

4.4-Results of Electron Microscopic

Electron microscopy:

In healthy individuals, ultrastructural examination of subsarcolemmal and intermyofibrillar mitochondria revealed well-defined, uniform cristae after staining with lead citrate and uranyl acetate (scale bar = 500 nm). Using the Zeiss Supra 55Vp (Germany) with a STEM detector, the results demonstrated that the mitochondria in non-diabetic individuals function normally. As in the figure (4-13).





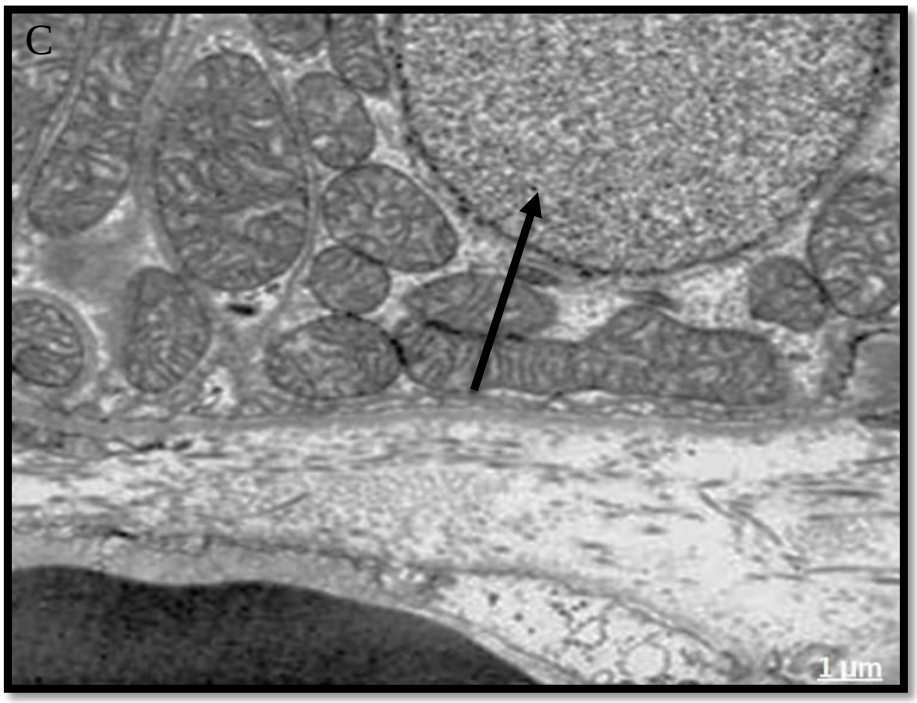
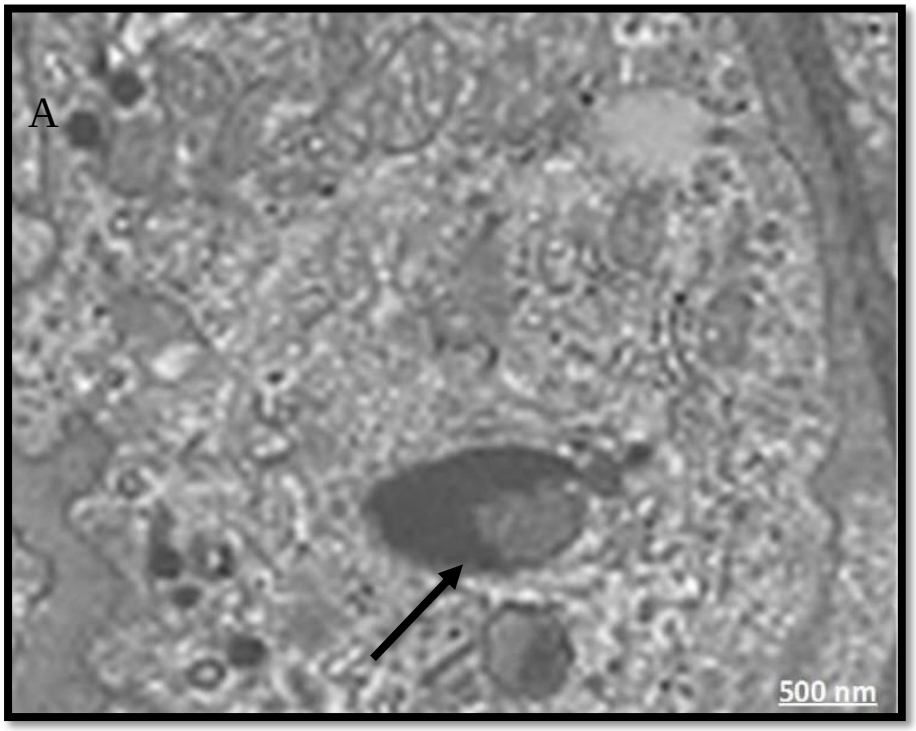


Figure 4-13: Stem detector, showed that mitochondria with non-diabetes function normally. Method of staining: Uranile acetate/ Lead citrate, Bar:2µm.

In diabetic patients, ultrastructural features of buccal cells revealed several abnormalities: loss of mitochondrial cristae, disrupted outer mitochondrial membranes, and hypertrophic endothelium. A clear disarray and reduction in the number of mitochondrial cristae were observed (scale bar = 500 nm). Utilizing the Zeiss Supra 55Vp (Germany) with a STEM detector, the results showed that individuals with diabetes had degenerated mitochondria that do not function normally Figure (4-14).



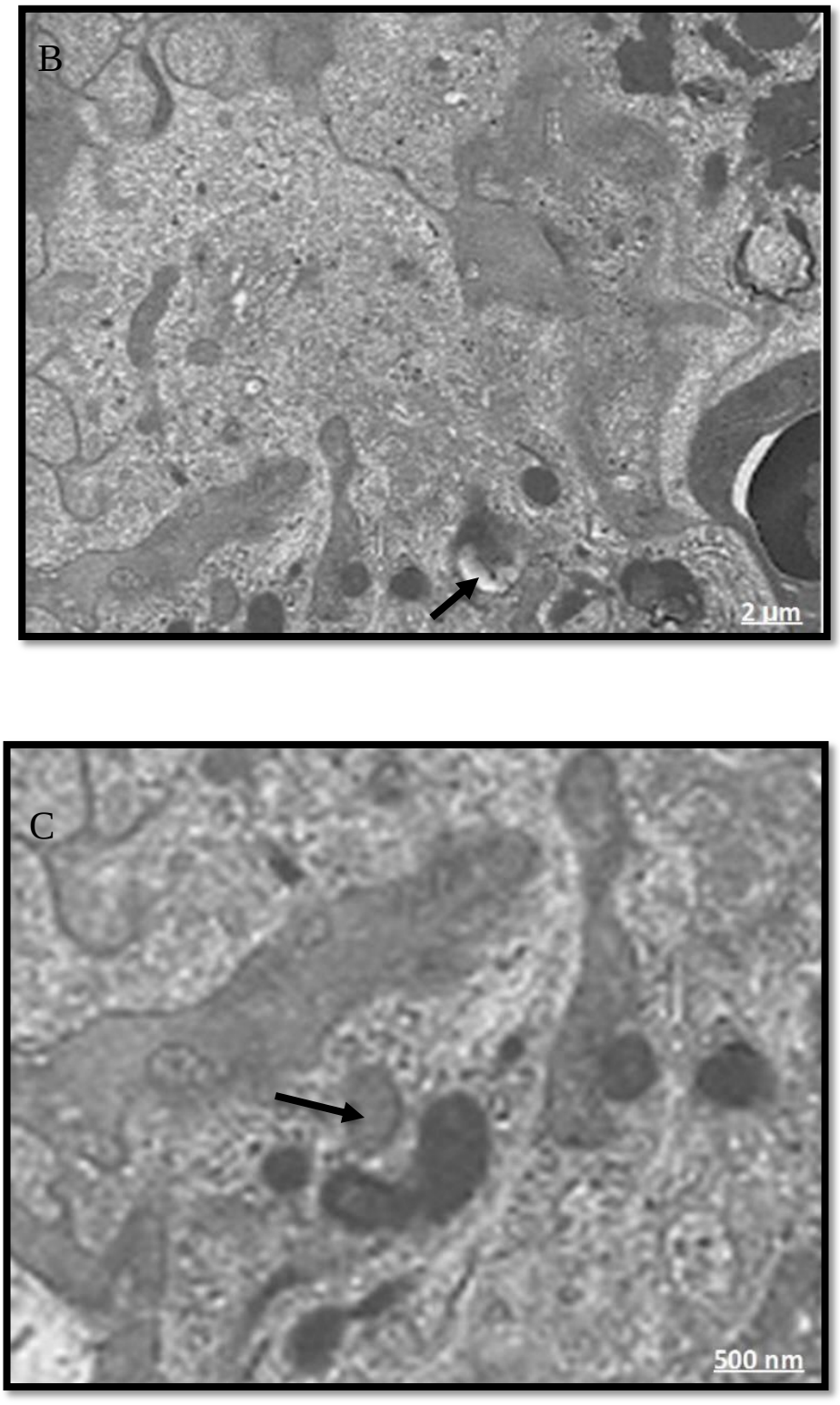


Figure 4-14: Stem detector, showed that mitochondria with diabetes had degenerated. Method of staining: Uranile acetate/ Lead citrate, Bar:2µm.

4.5-Results of Florescent Microscopic Study

After taking samples from the oral areas and staining them with fluorescent dyes, the current results of the fluorescent microscopic cell sections showed an increase in the release of mtROS in diabetic cells, and the fluorescent microscopic results also showed an increase in the release of ROS. (Mito Sox), ROS (Rodamen).

Other sections showed that the fluorescent microscopic cells increased the release of mitoTreaker in diabetic cells, and the fluorescent microscopic results also showed an increase in the release of ROS. (mitoTreaker). The cells show enlarged mitochondria with broken cristae; there are many enlarged mito chondria of different sizes and many degree

s of cristae disorganization as shown in the figure (4-15), (4-16), (4-17).

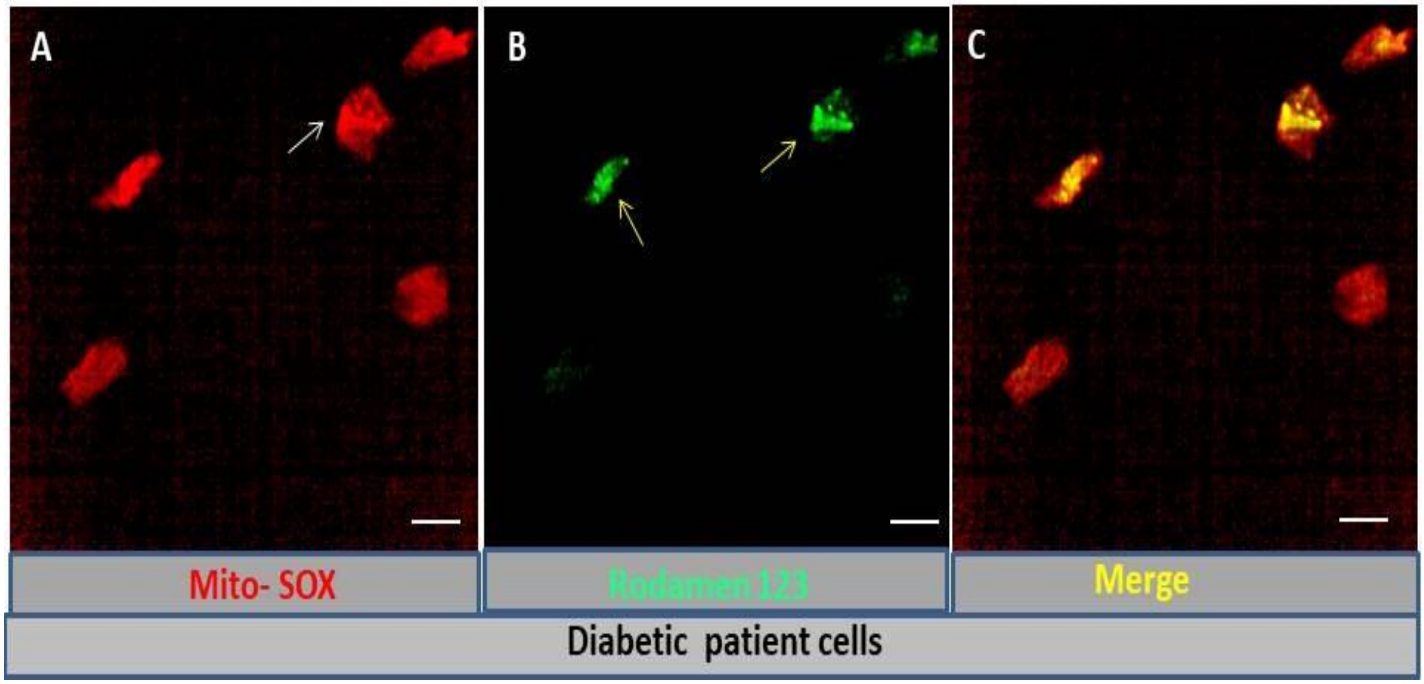


Figure 4-15: (A) Cell sections of florescent microscopy showed increase release of mtROS in diabetic patient cells. (B) Florescent microscopy

showed increased release of ROS. (Mito Sox) (red), ROS (Rodamen) (green)(C) Merge images A and B.

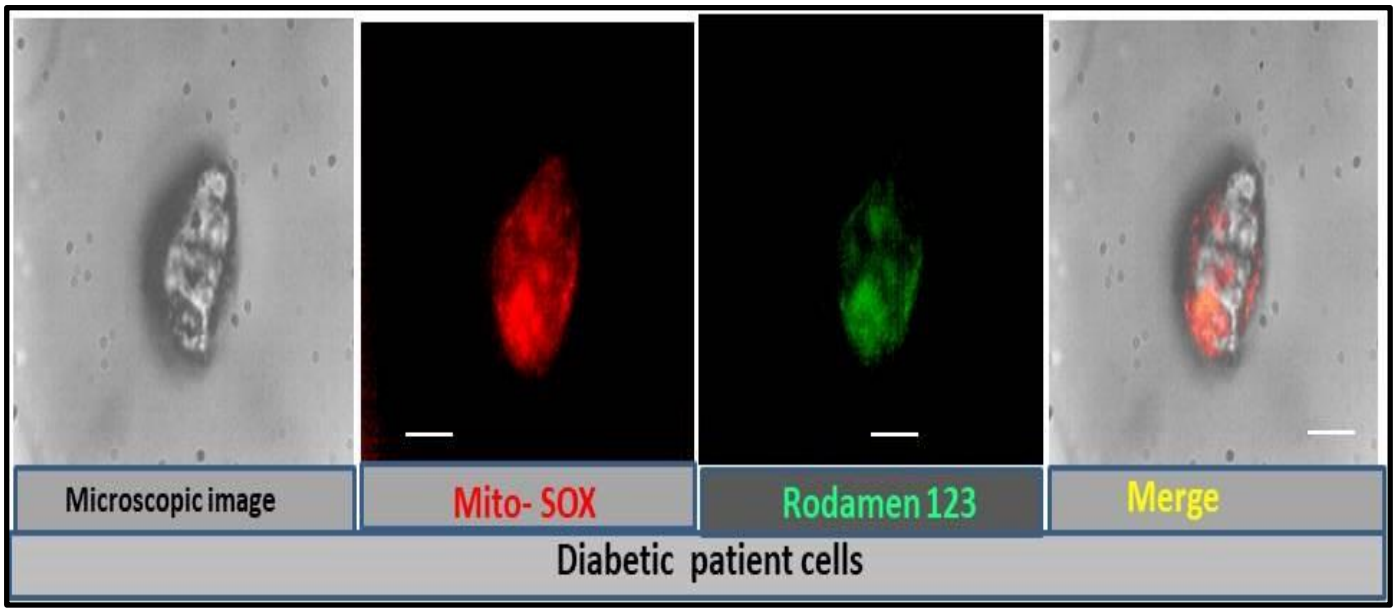


Figure 4-16: Cell sections of florescent microscopy showed an increase release of mtROS in diabetic patient cells Florescent microscopy showed an increased release of ROS. (Mito-Sox) (Red), ROS (Rodamen) (green).

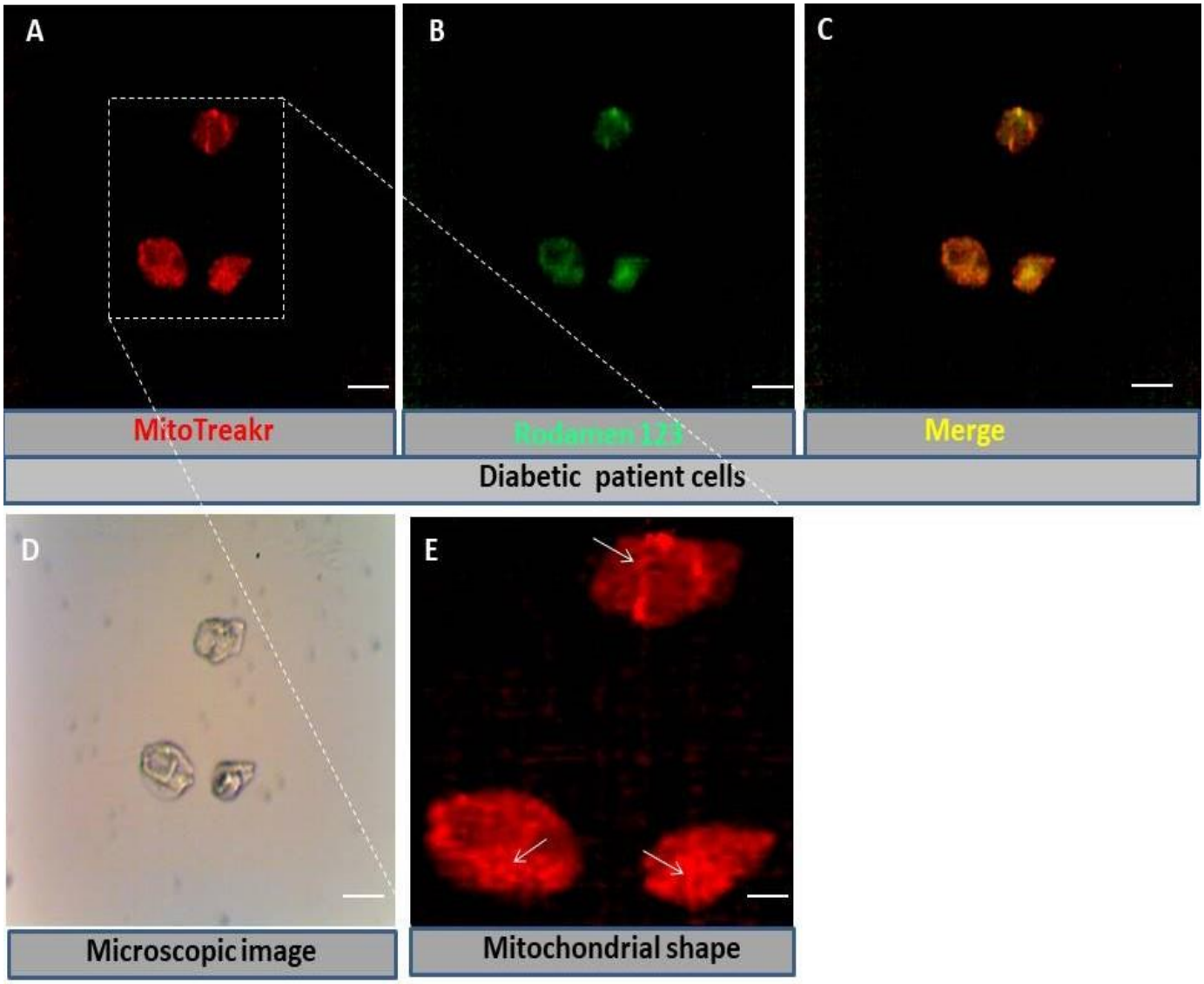


Figure 4-17: (A) Cell sections of florescent microscopy showed increased release of mitoTreaker in diabetic patient cells. (B) Florescent microscopy showed increased release of ROS. (mitoTreaker) (Red). ROS (Rodamen) (green). Cells exhibit enlarged mitochondria with broken cristae; there are multiple swelling mitochondria of different sizes and several degrees of cristae disarrangement (arrow)

Swamps were taken from the oral cavity for healthy people stained. Then they were dyed with a Mitotraacker dye as regular mitochondria appeared with a similar size and mitochondria swelling. The ROS evaluation also included natural expression. Figure(4-18)

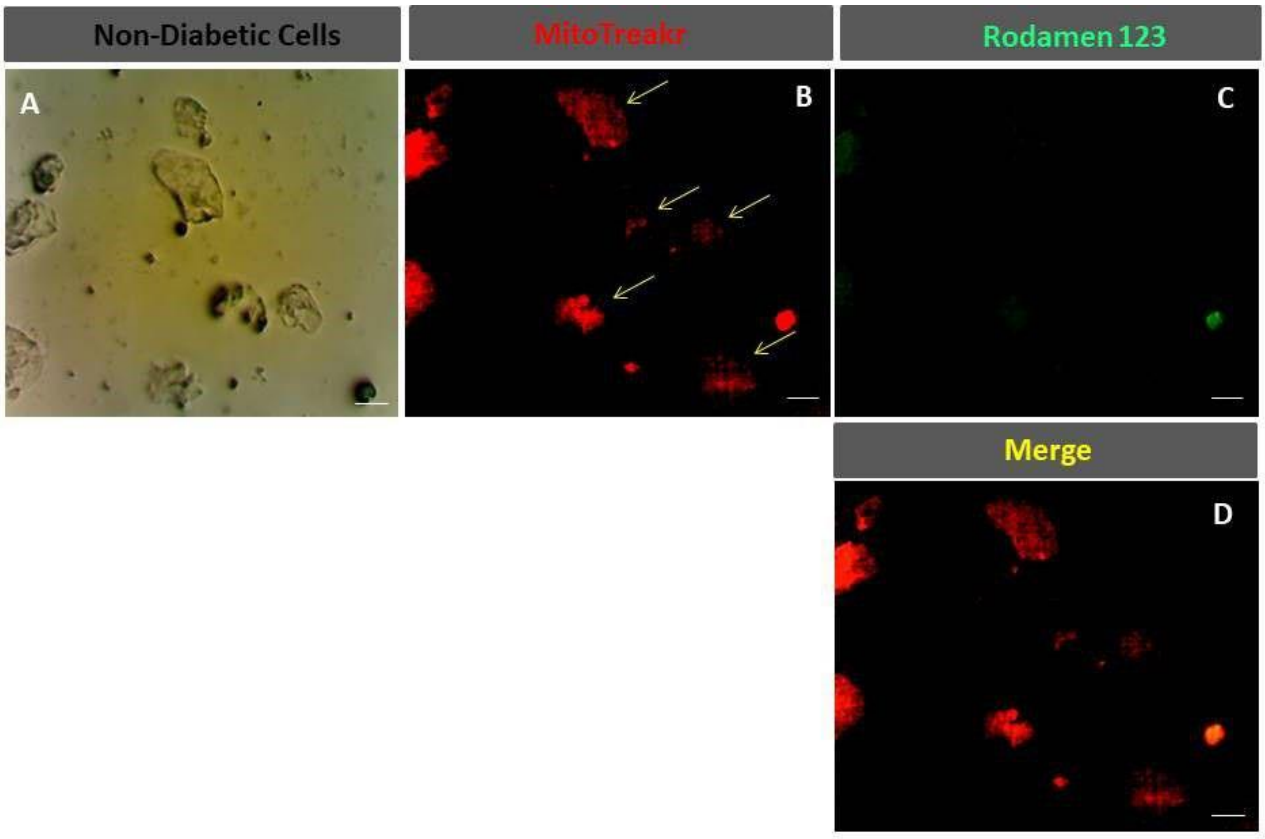


Figure 4-18: Images of non-diabetic oral cavity cells stained with. **A** MitoTracker dye. **B** images display normal mitochondria of similar size and multiple swelling mitochondria (arrows). **C** images included **an** assessment of ROS normal expression.

Chapter Five
Discussion

Discussion

The results revealed a significant variation in cell counts between oral sampling sites for both genders and different age groups, as these samples were taken by brushing and from four areas (tongue, cheek, gums, and palate). The results of the study also revealed a significant variation and statistically significant differences in the number of squamous cells falling from the oral cavity between participants with type 1 and type 2 diabetes, one of whom was insulin-dependent and the other was non-insulin-dependent and non-diabetic. Specifically, slides stained with hematoxylin-eosin and PAP and PAS stain showed a significant proliferation of cells in all oral sites in non-diabetic individuals, especially in the cheek area, as well as in diabetic individuals, accompanied by a decrease in cell counts in the gingival area in diabetic and non-diabetic individuals. My research was interpreted as adopting cell counts as a means of diagnosing diabetes.

Research that is consistent or inconsistent with my research indicates that in non-diabetic individuals, cell division occurs completely, that is, the cell divides into two daughter cells (Rappaport, 1986). The mechanism of cell division is that the interphase constitutes the largest part of the division in the cell life cycle, so that the daughter phase divides into two parts, first the cell doubles in preparation for division. Second is the young division that occurs after the interphase, but the mechanism of cell division in diabetic people differs from the mechanism of cell division in non-diabetic people. In non-diabetic people, division is faster and is renewed continuously, especially the epithelial cell mitosis, while in diabetic people, division occurs slowly or is absent as a result of high blood sugar, which leads to the

inability of cells to divide, and thus the number of cells in diabetic people is less than in non-diabetic people (Young et al.,1995). I expect that these results were the first to be scientifically proven, and therefore they are considered a patent, because until now no one has been able to diagnose diabetes by counting the cells falling from the lining of the mouth.

Since Hb1Ac provides an accurate and objective measure of glycemic control over the previous 3 months and is not affected by variables such as food or medication consumption, it was chosen for the current study as a method of measuring blood glucose level because it is a more reliable parameter (Kilpatrick, 2004). A cytomorphometric study was conducted in the Iraqi population to determine the oral signs and symptoms of type 2 and type 1 diabetes as well as the hyperglycemic status of the patient (Baban and Gharib, 2013). To our knowledge, this work is the first to investigate the morphological and cellular changes in the exfoliated cells of normal buccal mucosa and lateral border of the tongue in individuals with type 2 and type 1 diabetes.

There are several ways to diagnose diabetes by measuring HbA1c. Still, this method has a drawback, which is that the person must fast for at least 8 hours, because the diagnosis cannot be made in most patients who come for afternoon appointments or if they eat before the morning appointment. In addition, this is done by obtaining venous blood, and there is another way to diagnose diabetes: measuring the FPG level, as it requires only venous blood, which is easier than the HbA1c method (Gavin *et al*.,1997

5 - Cytomorphometrical Analysis of Parameters:

Cytomorphometric analysis of parameters: Cytomorphometric analysis of the study and control groups at the smear sites (oral mucosa, tongue, cheek, and gum) included determination of mean cytoplasmic diameter(CYD), nucleus diameter (ND), and nucleus-to-cytoplasm ratio (N : CR).

5 - 1 - Nuclear Diameter:

The study showed that there is a significant variation in the diameter of the nucleus between individuals with diabetes and non-diabetics according to age, as well as an increase in the diameter of the nucleus for individuals without diabetes compared to the diameter of the nucleus for individuals with diabetes, and a variation in the diameter of the nucleus across age groups and diabetes status according to statistical analysis. This result is consistent with the research by Prasad et al. (2010), who found that ND was affected by the degree of diabetes (i.e., the degree of glycemic control), as assessed by Hb1Ac.

In addition, it is consistent with another study by Sadia et al. (2017) who found that nuclear diameter showed a gradual increase in size from the control group to the uncontrolled diabetes group and produced similar results to this study for the ND variable. In addition, our results are consistent with those of other investigations (Jajarm et al., 2008, Shareef et al., 2008, Tozoglu and Bilge 2010; Hallikerimath et al., 2011; Suvarna et al., 2013). The diabetic group was found to have significantly greater nuclear

alteration. The first possible explanation for its increase in the research group is prolonged hyperglycemia, which may be due to inflammatory processes, aging-related effects, dryness/atrophy, and delayed keratinization of the oral epithelium. Glycosyl modifications are the cause of delayed keratinization.

Due to abnormal glycosylation of proteins, lipids, and nucleic acids in the basement membrane of the microvessel walls and major arteries, persistent hyperglycemia leads to a greater accumulation of advanced glycosylation end products. Delayed keratinization of the epithelium can be explained by increased narrowing of the arterial lumen, which reduces cell turnover and perfusion of the affected tissue. This delay in epithelial cell differentiation results in an increase in the number of mature cells, which present a large nucleus as a precursor (Alberti et al., 2003; Martin and Michael, 2003; Kumar et al., 2003; Jajaram et al., 2008). Dry mouth due to reduced salivary flow may be the second cause. Ultimately, dry mouth increases the damage to the oral mucosa, leading to cell loss. As a result, by increasing the proportion of active cell divisions – which consist of cells with large, clear nuclei – basal cells become more active to replace the lost cells (Suvarna et al., 2013).

5 - 2- Cytoplasm Diameter:

The present study also evaluated the cytoplasmic diameter (CyD). The results revealed that compared to non-diabetics and diabetics, there were statistically significant differences in the cytoplasmic diameters of non-diabetics and diabetics. This is similar to the findings of Prasad et al. (2010) who observed a clear and specific decrease in cytoplasmic diameter in

diabetic patients. This results also agree with Sherif *et al*., (2008). A similar study found a statistically significant decrease in cytoplasmic area, which may be a result of dehydration-induced cell shrinkage. This theory is supported by the findings of Ogden *et al*. (1999) who observed a decrease in cytoplasmic diameter in alcoholic patients and suggested that this may be due to their dehydration. Individuals with diabetes also experience a similar state of dehydration, which may be responsible for the decrease in cytoplasmic diameter.

This finding has also been suggested by the findings of Frost *et al*. (1997) who reported that actively expanding cells had a decrease in cytoplasmic volume with an increase in nuclear content due to replication. In another study by Sadia *et al*. (2017), they found a decrease in cytoplasmic diameter (CyD), but no significant difference was observed across all study groups. Alberti *et al*. ,(2003) also found a decrease in cytoplasmic area in the diabetic group but concluded that there was no significant difference in cytoplasmic area among diabetic patients. This finding is in contrast to the findings of Jajarm *et al*.,(2008) who reported a significant increase in cytoplasmic area in diabetic patients.

5 - 3- Nuclear: Cytoplasmic Diameter (N: C) ratio

Both type 1 and type 2 diabetic patients were found to have lower N: CR values in the present study. Compared to non-diabetics, a statistically significant increase in the mean N: CR values was observed for age groups (0-15) in exfoliated cells of oral mucosa. This phenomenon could be explained by the relatively greater increase in nuclear diameter compared to

cytoplasmic diameter, whereas no statistically significant differences were observed in age groups 16–70. The N: CR values were consistent with the study by Prasad et al. (2010) who found an association between high N: CR and severity of diabetes as determined by Hb 1Ac. This finding is also consistent with previous research (Alberti et al., 2003; Sharif et al., 2008), however, in contrast to the findings of Jagaram et al. (2008), who claimed that the mean N: CR in the diabetic group was significantly lower than that in the control group.

In diabetic patients, the ultrastructural features of cheek cells revealed several abnormalities: loss of mitochondrial peaks, rupture of mitochondrial outer membranes, and endothelial hyperplasia. Marked disorganization and a decrease in the number of mitochondrial peaks were observed. In healthy individuals, ultrastructural examination of mitochondria under the sarcoma and between muscle fibers revealed uniform and well-defined peaks after staining with lead citrate and uranyl acetate. As indicated in Figure (4-14).

The term programmed cell death was originally used to describe a specific type of cell death characterized by specific cellular morphological changes, including oral mucosal hemorrhage, cell shrinkage, and mitochondrial deformation (Kerr, 2002; Kerr et al., 1972; Taylor et al., 2008). While the functional interaction between the mitochondrial pathway and death receptor-mediated apoptosis is well established, recent evidence suggests that the mitochondrial pathway also communicates with death receptor-induced programmed necrosis (also called apoptosis). Typical forms of necrosis include the formation of intracellular vacuoles, swelling of organelles, and rupture of the plasma membrane (Chan, 2012). The various morphological changes that occur during apoptosis have been recognized by light microscopy and electron microscopy. Cell and senescence reduction

can occur during apoptosis by light microscopy. With cell size reduction, cells become smaller, the cytoplasm is denser, and organelles are more readily available. It is the result of chromatin condensation and is the most characteristic feature of apoptosis. Apoptosis involves single cells or small cell clusters on histological examination with hematoxylin and eosin staining. The apoptotic cell appears as a red or oval mass containing dense portions of violet nuclear chromosomes and dark eosinophilic convoluted cytoplasm. Electron microscopy can best describe the changes in subcellular compartments. Early during the chromatin condensation phase, electron-dense nuclear material is usually clustered peripherally beneath (Majno & Joris ,1995).

Chapter Six

Conclusions and Recommendations

Conclusions and Recommendations

6.1. Conclusions

1- The number of scaly cells in the cheek area in non-diabetic individuals was statistically higher than that in diabetic individuals, and the proportion of oral cells was increased in non-diabetic individuals compared to diabetic individuals.

2- The number of cells is directly proportional to age, especially in the age groups of 40-70 years in diabetic patients, as this group had the highest number and was most likely to continue treatment.

3-Hemotoxin-eosin staining gives the same results as PAP staining but is used for comparison in clarity, cost, and content CH.

4- There was a clear increase in the diameter of the nucleus with age between diabetics and non-diabetics.

5- The diameter of the cytoplasm of healthy people is relatively larger than the diameter of the cytoplasm of people with diabetes.

6- There were some differences in the nucleus-to-cytoplasm ratio between some age groups of diabetic and healthy subjects.

7-Evidence of changes such as increased MtRose and increased ROS in people with diabetes compared to people with non-diabetes.

8-Detection of mitochondrial structural features and the presence of changes and abnormalities in the mitochondria in people with diabetes compared to those healthy.

6.2. Recommendations

1. Using electronic research to count the number of individuals with cell numbers who are infected and those who are not.

2. Carrying out a cell analysis to ascertain the number of cells that have decreased in accordance with the applied therapies.

3. Conducting research on cells in the oral mucosa of people with diabetes to investigate various medical disorders including kidney failure..

4-Determining the mitochondrial ultrastructure in people with and without diabetes using electron microscopy.

5. Adoption of fluorescent microscopy for the purpose of diagnosing oral cells, detecting the rise in Ros details, and understanding the mitochondrial phenotype in individuals with and without diabetes.

References

References

- Abdulrahman, K. A., & Dphc, A. B. F. M. (2006). Diabetes mellitus and its oral complications: A brief review. *Pak Oral Dental J*, *26*, 97-100.

-Abraira, C., Duckworth, W., McCarren, M., Emanuele, N., Arca, D., Reda, D., & Henderson, W. (2003). Design of the cooperative study on glycemic control and complications in diabetes mellitus type 2: Veterans Affairs Diabetes Trial. *Journal of Diabetes and its Complications*, *17*(6), 314-322.

- Adams, D. (1976). Keratinization of the oral epithelium. *Annals of the Royal College of Surgeons of England*, *58*(5), 351.

- Ahmed N (2010). Chemical Biochemistry, IBMS, Oxford University, pp. 341.

- AlJulaih GH, &Lasrado S. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Aug 14, 2023. Anatomy, Head and Neck, Tongue Taste Buds.

- American Diabetes Association (ADA). Diagnosis and Classification of Diabetes Mellitus. Diabetes care. 2007; 30:S42-S47

- American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33: Suppl 1:S62-S69.

- Baldwin E. Oral health. Lancet. 2009; 373:628–9.

- Barrett, A. W., & Scully, C. (1994). Human oral mucosal melanocytes: a review. *Journal of oral pathology & medicine*, *23*(3), 97-103.

- Bearelly, S., & Cheung, S. W. (2017). Sensory topography of oral structures. *JAMA Otolaryngology–Head & Neck Surgery*, *143*(1), 73-80.

References

- Beers MH, Porter RS, Jones TV. The Merck Manual of Diagnosis and Therapies: Endocrine and Metabolic Disorders. Section 2. Chapter 13.Merck Research laboratories. Glasgow. Washington. 2006.133

- -Bogdan Calenic, Maria Greabu, Constantin Caruntu, Cristiana Tanase, Maurizio Battino Periodontology 2000 69 (1), 68-82, 2015

- Calenic, B., Ishkitiev, N., Yaegaki, K., Imai, T., Costache, M., Tovaru, M., ... & Parlatescu, I. (2010). Characterization of oral keratinocyte stem cells and prospects of its differentiation to oral epithelial equivalents. *Rom J Morphol Embryol*, *51*(4), 641-5.

- Chen J, Ahmad R, Li W, Swain M, Li Q. Biomechanics of the oral mucosa. J R Soc Interface. 2015 Aug 06;12(109):20150325.

- Collin HL, Niskanen L, Uusitupa M, Toyry J, Collin P, Koivisto A-M, et al. Oral symptoms and signs in elderly patients with type 2 diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90: 299–305.

- Defronzo RA, Ferrannini E, Keen H, Zimmet P. International textbook of diabetes mellitus, 3rd ed. John Wiley & Sons Ltd, 2004.

- Delong, L. & Burkhart, N. Endocrine disorders. In General and Oral Pathology 2008; pp 147-178, Philadelphia: Lippincott Williams &Wilkins.

- Dummett CO, Barens G. Oromucosal pigmentation: an updated literary review. J Periodontol. 1971 Nov;42(11):726-36.

References

- Eisen D. Disorders of pigmentation in the oral cavity. Clin Dermatol. 2000 Sep-Oct;18(5):579-87.

- Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Methods Mol Biol.

- Feller L, Masilana A, Khammissa RA, Altini M, Jadwat Y, Lemmer J. Melanin: the biophysiology of oral melanocytes and physiological oral pigmentation. Head Face Med. 2014 Mar 24;10:8.

- Fleisch L, Austin JC. A histologic study of the response of masticatory and lining mucosa to mechanical loading in the vervet monkey. J Prosthet Dent. 1978 Feb;39(2):211-6.

- Frost JK. Pathologic processes affecting from inflammation to cancer. In: Bibbo M, editor. Comprehensive cytopathology. Philadelphia, USA: WB Saunders Co; 1997. p. 84- 5.

- Gaballah KY, Rahimi I. Can the presence of oral Fordyce's granules serve as a marker for hyperlipidemia? Dent Res J (Isfahan). 2014 Sep;11(5) :553-8.

- García-Caballero L, Caneiro J, Gándara M, González-Ortega N, Cepeda-Emiliani A, Gude F, Collado M, Beiras A, Gallego R. Merkel cells of human oral mucosa express the pluripotent stem cell transcription factor Sox2. Histol Histopathol. 2020 Sep;35(9):1007-1012.

- Gavin III, J. R., Alberti, K. G. M. M., Davidson, M. B., & DeFronzo, R. A. (1997). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care*, *20*(7), 1183.

References

- Gonsalves, W. C., Chi, A. C., & Neville, B. W. (2007). Common oral lesions: Part I. Superficial mucosal lesions. *American Family Physician*, *75*(4), 501-506.

- Groeger S, Meyle J. Oral Mucosal Epithelial Cells. Front Immunol. 2019;10:208.

- Guyton C, Hall JE. Textbook of Medical Physiology. 12th ed. ElsevierSaunders. Philadelphia, 2012.

- Hanas R& , John G, International HBA1c Consensus Committee.Consensus statement on the worldwide standardization of the hemoglobin A1C measurement. Diabetes Care 2010; 33:1903-4.

- Hanson RL, Charles MA, Pettitt DJ, Bennett PH. Which test for diagnosing diabetes? Diabetes Care. 1995; 18:1042-1044.

- Horn OK, Jacobs-Whyte H, Ing A, Bruegl A, et al. Incidence and prevalence of type 2 diabetes in the First Nation Community of Kahnawá:ke, Quebec, Canada, 1986-2003. Can J Public Health. 2007; 98(6):438-43

- Hoseinpour, H., Karimi, K., Zilouei, H., & Taherzadeh, M. J. (2010). Simultaneous pretreatment of lignocellulose and hydrolysis of starch in mixtures to sugars. *BioResources*, *5*(4), 2457-2469.

- IJssennagger, N., Rijnierse, A., de Wit, N., Jonker-Termont, D., Dekker, J., Müller, M., & Van Der Meer, R. (2012). Dietary haem stimulates epithelial cell turnover by downregulating feedback inhibitors of proliferation in the murine colon. *Gut*, *61*(7), 1041-1049.

- Kilpatrick ES. HbA1c measurement. J Clin Pathol. 2004; 57:3445.

References

- Kosicki DM, Riva C, Pajarola GF, Burkhardt A, Grätz KW: OralCDx brush biopsy-a tool for early diagnosis of oral squamous cell carcinoma. Schweiz Monatsschr Zahnmed 2007, 117, 222–227.

- Kumar PJ & Clark M (2007). Diabetes mellitus and other disorders of metabolism. In: Kumar PJ, Clark M, editors. Textbook of Medicine. Saunders: London; pp. 1069-1122., Textbook of Medicine. Saunders: London ed., pp. 1069-1122.

- Kurn H, Daly DT. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Feb 17, 2023. Histology, Epithelial Cell.

- Kydd WL, Daly CH. The biological and mechanical effects of stress on oral mucosa. J Prosthet Dent. 1982 Mar;47(3):317-29.

- Laugerette F, Gaillard D, Passilly-Degrace P, Niot I, Besnard P. Do we taste fat? Biochimie. 2007 Feb;89(2):265-9.

- Liang, J., Balachandra, S., Ngo, S., & O'Brien, L. E. (2017). Feedback regulation of steady-state epithelial turnover and organ size. *Nature*, *548*(7669), 588-591.

- Maley BL, Oates TW. Diabetes mellitus and periodontal diseases. AAP Commissioned review. J Periodontal 2006; 77:1289-1303.

- Marshall SM. Standardization of HbA1c: good or bad? Nat.Rev.Endocrinol. 2010; 6: 408-411.

- Martin S Greenberg, Michael Glick. Burket's Oral Medicine – Diagnosis & Treatment. 10th Edition. B C Decker Inc: Elsevier India; 2003

References

- Meleti M, Vescovi P, Mooi WJ, van der Waal I. Pigmented lesions of the oral mucosa and perioral tissues: a flow-chart for the diagnosis and some recommendations for the management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 May;105(5):606-16.

- Merz, C. N. B., Buse, J. B., Tuncer, D., & Twillman, G. B. (2002). Physician attitudes and practices and patient awareness of the cardiovascular complications of diabetes. *Journal of the American College of Cardiology*, *40*(10), 1877-1881.

- Nakamura, T., Imai, Y., Matsumoto, T., Sato, S., Takeuchi, K., Igarashi, K., ... & Kato, S. (2007). Estrogen prevents bone loss via estrogen receptor α and induction of Fas ligand in osteoclasts. *Cell*, *130*(5), 811-823.

-Ogden GR, Cowpe JG,& Green M: Cytobrush and wooden spatula for oral exfoliative cytology. A comparison. ActaCytol 1992, 36, 706–710.

- Otsuka-Tanaka Y, Oommen S, Kawasaki M, Kawasaki K, Imam N, Jalani-Ghazani F, Hindges R, Sharpe PT, Ohazama A. Oral lining mucosa development depends on mesenchymal microRNAs. J Dent Res. 2013 Mar;92(3):229-34.

- Prasad H, Ramesh V, and Balamurali P. Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J Cytol 2010; 27: 113.

- Rappaport, T. S. (1986). *Wireless communications: principles and practice*. Cambridge University Press.

References

- Righi A, Betts CM, Marchetti C, Marucci G, Montebugnoli L, Prati C, Eusebi LH, Muzzi L, Ragazzini T, Foschini MP. Merkel cells in the oral mucosa. Int J Surg Pathol. 2006 Jul;14(3):206-11.

- Rivard, N., Boucher, M. J., Asselin, C., & L'Allemain, G. (1999). MAP kinase cascade is required for p27 downregulation and S phase entry in fibroblasts and epithelial cells. *American Journal of Physiology-Cell Physiology*, *277*(4), C652-C664.

- Rother KI "Diabetes treatment-bridging the divide". The New England Journal of Medicine 2007 April; 356(15): 1499 - 501.

- Saini R, Al-Maweri SA, Saini D, Ismail NM, Ismail. Oral mucosal lesions in nonoral habit diabetic patients and association of diabetes mellitus with oral precancerous lesions. Diabetes Res Clin Pract 2010; 89:320–6.

- Sandberg GE, Sundberg HE, Fjellstrom CA, Wikblad KFl. Type 2 diabetes and oral health: a comparison between diabetic and non-diabetic subjects. Diabetes Res Clin Pract 2000; 50(1): 27-34.

- Ship JA. Diabetes and oral health: An overview. J Am Dent Assoc. 2003; 134: 4S–10S

- Silvio E., Inzucchi M.D. Diagnosis of Diabetes. The New England Journal of Medicine. N ENGL J MED 367; 6 NEJM. ORG, 2012; 9:542-550.

- Skamagas, M., Breen, T. L., & LeRoith, D. (2008). Update on diabetes mellitus: prevention, treatment, and association with oral diseases. *Oral diseases*, *14*(2), 105-114.

References

- Squier CA, Finkelstein MW. Oral mucosa. In: Oral histology, development, structure and function. St. Louis (MO): CV Mosby; 1998. p. 345–85

- Squier CA, Kremer MJ. Biology of oral mucosa and esophagus. J Natl Cancer Inst Monogr. 2001;(29):7-15.

- Suvarna M, Anuradha C, Kumar KK, Sekhar PC, Chandra KLP, Reddy BVM. Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. J NTR Univ Health Sci 2013;1:33- 7.

- Thomas AJ, Erickson CA. The making of a melanocyte: the specification of melanoblasts from the neural crest. Pigment Cell Melanoma Res. 2008 Dec;21(6):598-610.

- Tungare S, Paranjpe AG. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Sep 19, 2022. Drug-Induced Gingival Overgrowth.

- Von Herbal, A., & Rudi, J. (2000). Role of apoptosis in gastric epithelial turnover. *Microscopy Research and Technique*, *48*(5), 303-311.

-Wang SS, Tang YL, Pang X, Zheng M, Tang YJ, Liang XH. The maintenance of an oral epithelial barrier. Life Sci. 2019 Jun 15;227:129-136.

-Wang YP, Chen IC, Wu YH, Wu YC, Chen HM, Yu-Fong Chang J. Langerhans cell counts in oral epithelial dysplasia and their correlation to clinicopathological parameters. J Formos Med Assoc. 2017 Jun;116(6):457-463.

-Wertz PW. Lipids and the Permeability and Antimicrobial Barriers of the Skin. J Lipids. 2018;2018:5954034.

References

- World Health Organization. Global Prevalence of Diabetes: Estimates for the Year 2000 and Projections for 2030. Geneva: World Health Organization; 2009.

- World Health Organization: Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva, World Health Org., 1999.

- Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. J Biol Chem. 2007 Sep 21;282(38):27557-61.

- Young, E. D., Shahar, A., & Schlichting, H. E. (1995). Earth is shaped by primordial H2 atmospheres. *Nature*, *616*(7956), 306-311.

. -Ogden GR, Cowpe JG, Wight AJ. Oral exfoliative cytology: a review of methods of assessment. J Oral Pathol Med 1997b, 26:201-5

-Alberti S, Spadella TC, Francbiscbone TRCG, Assis GF, Cestari TM, Taveira LAA. Exfoliative cytology of the oral mucosa in type II Diabetic patients: Morphology and cytomorphometry. J Oral Pathol Med 2003; 32:538-543.

Auluck A. Diabetes mellitus: An emerging risk factor for oral cancer? J Can Dent Assoc 2007; 73:501-3.-

Braunwald E, Fauci A, Kasper D. Harrison's principles of internal Medicine. 16th Edition. Mc Graw Hill Company; 2003.

-Chan, K. M., Satterfield, T., & Goldstein, J. (2012). Rethinking ecosystem services to better address and navigate cultural values. *Ecological economics*, *74*, 8-18.

References

-Choi, A. M., Ryter, S. W., & Levine, B. (2013). Autophagy in human health and disease. New England Journal of Medicine, 368(7), 651-662.

-Chomkhakhai U, Thanakun S, Khovidhunkit S-P, Khovidhunkit W, Thaweboon S. Oral health in Thai patients with metabolic syndrome. Diabetes Metab Syndr 2009; 3:192–7.

-Cowpe JG, &Longmore RB. Nuclear area and fulgent DNA content of normal buccal mucosal smears. *J Oral Pathol.* 1981;10:81–6

-Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: An age, site, and sex-related survey. J R Soc Med 1985; 78:995- 1004

-D'Emden MC, Shaw JE, Colman PG, et al, Australian Diabetes Society; Royal College of Pathologists of Australasia; Australasian Association of Clinical Biochemists. The role of HbA1c in the diagnosis of diabetes mellitus in Australia. Med J Aust. 2012; 197(4):220–221.

-Das Bijoy Kurnar&, Mallik NC. The diagnostic perspectives of oral exfoliative cytology- an overview JIDA. 2000;71:7–9

-Dragh, M. A., Xu, Z., Al-Allak, Z. S., & Hong, L. (2017). Vitamin K2 prevents lymphoma in Drosophila. Scientific reports, 7(1), 17047.

-Eduardo Jose Caldeira, Progresso Jose Garcia, Elaine Minatel1, Jose Angelo Camilli, Valeria Helena Alves Cagnon. Morphometric analysis and ultrastructure of the epithelium of the oral mucosa in diabetic autoimmune NOD mice. Braz. J. Morphol. Sci. 2004; 21(4):197-205.

References

-Endo H, Rees TD, Koyama K, Matsue M, Yamamoto H: Use of oral exfoliative cytology to diagnose desquamative gingivitis: a pilot study. Quintessence Int 2008, 39, 152–161.

-Glim, J. E., van Egmond, M., Niessen, F. B., Everts, V., & Beelen, R. H. (2013). Detrimental dermal wound healing: what can we learn from the oral mucosa?. Wound Repair and Regeneration, 21(5), 648-660.

-Gururaj N, Sivapathasundharam B, Sumathy N. Cytologic findings in iron deficiency anemia. Ind J Dent Res 2004; 15(4):126-128.

-Hallikerimath S, Sapra G, Kale A and Malur P. Cytomorphometric analysis and assessment of Periodic Acid Schiff positivity of Exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients. Acta cytologica 2011; 55: 197-202.

-Ilayaraja V, Priyadharshini TK, Ganapathy N, Yamunadevi A, Dineshshankar J, Maheswaran T. Exfoliative cytology for age estimation: A correlative study in different age groups. J Indian Acad Dent Spec Res 2018;5:25–8.

-Jajarm HH, Mohtasham N, Moshaverinia M, Rangiani A. Evaluation of oral mucosa epithelium in type II Diabetic patients by an exfoliative cytology method. Journal of Oral Science 2008; 50(3): 335-340.

-James R. Gavin, K.G.M.M. Alberti, Mayer B. Davidson, Ralph A. DeFronzo, Allan Drash, Steven G. Gabbe, et al. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003; 26(1): S5-S20

References

-Kerr, B., Riley, M. A., Feldman, M. W., & Bohannan, B. J. (2002). Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors. *Nature*, *418*(6894), 171-174.

-Kerr, John FR, Andrew H. Wyllie, and Alastair R. Currie. "Apoptosis: a basic biological phenomenon with wideranging implications in tissue kinetics." *British journal of cancer* 26.4 (1972): 239-257.

-Kujan O, Desai M, Sargent A, Bailey A, Turner A, Sloan P: Potential applications of oral brush cytology with liquid-based technology: results from a cohort of normal oral mucosa. Oral Oncol 2006, 42, 810–818.

-Kumar V, Cotran RS, Robbins SL (2003) Robbins basic pathology. 7th ed, Saunders, Philadelphia, 42-1198

-Kumaresan, G., & Jagannathan, N. (2014). Exfoliative cytology – A predictive diagnostic tool. Int J of Pharma and Pharmac Scien, 6 (5), 1-3.

-Lamster IB, Lalla E, Borgnakke WS, Taylor GW.The relationship between oral health and diabetes mellitus. J Am Dent Assoc 2008; 139:1924.

-Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. In *Manual of histologic staining methods of the Armed Forces Institute of Pathology* (pp. xii-258).

-Majno, G., & Joris, I. (1995). Apoptosis, oncosis, and necrosis. An overview of cell death. *The American journal of pathology*, *146*(1), 3.

-Mehrotra R. Oral Cytology: A Concise Guide. Springer 2012a

-Mehrotra R. The role of cytology in oral lesions: a review of recent improvements. Diagn Cytopathol 2012b; 40: 73-83.

References

-Mizushima, N., Levine, B., Cuervo, A. M., & Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. nature, 451(7182), 1069-1075.

-Nall, R. (2018). An overview of diabetes types and treatments. *Newsletter on Health. Medical News Today, 1*(1), 1-5.

-Ogden GR, Wight AJ, Cowpe JG. Quantitative oral exfoliative cytology.Effect of alcohol on normal buccal mucosa. Anal Quant Cytol Histol 1999; 21:126- 30

-Ogden GR: The future role for oral exfoliative cytology – bleak or bright? Oral Oncol 1997a, 33, 2–4. 1.9.3. Areas for Collecting Smear

-Ogden, G. R., Cowpe, J. G., & Wight, A. J. (1997). Oral exfoliative cytology: a review of methods of assessment. *Journal of oral pathology & medicine*, *26*(5), 201-205.

-Quirino, M. R., Birman, E. G., & Paula, C. R. (1995). Oral manifestations of diabetes mellitus in controlled and uncontrolled patients. *Braz Dent J*, *6*(2), 131-6.

-Rajput DV,& Tupkari JV: Early detection of oral cancer: PAP and AgNOR staining in brush biopsies. J Oral Maxillofac Pathol 2010, 14, 52–58

-Ramaesh T, Mendis BR, Ratnatunga N, Thatti RO. Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma J Oral Pathol Med. 1998;27:83–6.

-Ramaesh T, Mendis BR, Ratnatunga N, Thatti RO. Diagnosis of oral premalignant and malignant lesions using cytomorphometry Odontostomatol Trop. 1999;85:23–8

Reboiras-López M, Pérez-Sayáns M, Somoza-Martín J, Antúnez-López J, Gándara-Vila P, Gayoso-Diz P, Gándara-Rey J, García-García A: Comparison of three sampling instruments, Cytobrush, Curette and OralCDx, for liquid-based cytology of the oral mucosa. Biotech Histochem 2012, 87, 51–58.

-Renwik EL: Papanicolau staining. Supervisor of cytology technicians. Hosp. Nac. Prof. Dr. A. Posadas (Prof. Dr. A. Posadas National Hospital), El Palomar – Buenos Aires – Republic of Argentina, 07.27.2009.

Sacks DB. Global harmonization of hemoglobin A1c. Clin Chem. 2005; 51(4):681-683.

-Sadia Sharif, Naureen Sarwar, BushraNisar, Muhammad Khalid Masood, Asim Hameed. Detection of Cytomorphological Changes in Buccal Mucosal Cells of Type 2 Diabetic Patients Using Oral Exfoliative Cytology. ANNALS VOL 23, ISSUE 2, APR. – JUN. 2017

-Saudek CD, Herman WH, Sacks DB, et al. A new Look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab. 2008;93(7):2447-253.

-Scheifele C, Schmidt-Westhausen AM, Dietrich T, Reichart PA: The sensitivity and specificity of the OralCDx technique: evaluation of 103 cases. Oral Oncol 2004, 40, 824–828.

-Selvin E, Steffes MW, Zhu H, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med. 2010; 362(9): 800– 811.

-Shareef BT, Ang KT, Naik VR. Qualitative and quantitative exfoliativecytology of normal oral mucosa in type II Diabetic patients. Med Oral Patol Oral Cir Bucal 2008; 13:E693-6

-Shoback, edited by David G. Gardner, Dolores. Greenspan's Basic & Clinical Endocrinology (9th ed.). New York: McGraw-Hill Medical. Pp. Chapter 17. 2011.

-Shrimali L, Astekar M, Sowmya GV. Correlation of oral manifestations in diabetes mellitus. Int J Oral Max Pathol. 2011; 27

Skamagas M, TL Breen, D LeRoith. Update on diabetes mellitus: prevention, treatment, and association with oral diseases. Oral Diseases 2008; 14:105– 114.

-Soubannier, V., McLelland, G. L., Zunino, R., Braschi, E., Rippstein, P., Fon, E. A., & McBride, H. M. (2012). A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. Current Biology, 22(2), 135-141.

-Sugerman PB, Savage NW. Exfoliative cytology in clinical oral pathology. Aust. Dent J. 1996; 41(2):71-74.

-Sultanpur CM, Deepa K, Kumar SV. Comprehensive review on HbA1cin diagnosis of diabetes mellitus. Internat. Pharmace Scien Rev & Res.2010; 3(Issue 2):119-124.

References

-Sumanthi J, Reddy GS, Anuradha CH, Sekhar PC, Prasad LK, Reddy BV. A study on histomorphometric analysis of exfoliative buccal cells in iron deficiency anemia patients. *Contemp Clin Dent.* 2012;3:S156–9.

-Taylor GW, Borgnakke WS. Periodontal disease: Associations with diabetes, glycemic control and complications. Oral Dis 2008; 14:191–203.

-Tozoğlu Ü and Bilge OM. Exfoliative Cytology of Type 1 Diabetic Patients. Eur J Gen Med 2010; 7: 264-268.

-Vernillo A. T. "Dental considerations for the treatment of patients with diabetes mellitus," Journal of the American Dental Association 2003; vol. 134, pp. 24S–33S.

-Willis AM, Coulter WA, Fulton CR, Hayes RJ, Bell PM, Lamey PJ. Oral candidal carriage and infection in insulin-treated diabetic patients. Diabet Med 1999; 16:675–9.

-Yadav, A., Jaggi, S. (2015). Buccal micronucleus cytometry assay- A Biomarker of Genotoxicity. J of Bio & Diag, 6, 2-5.

تهدف الدراسة إلى التعرف على التغيرات الظاهرية في الخلايا الظهارية الفموية باستخدام الخلايا التقشرية من مرضى السكري من النوع الأول والثاني ومقارنتها مع الأشخاص الأصحاء، ومعرفة العلاقة بين عدد الخلايا المحسوبة من الغشاء المخاطي الفموي والمناطق المختلفة من تجويف الفم ومعدل الإصابة بالسكري؛ وفي هذه التجربة تراوحت أعمار الذكور والإناث المستخدمين في الدراسة من 1-70 سنة. وبعد الحصول على موافقة المريض وموافقة لجنة أخلاقيات البحث العلمي في جامعة ميسان، تم جمع العينات من مركز السكري والغدد الصماء في محافظة ميسان، واستغرقت الفترة من 24\10 أكتوبر إلى 1\6 يونيو، وتم أخذ عينات من أربع مناطق من الغشاء المخاطي الفموي (الخد واللثة واللسان والحنك) لجمع هذه العينات. شملت هذه الدراسة 100 شخص، تم تقسيمهم إلى مجموعتين، المجموعة الأولى (مجموعة التحكم السليمة) تكونت من 50 شخصًا، بينما كان هناك 50 شخصًا في المجموعة الثانية.

تم تشخيص مرض السكري بناء على الأطباء الذين أجروا الفحص التشخيصي في مركز السكري، وتحليل السكر التراكمي في الدم، والتحليل السريع، حيث تم أخذ عينات دم حوالي 5 مل من كل شخص للفحص. تم أخذ العينات في الصباح من الأشخاص الذين تم قياس مستويات السكر في الدم لديهم. بعد أخذ العينات تم صبغها بعدة صبغات: Papas، hematoxylin، eosin، MitoTracker، DCFH-DA، و MitoSox.

وتشير نتائج الدراسة الحالية إلى وجود فرق معنوي عند مستوى (0.05) في عدد الخلايا الحرشفية المتساقطة من تجويف الفم بين مرضى السكري من النوع الأول والثاني مقارنة بالأفراد الأصحاء.

حيث زاد عدد الخلايا في منطقة الخد، في حين لوحظ انخفاض في منطقة اللثة. وأظهرت النتائج فروقاً معنوية في عدد الخلايا المرصودة في كافة مناطق تجويف الفم، وتم تشخيص عدد الخلايا في الغشاء المخاطي للفم في مناطق الخد واللثة واللسان والحنك حسب الفئات العمرية وبناءً على مستوى السكر والسكر التراكمي. ومن نتائج هذا الجدول يتضح أن هناك علاقة إحصائية معنوية عند مستوى $P \geq 0.05$ بين عدد الخلايا المحسوبة من تجويف الفم ومستوى السكر حسب الفئات العمرية.

وأظهرت النتائج الحالية وجود فرق معنوي مقداره 0.05 بين أقطار نوى الأشخاص حسب العمر بين مرضى السكر وغير المصابين بالسكري، حيث ارتفع معدل ND في المجموعة غير المصابة بالسكري بشكل معنوي مقارنة بالمجموعة الأخرى، وبلغ مستوى نسبة العمر (0.417)، في حين بلغ مستوى نسبة المعنوية لدى مرضى السكر (0.174). كما أظهرت النتائج أن الفئات العمرية 0-15 و46-70 لم يكن لها فروق ذات دلالة إحصائية، أي أن A = B، في حين أن الفئات العمرية 16-30 و31-45 لها فروق ذات دلالة إحصائية، حيث أن A ≥ B. وأظهرت النتائج الحالية وجود فروق ذات دلالة إحصائية في أقطار السيتوبلازم في جميع الفئات العمرية، حيث أن A ≥ B عند مقارنة المجموعة الضابطة بالمجموعة المصابة بالسكري، حسب الفئات العمرية. أظهرت النتائج الحالية أن هناك زيادة ذات دلالة إحصائية في نتائج نسبة النواة إلى السيتوبلازم في مرضى السكري وغير المصابين بالسكري في الفئات العمرية 0-15 عامًا، حيث كانت أكبر من B، مما يدل على اختلاف كبير 0.05، في حين لم تتأثر بقية الفئات العمرية من 16 إلى 70 عامًا بشكل كبير.

في الأشخاص غير المصابين بالسكري، كشف الفحص الدقيق للبنية الدقيقة للميتوكوندريا تحت الساركوما وبين الألياف العضلية عن قمم محددة جيدًا وموحدة بعد التلوين باستخدام كاشف سترات الرصاص وأسيتات اليورانيل أظهرت النتائج أن الميتوكوندريا في الأشخاص غير المصابين بالسكري تعمل بشكل طبيعي. في الأشخاص المصابين بالسكري، كشفت السمات المجهرية لخلايا الخد عن العديد من التشوهات: فقدان قمم الميتوكوندريا، وتمزق الأغشية الخارجية للميتوكوندريا، وفرط تنسج الخلايا البطانية. لوحظ فوضى واضحة وانخفاض في عدد قمم الميتوكوندريا. أظهرت النتائج أن مرضى السكري لديهم ميتوكوندريا متدهورة لا تعمل بشكل طبيعي.

أظهرت النتائج الحالية لمقاطع الخلايا المجهرية الفلورية زيادة في إطلاق mtROS في الخلايا المصابة بالسكري، وأظهرت النتائج المجهرية الفلورية أيضًا زيادة في إطلاق ROS.

أظهرت مقاطع الخلايا المجهرية الفلورية الأخرى زيادة في إطلاق mitoTreaker في الخلايا المصابة بالسكري، وأظهرت النتائج المجهرية الفلورية أيضًا زيادة في إطلاق ROS. (mitoTreaker). تظهر الخلايا ميتوكوندريا متضخمة مع كريستا مكسورة هناك العديد من الميتوكوندريا المتضخمة بأحجام مختلفة ودرجات متفاوتة من التكسير.

وزارة التعليم العالي والبحث العلمي
جامعـــــــة ميســـــان
كليـــة العلـــــــوم
قســم علـــوم الحيـــاة

دراسة خلويه مقارنه للغشاء المخاطي الفمي للسكان الاصحاء والمصابين بالسكري في محافظة ميسان

دراسة مقدمة

إلى مجلس كلية العلوم / جامعة ميسان

هي جزء من متطلبات درجة الماجستير في علم الأحياء

من قبل

نور سعيد عنيد

بكالوريوس العلوم. علم الأحياء (2019 - 2020)

تحت الإشراف

الأستاذ المساعد الدكتور علي خلف علي

الأستاذ المساعد الدكتور ميثم عبد الكاظم دراغ

1446هـ 2024م