Ministry of Higher Education
and Scientific Research
University of Misan
College of Science
Department of Biology

Comparative Exfoliative Cytological Analysis of Oral Mucosa in Patients with Metabolic Diseases in Maysan Province

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

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B.Sc. Biology (2022)

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2026 A.D. 1447 A.H.

بسم الله الرحمن الرحيم

{خَلَقَ السَّمَاوَاتِ وَالْأَرْضَ بِالْحَقِّ وَصَوَّرَكُمْ فَأَحْسَنَ صُوَرَكُمْ ۖ وَإِلَيْهِ الْمَصِيرُ}

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Supervisor's Certificate

We certify that this thesis entitled "**Comparative Exfoliative Cytological Analysis of Oral Mucosa in Patients with Metabolic Diseases in Maysan Province** " has been prepared under our supervision at the college of science , university of misan ; as a partial fulfillment of the requirements for the degree of Master of Biology.

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Dedication

To Al-Hussein ibn Ali (peace be upon him), the revolutionary martyr, I dedicate this humble part of my work.

To those who instilled in my heart a love of knowledge and were a guiding light on my path...

To those who taught me patience and steadfastness and were my endless support...

To my dear parents,

To my brothers and sisters,

To my entire family,

I dedicate to you the fruits of my labor and years of toil... You are the ones who truly deserve this accomplishment.

Siddiqa Hassan

Acknowledgment

*Praise be to **God**, Lord of the Worlds, by whose grace good deeds are accomplished, and by whose favor blessings are bestowed. I first express my gratitude and appreciation to God Almighty, who has bestowed upon me His help, success, and patience, enabling me to complete this research despite all the difficulties.*

*I would like to extend my sincere thanks and gratitude to my esteemed supervisor, Professor **Dr. Ali Khalaf Ali**, for his support, guidance and advice during the research period. I wish him continued health and wellness.*

*Many thanks extended to the **college of science Misan university** for providing the chance to get the degree of Master of Biology .*

I would also like to extend my thanks and appreciation to the Diabetes and Endocrinology Center, Al-Hakim Teaching Hospital, and the laboratories, especially the NASA Laboratory, for their assistance in collecting my study samples and completing this work.

*I would like to express my sincere gratitude to **Professor Dr. Muslim Abdulrahman Mohamed al tooma** for his valuable assistance and expert interpretation of the transmission electron microscopy (TEM) results. His scientific insight and guidance greatly contributed to the accurate analysis and understanding of the findings of this study. I would like to sincerely thank A**ssistant Professor Sadeq Sabeeh Kareem** for teaching me how to measure the diameters of the nucleus and cytoplasm.*

*Finally, I extend my thanks and gratitude to my generous **family** for their continuous support and encouragement during my studies, especially **my parents, my sister , Ms. Zahraa**, and everyone who helped me from friends and colleagues during my academic career. I ask God to make this effort sincere for His sake and to reward everyone who contributed to it with the best reward.*

Siddiqa Hassan

SUMMARY

Summary

The current study, using exfoliative cytology, aimed to investigate oral cytological changes at the cellular and molecular level and their relationship with metabolic diseases and their development. Cell counts were evaluated in buccal smears, and nuclear morphological changes, cytomorphological changes, and their association with changes in insulin levels, fasting glucose, glycated hemoglobin, body mass index (BMI), anti-Tissue transglutaminase, and insulin resistance were studied. Samples were collected from December 2024 to May 2025 from the Diabetes and Endocrinology Center, Al-Hakim Teaching Hospital, and private laboratories. The whole sample is 60 participants of both genders, aged (20 - 60) years, the samples were stained using H&E, PAS and PAP, divided into four equal groups (15 for each group) : Control group (healthy), Type 2 diabetes group, Celiac group and Obesity group (BMI greater than 30 kg/m2) .

The results revealed a significant decrease ($p \leq 0.05$) in oral epithelial cell count when comparing the different groups with the control group. There was also a change in the cell count with age, and there was also a relationship between the cell count and the biochemical parameters. The results of the cytomorphological study showed that the diameter of the nucleus was no significantly increased ($p \leq 0.05$) in the different groups when compared with the control group, while the diameter of the cytoplasm and the ratio of the nucleus to the cytoplasm showed a significant increase ($p \leq 0.05$) in the different groups when compared with the control group. Additionally, study of nuclear morphological changes, there is a significant increase ($p \leq 0.05$) when comparing the results of the different groups with the control group, there is also a relationship between these changes and biochemical standards. As for the biochemical criteria, there was a significant increase ($p \leq 0.05$) in the different groups compared to the control group.

The results also revealed a difference in the intensity of cell staining in the different groups compared to the control group. The cellular changes may be

explained by effects of increased fat mass, hyperglycemia, insulin resistance and anti-Tissue transglutaminase their impact on cell cycle, tissue regeneration, cytomorphological and nuclear morphological changes in each of the diabetic, celiac and obesity groups.

In conclusion, oral exfoliative cytology is a low-cost, non-invasive, and safe technique that can be used in the initial diagnosis of metabolic diseases. It also helps predict insulin resistance and assess changes in nucleus morphology. All groups participating in the study showed a significant decrease in cell counts.

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List of Abbreviations

	Abbreviations
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CT	Computed Tomography
CYD	Cytoplasm Diameter
DCCT	Diabetes Control and Complications Trial

DEXA	Dual-Energy X-ray Absorptiometry
DPX	Dibutylphthalate Polystyrene Xylene
EDTA	Ethylenediaminetetraacetic Acid
EGF	Epidermal Growth Factor
FPG	Fasting Plasma Glucose
H &E	Hematoxylin & Eosin
HbA1c	Glycated Hemoglobin A1c
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IGT	Impaired Glucose Tolerance
IL-2	Interleukin - 2
IL-6	Interleukin - 6
IR	Insulin Resistance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
N/C	Nuclear/Cytoplasmic ratio
ND	Nuclear Diameter
PAP	Papanicolaou stain
PAS	Periodic Acid–Schiff
RBS	Random Blood Sugar
RMP	Revolutions Per Minute
ROS	Reactive Oxygen Species
SD	Standard Deviation
tTG	Tissue Transglutaminase
TNF-α	Tumor necrosis factor-α
WHO	World Health Organization

Chapter one

Introduction

Introduction

Metabolic diseases are one of the factors that pose a major threat to public health in the world and include diabetes, obesity, hyperlipidemia, non-alcoholic fatty liver disease, high blood pressure, celiac disease and other diseases (Loyola-Rodriguez *et al*., 2011), Metabolic diseases can be defined as a group of disorders that occur in the metabolism of fats, carbohydrates and proteins, which lead to a deficiency or accumulation of certain substances in the body, causing various health effects (Moldakozhayev & Gladyshev, 2023).

Some of these diseases are associated with a wide range of systemic complications that extend their impact to the mouth, as the oral cavity is a vital tissue that plays an important role in maintaining oral health and serves as an early indicator of some systemic diseases. Many systemic conditions cause changes at the cellular and molecular level. These changes in the oral mucosa are common effects that may not be clinically noticeable, but they can be detected by non-invasive cytological examination. Many systemic conditions, such as diabetes, celiac disease and obesity , disrupt the biological balance of the cells that make up this mucosa, leading to notice changes that can be detected through cytological studies (Taylor & borgnakke, 2008).

Diabetes is a chronic condition affecting glucose metabolism, and it is associated with various changes in oral tissues, including ulcers, dry mouth, and gum infections. On the other hand, celiac disease is an autoimmune disorder triggered by gluten, primarily affecting the digestive system, but it can also lead to oral mucosal changes due to malabsorption and nutrient deficiencies (Kumar *et al*., 2014) and Obesity is a chronic inflammatory disease that is caused by a defect in fat mobilization and accumulation within the adipose tissue (Weisberg *et al*., 2003), which is characterized by an increase in the size and number of fat cells, along with the presence of chronic inflammation represented by the

infiltration of inflammatory cells and the activation of the inflammatory cytokine network (Wellen & Hotamisligil, 2003), and studies indicate poor oral health in people suffering from obesity, such as tooth loss, gingivitis, and Xerostomia (Östberg *et al*., 2012).

The changes at the cellular and molecular level caused by these complications caused by metabolic diseases affect the regeneration of tissues, repairing damaged tissue and replacing it through division in a process known as regeneration, which occurs in the cells of the mucous membrane every 14 to 21 days and varies from one area to another depending on the surface of the area exposed to mechanical stress, this renewal process begins from the basal layer, which is mainly composed of mitotic cells that undergo proliferation, then the differentiation process, and finally migration. Control of this process is not sufficiently controlled, there are several factors that affect cell renewal, including adrenergic factors, prostaglandins, growth factors, mesenchymal factors, estrogen, insulin and vitamin A. Any defect that affects the control of this process leads to a similar process, which is the proliferation of cancer cells, which must be detected and treated early, This defect is reflected in the cell itself, thus causing damage to the tissue, for example, impaired cell turnover and renewal, increased appearance of nuclear abnormalities that are considered an indicator of early programmed cell death, and Cytomorphometric changes. (Matsuda, & Mori, 2003; Wang *et al.,* 2019 ; Brizuela & Winters, 2021).

A useful and non-invasive method for identifying cellular changes that can provide important details about how systemic disorders like diabetes, celiac disease, and obesity affect oral health is cytological analysis of the oral mucosa (Ducatman, 2020).

Exfoliative cytology, which is predicated on the phenotypic assessment of epithelial cells following fixation and staining, is used to accomplish this (Rajput & Tupkari, 2010).

Aimes of study:

The present study was designed to compare buccal smears from four groups: control group, patients with type 2 diabetes, patients with celiac disease, and patients with obesity.

The main aims were:

1. Evaluating the relationship between changes in cell counts and age in people with metabolic diseases and control groups.
2. To study and compare Cytomorphometric parameters in buccal smear with control group.
3. To study nuclear morphological changes in buccal smears, and to evaluate their relationship with changes in cell counts in comparison with the control group.
4. Evaluation of biochemical parameters and their impact on cellular changes in the oral mucosa in patients with and without metabolic diseases.
5. To Study the cell’s ultrastructure and the alterations that reflect its functional and pathological states.

Chapter Two

Literature Review

2.1 Human Oral Mucosa

The oral cavity is lined with a mucous membrane that extends anteriorly at the vermilion border and ends at the pharyngeal mucosa. The oral mucosa is continuous with digestive tract with the connection of the oral mucosa being severed by the teeth to which it is attached (Kumar, 2023). The oral mucosa is classified into three types to reflect the different functions it performs and its ability to resist friction. The first type is called the masticatory mucosa, which is tightly attached to the periosteum, as this is suitable for its function in the process of mastication, which is present in the hard palate and gums, the second type of oral mucosa is Specialized mucosa on the dorsal surface of the tongue has many papillae and taste buds and the lining mucosa, which is attached in a more flexible form to allow it to expand into other areas of the oral cavity in which it is located, except in the dorsal of the tongue (Ovalle & Nahirney, 2013).

2.1.1 Histological Structure of Oral Mucosa

The whole oral mucosa is covered in stratified squamous epithelium. The location within the oral cavity and the mechanical and functional needs of the area determine the thickness and degree of keratinization of this highly organized, avascular, semi-permeable tissue. An interdigitated contact connects the lamina propria to the epithelium. The undulating projections of the deeper layer of the epithelium, known as rete pegs, are connected to the underlying papillary projections of the lamina propria. These two tissues are separated by a non-cellular foundation membrane to which the epithelium is firmly linked. The basement membrane supports and connects the connective tissue to the epithelium. Based on their functional composition, clinical traits, and histology, three different types of oral mucosa may be identified. The mucosa lining the mouth's moveable tissues is referred to as lining or movable mucosa. This mucosa is found on the soft palate, cheeks, lips, vestibular fornix, and floor of the mouth.

The lining mucosa is covered by an epithelium called non-keratinized stratified squamous epithelium (Meyle & Groeger, 2019). The term "masticatory mucosa" describes the stiff mucosa of the hard palate and gingiva that is securely attached to the underlying bone. The capacity of the masticatory mucosa to tolerate the stress it undergoes during mastication is due to the keratinized or para-keratinized stratified squamous epithelium covering these surfaces.

Additionally, a particular mucosa on the dorsum of the tongue has a squamous stratified epithelium that may or may not be keratinized. This name comes from its unique feature of having several lingual papillae and taste buds that facilitate taste perception. This mucosa is sometimes called masticatory mucosa because the dorsum of the tongue actively participates in mastication. Four layers make up the oral epithelium in keratinized oral mucosa, also known as the masticatory mucosa. Beginning with the deeper layer, we found the stratum spinosum, stratum granulosum, and stratum corneum. The stratum filamentosum and stratum distendum are the two layers above the stratum basale where the epithelium is nonkeratinized, like the lining mucosa (Meyle & Groeger, 2019). Furthermore, the non-keratinized epithelium lining the mucosa lacks the granular layer, and the spinous layer is generally thought to be thinner. (Tanaka-Otsuka *et al*., 2013).

2.1.1.1 keratinized Oral Epithelium

The skin and oral mucosa are lined by keratinocytes, which are the main component of stratified squamous epithelium. In contrast to skin keratinocytes, oral cells are constantly exposed to the epidermal growth factor (EGF) present in saliva, which in turn increases their rate of cell division and accelerates the wound healing process (Cabunac *et al*.,2016). The oral epithelium of keratinized oral mucosa, such as the masticatory mucosa, is composed of four layers. The stratum basale was the first one we discovered in the deeper layer, followed by the stratum

spinosum, stratum granulosum, and stratum corneum (Meyle & Groeger, 2019). Areas of oral mucosal tissue contain different proportions in terms of distribution and density of keratinocyte stem cells, as keratinocyte stem cells are less present in the oral epithelium than in the skin (Terskikh *et al*.,2012).

2.1.1.2 Nonkeratinized Oral Epithelium

In the oral epithelium, there are small clusters of cells that lack cytokeratin filaments, so they do not have the ability to keratinize. They are called non-keratinocytes and are often referred to as clear cells because they appear to have a halo around the nucleus. They constitute approximately 10% of the cells in the oral epithelium. Unlike keratinized cells, these cells do not exhibit mitotic activity, do not undergo maturation or cell shedding, are not arranged in layers, and do not form desmosomal filaments with keratinized cells. In non-keratinized epithelial tissues, there are three layers: stratum filamentosum , distendum, and the spinous layer is usually thinner, and the granular layer is absent in these tissues (Otsuka-Tanaka *et al.,* 2013).The oral epithelium contains a group of non-keratinized cells, namely melanocytes that produce melanin, Markel cells that function as special nerve receptors that are sensitive cells, Langerhans cells that participate in the immune response and inflammatory cells, especially lymphocytes, some of which have a defensive function. Non-keratinized cells migrate from the neural crest or bone marrow to the oral epithelium (Hand& Frank, 2014 ; Kumar, 2023).

2.1.2 Functions of Oral Mucosa:

2.1.2.1 Protection

The oral mucosa acts as a strong scaffold that separates the deeper tissues and organs in the mouth from direct exposure to environmental factors. The mouth is the first station of digestion. When eating, chewing and biting processes break

down solid particles using mechanical forces such as pressure, tension, and stretching. Soft tissues are exposed to these mechanical forces. The lamina propria, rich collagen and elastin fibers, protects these tissues. Collagen fibers provide rigidity and strength to resist pressure and stretching during chewing, while elastin gives flexibility to the mucosa and allows it to stretch and return to its normal position without tearing, The stratum corneum, acts as a physical barrier to prevent the infiltration of microorganisms and harmful substances into the deeper tissues, as it gives a keratinized characteristic to some areas of the mouth that it covers from the outside, such as the gums and the roof of the mouth, to make them more resistant to friction and pressure resulting from chewing (Squier & Brogden, 2010) .

2.1.2.2 Sensory receptors

The oral mucosa contains many receptors that respond to various stimuli spread throughout the pharynx and epiglottis, The function of these receptors is to sense heat, touch, and pain, The mouth also contains taste buds, which transmit the sensation of whether the substance entering the mouth is sweet, bitter, salty, or sour. It also tastes fatty, the sensory function is considered important because it is important in providing information about events within the oral cavity and transmitting it to the brain (Laugerette *et al*., 2007).

2.1.2.3 Glandular Activity

Saliva is the primary secretion produced by the major salivary glands, whose secretion is linked to the oral mucosa, which in turn works to maintain the moisture of the surface of the oral cavity. The salivary glands that produce saliva are far from the oral mucosa, Salivary gland function is impacted in some pathological situations, and when taking medications that reduce salivary secretion, these effects spread to the mouth and can result in a variety of oral

health issues, such as dry mouth, tooth decay, fungal infections, oral infections, and other complications (Arany *et al*., 2021 ; Nanci, 2024).

2.2 Metabolic Disease

2.2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder characterized by a high blood sugar level resulting from a defect in insulin secretion or function or both, where insulin is an important anabolic hormone that has a role in any carbohydrates, fats and proteins (Poznyak *et al*.,2020). in 2016, the World Health Organization announced the death of 1. 5 million people, and 48% of the deaths were before the age of 70. Diabetes is associated with a high rate of morbidity due to a wide range of complications including retinopathy, neuropathy, nephropathy and cardiovascular disease (Fowler, 2008), and in addition to these known complications, there is research indicating oral complications in patients with diabetes (Ship, 2003 ; D'Aiuto *et al*., 2017).

2.2.1.1 Epidemiology of Diabetes Mellitus

According to the World Health Organization (WHO) in 2016, the global diabetes prevalence rate is about 9%, and according to data from the International Diabetes Federation in 2017, 8.8%, while the prevalence rate in the Middle East and North Africa was estimated at 9.2%. Over the past 30 years, the world has witnessed a continuous increase in the prevalence of diabetes, especially in middle and low-income countries, where the highest growth rates were recorded, and also recorded an increase in the incidence of type 2 diabetes in children, due to changing lifestyles in the direction of reduced physical activity and eating high-calorie foods (Hu, 2011) where diabetes is a disease burdened by health and economic burdens In 2017, the proportion of diabetes-related deaths among adults aged 20 to 79 years was 10.7% in the MENA region. In the Middle East

and North Africa region, 373,557 deaths were caused by diabetes in 21 countries, including Iraq. 51.8% of these deaths were among people aged 60 years according to data from the International Diabetes Federation in 2017 and the number of diabetic patients in Iraq is estimated at about 1.4 million people. In a local study, the prevalence of diabetes by age appeared 5400 people in the city of Basra, which is 9.7% among people aged 19 to 94 years (Mansour *et al*., 2014). There is still a lack of studies on diabetes in Iraq, and this makes it difficult to accurately assess the reality of the disease.

2.2.1.2 Diagnosis of Diabetes Mellitus

When people show symptoms such as high glucose levels for a long period, frequent urination, excessive hunger, and a strong desire to drink large amounts of water, they should undergo the following tests to detect diabetes, as the above symptoms are considered an initial sign of type 2 diabetes (Alam *et al*., 2021), Glycosylated hemoglobin(HbA1c) according to the World Health Organization in 2006 is a test that gives an indication of the patient's average plasma glucose concentration over the past three months, People with type 2 diabetes show a level of (≥6.5%) according to the DCCT standard, Oral Glucose Tolerance Test (OGTT) Measures the ability of the body's cells to absorb glucose after ingesting a specific amount of sugar, A 75-gram dose of glucose is given orally, and the plasma glucose level is measured two hours later If the result is ≥ 11.1 mmol/L, the person is diagnosed with diabetes(Vijan, 2010) ,Fasting Plasma Glucose (FPG) test is a routine and reliable way to diagnose diabetes, a person is diagnosed with diabetes if the fasting glucose result is ≥ 7.0 mmol/L, Impaired Glucose Tolerance (IGT) If the plasma glucose reading two hours after eating 75 grams of glucose is between 7.8-11.0 mmol/L, considered pre-diabetic or have IGT and Random Blood Sugar (RBS) is used as a Prognostic Marker to determine the likelihood of developing diabetes, especially in urgent or non-fasting situations (Siu, 2015).

Both fasting blood glucose and fasting insulin levels are measured to calculate the HOMA-IR index, which is used to assess insulin resistance. This index is based on a simple mathematical formula, the higher the number, the greater the cells' resistance to the effects of insulin. Insulin resistance is believed to be a major contributing factor to type 2 diabetes and metabolic syndrome (Sharma *et al.,* 2021).

2.2.2 Celiac Disease

It is a systemic disease of immune origin characterized by an abnormal response to the ingestion of gluten, which leads to damage to the epithelial cells of the small intestine. These cells play essential roles in enzymatic digestion, nutrient absorption, regulating the immune response, and maintaining the intestinal barrier Villus atrophy and lymphatic infiltration into the microvascular mucosa often occur, thus, chronic inflammation and tissue damage resulting from celiac disease disrupt the complex network of metabolic processes within epithelial cells that support these functions. This, in turn, impairs their ability to perform their primary functions (Kagnoff,2007; McCreery *et al*., 2025). Gluten is a protein complex found in wheat, Barley, Rye and Triticale; the active part of gluten is specific compounds such as gliadin found in wheat. This is a protein molecule that is abnormally rich in amino acids glutamine and proline, due to these two acids, the intestines of people with a genetic predisposition cannot fully digest these proteins by intestinal enzymes (Silano *et al*., 2009). As a result of the incomplete digestion of these protein molecules, a mixture of peptides is formed that plays a role in triggering an immune response in the body, for example, increasing intestinal permeability and activating the innate and adaptive immune response, these responses may be like exposure to intestinal pathogens such as harmful bacteria and viruses (Picarelli, 1999 ; Shan *et al*., 2002).

2.2.2.1 Epidemiology of Celiac

It is one of the most common autoimmune diseases worldwide (Rubio– Tapia *et al*., 2009), with a prevalence rate of 0.5% to 1% of the population, with notable geographical and ethnic variations (Barada *et al*., 2010 ; Kang *et al*., 2013) in Western countries such as the United States, Ireland, and Italy. Its prevalence rate reaches 0.4% to 1% according to serological studies, while in Switzerland, the highest prevalence rate was recorded, approximately 0.76%, while in other European countries, the prevalence rate ranged from 0.05% to 0.1% (Fasano *et al*., 2003; Irvine *et al*., 2017). Due to the high consumption of wheat and barley and a higher frequency of DR3-DQ2 haplotypes, its prevalence in the Middle East and North Africa, as some studies have shown, was approximately 0.6% to 1% (Bdioui *et al*., 2006). Studies have also shown that women are more susceptible to infection than men, i.e. 66.6% in females compared to 33.3% in males (Al-Hussaini *et al*., 2017 ; Catassi, 2017). The highest incidence was recorded among high-risk groups such as type 1 diabetes, Down syndrome, autoimmune thyroiditis, Turner syndrome, and immunoglobulin IgA deficiency. Despite the existence of highly sensitive and accurate serological tests, many cases remain undiagnosed due to the diversity of symptoms and their occasional absence (El-Metwally *et al*., 2020).

2.2.2.2 Diagnosis of Celiac

Diagnosing celiac disease is difficult due to the high prevalence of atypical clinical patterns. Clinical symptoms associated with the intestines include chronic diarrhea, abdominal pain, weight loss, malabsorption, anemia, and failure to thrive. Atypical clinical symptoms, such as dermatitis herpetiformis, idiopathic hepatitis, and enamel defects, all overlap with symptoms of other diseases, making it difficult to diagnose easily (Lucchese *et al*., 2023). There are several criteria that are relied upon to diagnose the disease, including case identification,

serological tests, and histological evaluation of a small bowel biopsy. Serological tests are used as a non-invasive first step in diagnosis. The most accurate sensitivity test is IgA antibodies against tissue transglutaminases, which have a specificity ranging from 91% to 100% and an approximate sensitivity of 77% to 100%. The second test is IgA antibodies against endomysium, which has a greater specificity (approximately 100%) but is less sensitive (Leeds *et al*., 2008). Compared to anti-tissue transglutaminase (tTG) IgA antibodies, although these serological tests are accurate, a small bowel biopsy is the gold standard for diagnosis, as it predicts the presence of intraepithelial lymphocytic infiltration, crypt hyperplasia, and partial or complete atrophy of the villi. However, lymphocytic infiltration alone without apparent atrophy is specific and may result from other causes, such as the use of nonsteroidal anti-inflammatory drugs (Catassi & Fasano, 2010 ; Compilato *et al*., 2010).

2.2.3 Obesity

Obesity is characterized by an increase in fat mass and is defined as a body weight gain exceeding 20% of an individual's optimal weight (Britannica, 2021).

In advanced stages, obesity can be defined as is a chronic inflammatory disease that is caused by a defect in fat mobilization and accumulation within the adipose tissue (Weisberg *et al*., 2003), which is characterized by an increase in the size and number of fat cells, along with the presence of chronic inflammation represented by the infiltration of inflammatory cells and the activation of the inflammatory cytokine network (Wellen& Hotamisligil,2003), It is believed that accumulated fat contributes to pro-oxidative conditions and chronic inflammation, where excessive accumulation of fat leads to an energy imbalance, causing an excess of energy intake in exchange for a decrease in energy expenditure (Lobato *et al*., 2012) and this is the result of several factors, including increased consumption of fat-rich foods, low physical activity (Korita *et al*.,

2013) nutritional and hormonal status, genetic, environmental, cultural and economic factors (Pérez-Escamilla *et al.,* 2012 ; Ates *et al*., 2019 ; Bego *et al*., 2019). All these factors make obesity a cause of many major metabolic diseases, especially diabetes, cardiovascular disease, hypertension and non-alcoholic fatty liver disease (Eckel *et al*., 2005). Obesity also leads to other diseases, such as neurodegenerative diseases, airway disorders and cancer, and these diseases pose a great danger and cause morbidity and mortality (Cao, 2014).

2.2.3.1 Epidemiology of Obesity

In recent years, obesity has become a global epidemic, not only in developed countries, but also in developing countries. Current estimates indicate that more than a billion people around the world suffer from obesity or overweigh(Blüher, 2019 ; chooi *et al*., 2019). In the Middle East, obesity and overweight rates among adults range from 25% to 81.9%, depending on the country. In Iraq, local studies have recorded high rates of obesity and overweight in Erbil in 2017: 33.4% overweight and 40.9% obesity (Shabu, 2019). In Basra Governorate, between 2003 and 2010, the rate of overweight to obesity was 55.1% (Mansour *et al*., 2014). In a study Pengpid & Peltzer (2021) the results indicate that two-thirds of adults in Iraq suffer from obesity and overweight.

2.2.3.2 Diagnosis of Obesity

Obesity is diagnosed according to the World Health Organization based on the body mass index (BMI), which is a measure calculated by dividing the body weight into kilograms by the square of the height in meters. It is considered a simple, basic tool widely used to determine the level of overweight and obesity in adults, as it is linked to the risk of chronic diseases and increased mortality rates. According to the World Health Organization in 2021, if the body mass index is less than 18.5 kg/m², the person is underweight, 18.5 to 24.9 kg/m² is considered normal weight, and 25 to 29.9 kg/m² is considered overweight. While

if the body mass index is more than 30 kg/m², it is considered obese. Obesity is classified into three degrees according to the World Health Organization in 2004: the first degree: 30 to 34.9 kg/m², the second degree: 35 to 39.9 kg/m², and third degree is greater than 40 kg/m². Despite its widespread use, BMI has some limitations. It does not differentiate between fat mass and muscle mass, which can lead to misclassification of some athletes or older individuals. The sensitivity of BMI also varies depending on ethnicity. For example, studies show that individuals of Asian descent may have higher body fat percentages at the same BMI value compared to Western populations, leading some Asian guidelines to propose lower thresholds for diagnosing obesity (WHO Expert Consultation, 2004; WHO, 2011),For this reason, additional measurements are recommended to assess fat distribution, particularly visceral fat, which is associated with metabolic complications. Among these measures are Waist circumference: ≥ 102 cm for men and ≥ 88 cm for women Waist-to-Hip Ratio (WHR): > 0.95 for men and > 0.80 for women (WHO, 2008) These indicators are more accurate tools for identifying abdominal obesity, which has been shown to be closely associated with the risk of insulin resistance, metabolic syndrome, and cardiovascular disease. In addition to BMI, waist circumference, and waist-to-hip ratio, other more advanced techniques have been used to assess body composition with greater accuracy, including bioelectrical impedance analysis (BIA), dual-energy x-ray absorptiometry (DEXA), computed tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS). These techniques identify and measure the volume and mass of various body components, such as subcutaneous fat, visceral fat, fat around the heart and coronary vessels, and lean tissue such as bone marrow and skeletal muscle (Karlsson *et al*., 2013 ; Neamat-Allah *et al*., 2014). One of the most accurate techniques for determining the total amount of fat is MRI. Despite the accuracy of these techniques, their use is limited due to the complexity of the technical procedures, the need for advanced and expensive equipment, the long time and effort required to interpret the results,

and the lack of standardized recommendations (Wald *et al*., 2012 ; Nimptsch *et al*., 2019).

2.3 Oral Exfoliative Cytology

In the normal state, the oral epithelium is rapidly renewed, which results in continuous cell shedding. These renewed and shedding cells can be used to diagnose systemic and local diseases by studying the morphology differences of cells. These differences can reflect the various biological processes in the oral cavity. Furthermore, this cytological analysis is called exfoliation cytology, as cytology is concerned with studying cells at the morphology level, which reflects the biological behavior of tissues and the genetic and molecular biology of cells. Thus, exfoliation cytology can be defined as a technique for microscopic examination of cells that have fallen or shed from the surface of the oral epithelium. Exfoliation occurs naturally and continuously in oral cells, or it may result from the use of a tool that scrapes the surface of the tissue, such as a cytobrush or metal spatula, or through washing, as these cells are more useful because they are younger than the cells that shed spontaneously. In the normal state, cells also fall from the deeper layers of the oral epithelium, as the cells of the deep layers are tightly interconnected with each other, and when the epithelium is exposed due to a disease or exposure to abnormal reactions, these cells weaken and lose their cohesion, and this may be behind the shedding of deep cells along with the superficial cells (Aneja *et al*., 2017 ; Beeula *et al*., 2020) .

This technique is based on the shedding cells obtained by exfoliating them using a cytological brush or plastic spoon, then fixing them on a glass slide and staining them using different tissue dyes. Through exfoliation cytology, details of the nucleus and cytoplasm can be determined, including nucleus size, nucleus diameter, cytoplasmic diameter, and the ratio of nuclear to cytoplasmic diameter. It is also possible to evaluate nuclear changes that indicate dysplasia or early

cancer of the cells (Rajput & Tupkari, 2010). The use of exfoliation cytology was limited due to the nature of false negatives and interpretations, but this was overcome by the introduction of quantitative methods such as image analysis systems, especially in the evaluation of cellular and morphological changes (Gururaj *et al*., 2004). In previous studies conducted using exfoliation cytology, it played a role in evaluating morphological changes in the oral mucosa, for example, in the study conducted by Alberti *et al.,* (2003) on people with type 2 diabetes, there were changes in both the morphology of the oral mucosa and changes in the nucleus diameter and diameter of Cytoplasm and the ratio of the diameter of the nucleus to the cytoplasm were observed using a smear. In a study conducted by Mina *et al*., (2008) on people with celiac disease, exfoliation cytology played a role in determining the morphology of the mucous membrane. In the study of Tandon *et al*.,(2020)which was conducted on people with diabetes and people with obesity at the same time, exfoliation cytology also played a role in determining morphological changes, nuclear changes.

2.3.1 Advantages and Disadvantages of Exfoliative Cytology

It is a non-surgical technique that is quick, simple, painless, requires less equipment, and is inexpensive. It helps verify the results of negative biopsies. It is useful in post-treatment follow-up and detecting cancer recurrence in previously treated cases. It is useful in comprehensive examination and through it, post-biopsy complications can be identified. Despite these advantages that help in rapid and early diagnosis of disease conditions, this technique cannot be adopted as an alternative examination method to biopsy. This technique is not reliable, in addition to its use for epithelial cells only. It is rarely used in diagnosing CT lesions, and interpretation requires a skilled and experienced specialist in cytopathology (Sivapathasundharam & Kalasagar, 2004 ; Maloth *et al*., 2017).

2.4 Metabolic Disease Effect on Oral Mucosa:-

2.4.1 In Diabetes

Verhulst *et al*., (2019) explained that diabetes is associated with several microvascular and macrovascular complications represented by retinopathy, neuropathy, kidney disease, and cardiovascular diseases. The reason behind these complications is the disturbance of metabolic and hemodynamic processes, including hyperglycemia, insulin resistance, dyslipidemia, hypertension, and immune system disorders. These disorders play a role in preparing harmful mechanisms, the most important of which is the production of reactive oxygen species, the occurrence of inflammation, and ischemia. All these disorders cause damage to the vascular endothelium and nerve cells. This explains why areas with a large blood supply and dense innervation, such as the eyes, kidneys, and nerves, are affected. The oral cavity is also susceptible to damage because it is characterized by rich perfusion and extensive innervation. Therefore, oral complications are expected to occur as well, the most important of which are: Periodontitis, dry mouth, decreased saliva, atrophied, reddened, and thin mucous membranes, and increased irritation and friction. All these oral manifestations result from poor blood sugar control, a problem that people with diabetes suffer from (Löe,1993; Guggenheimer & Moore,2003; Jajarm *et al*., 2008). This indicates that blood sugar has a major impact, as hyperglycemia plays a major and prominent role in increasing oxidative stress in both gingival tissue, saliva and serum (Bullon *et al*.,2014) a decrease in antioxidants such as reduced glutathione (Barnes *et al*.,2014; Arana *et al*., 2017), and activation of harmful cellular pathways such as the accumulation of products , Advanced glycosylation leads to increased inflammation, impaired tissue healing, and the breakdown of ligaments and alveolar bone (Lalla *et al*., 2000; Zizzi *et al*.,2013) Immune dysfunction also plays a role in triggering chronic inflammation, which in turn leads to excessive production of inflammatory cytokines by neutrophils,

macrophages, and T cells. This inflammation is linked to metabolic changes in diabetes (Beckman *et al*., 2002 ; Karima *et al*., 2005 ; Gyurko *et al*., 2006).

2.4.2 In Celiac

Oral symptoms appear in people with celiac disease with or before intestinal symptoms due to intestinal malabsorption or because of drug therapy (Lourenço *et al.,* 2010). the inflammatory oral manifestations include swelling and damage to the oral mucosa, often accompanied by erosions and swelling of the cheeks and lips, ulcers in the lips, inflammation in the corners of the mouth, and damage to the integrity of the oral epithelial barrier. The mouth is an important source of biomarkers that help diagnose celiac disease (Bucci *et al*., 2006 ; Logan, 2010 ; Mejia, 2017 ; Sanchez-Solares *et al*., 2021).

2.4.3 In Obesity

Obesity is a global epidemic, and there has been a link between obesity and other diseases, including diabetes and cardiovascular disease (Grundy, 2012). Poor oral health has also been linked to obesity (Mathus-Vliegen *et al*., 2007), as it has been found that obese people suffer from oral health problems, including gingivitis, tooth loss and tooth decay. They can also suffer from inflammation of the mucous membrane because of dryness and causing atrophy (Forslund *et al*., 2002; Östberg *et al*., 2009; Sede & Ehizele, 2014) .

2.5 Ultrastructure Change in Oral Mucosa

The fundamental defect that alters any cell begins at the molecular level, thus leading to a series of interactions that regulate the cell. The overall biological activities of the cell are best reflected at the nucleus level, while functional activities are reflected in the cytoplasm (Sivapathasundharam & Kalasagar, 2004). Mitochondria are double-membrane organelles that perform multiple essential functions through thousands of proteins that encode both the nuclear and

mitochondrial genomes (Mootha *et al*., 2003; Pagliarini *et al*., 2008). They are considered the powerhouse of energy generation via oxidative phosphorylation and have other essential functions, including the production of reactive oxygen species with important signaling functions, the oxidative catabolism of amino acids, ketone formation, the urea cycle, and other vital functions (Starkov, 2008; Murphy, 2009). This multiplicity of functions causes variation and difference in pathophysiology and its severity, and the emergence of many diseases that arise from primary or secondary changes in specific mitochondrial pathways.

The health of cells and their dysfunction in tissues is due to the health and presence of mitochondria in terms of function, safety and abundance within the cells. Studying and monitoring changes in mitochondria may contribute to determining the mechanism of development of conditions associated with chronic inflammation, aging, neurodegenerative disorders and metabolic diseases (Morandini & Ramos-Junior, 2024). The characteristic conditions of metabolic diseases are the inability to control the regulation of cells' consumption of excess energy. This deficiency in the body's natural energy regulation, caused by insulin resistance, causes mitochondria to continue their activity to get rid of the unrestrained flow of metabolites. Thus, the attempt of mitochondria to deal with this excess leads to the development of disease, which negatively affects their normal form (Galloway & Yoon, 2013).

Also, Prasun (2020) was that mitochondria play a role in metabolic problems such as obesity and type 2 diabetes, as well as the syndrome. Mitochondrial dysfunction leads to oxidative stress and systemic inflammation that is observed in the above-mentioned pathological conditions. The center of energy metabolism via oxidative phosphorylation is the mitochondria, so it is natural for them to have a role in causing diseases.

2.5.1 Mitochondria in Diabetes

Maintaining mitochondrial function means maintaining normal glucose-stimulated insulin secretion from pancreatic beta cells. Genetic defects in mitochondrial DNA affect or disrupt mitochondrial function, causing a form of insulin-dependent diabetes like type 1 diabetes. Thus, mitochondrial dysfunction causes insulin resistance and type 2 diabetes (Luft, 1994; Lowell & Shulman, 2005). In previous studies conducted by taking a biopsy from People with type 2 diabetes and obesity may have fewer mitochondria in their muscles, maybe because of reduced expression of nuclear encoded mitochondrial biogenesis-regulating genes, including PGC-1b and peroxisome proliferator-activated receptor coactivator 1α [PGC-1α and PGC-1β] (Wu *et al*.,1999; St-Pierre *et al*., 2003). In different physiological conditions and in various tissues, mitochondria are exposed to varying concentrations of oxygen. Mitochondria regulate oxygen and phosphorus reactions with high precision, and any malfunction in their function negatively affects cellular function, as mitochondria produce an increase in reactive oxygen species resulting from tissue injury with large amounts of blood sugar levels, as in the case of diabetes (Ha *et al*., 2008).

2.5.2 Mitochondria in Celiac

In celiac disease patients, symptoms may appear outside the intestine as a defensive response to oxidative stress, which plays a role in causing gliadin toxicity (Piatek-Guziewicz *et al*., 2017), which results from a dysfunction in the mitochondrial function, which leads to its activation. A decrease in reactive oxygen species has a positive effect on mitochondria, especially stimulating mitochondrial biogenesis, while an increase in reactive oxygen species leads to programmed cell death. Oxidative stress associated with the disease affects the level of mitochondrial DNA, and therefore it is believed that the dysfunction in

mitochondrial function is the result of defects in mitochondrial DNA (Storz *et al*., 2005; Malik & Czajka, 2013).

2.5.3 Mitochondria in Obesity

As for obese people, the increase in free fatty acids stimulates the mitochondria to release more reactive oxygen species (ROS), and thus the mitochondria lose their polarity and become fragmented and eventually produce various metabolites that stimulate exocytosis and increase the secretion of basal insulin, this leads to the imposition of insulin (Las *et al*., 2020; Masschelin *et al.,* 2020). in a study by Kelley and his colleagues (2002), which showed that the size of mitochondria was smaller in people with obesity and type 2 diabetes compared to healthy people.

Chapter Three

Materials

&

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
Acidic alcohol	Dow Chemical	USA
Orange G	Avonchem	UK
Eosin 50	Avonchem	UK
Ethanol (absolute 99%)	BDH	England
Fasting Insulin	Roche	Germany
Acetone 100%	Mtedia	USA
Hematoxylin & Eosin	Thomas Baker	India
DPX	Loba chemie	India
Periodic acid	Abcam	China
Glacial acetic acid	Thomas Baker	India
Xylene	Alpha chemika	India
Phosphate Buffered Saline (10x)	Loba_chemie	India
Glutaraldehyde 3%	Sigma_Aldrich	Germany
Paraformaldehyde 2.5%	HiMedia	India
Schiff's Reagent	Avonchem	UK
HbA1c	Roche	Germany
Fasting Glucose	Roche	Germany

3.1.2. Instruments

Table (3-2) : List of instruments Manufacture and Countries used in this study

Instrument	Company	Country
Cyto Brush	DMK KOLDING	China
Glass Slide	Guide medlab	China
Cover Slips	Guide medlab	China
rubber gloves	Tglassco	India
Glass Coplin jars	Binder	USA
Plastic jars	LG	USA
Plastic slides container.	Leitz	Germany
An eyepiece microcoulometer	Binder	Germany
Gel Tube	TRUST LAB	China
EDTA Tube	TRUST LAB	China
Syringe	DMK KOLDING	China
Acular stage	LED	USA

3.1.3. Apparatus

Table (3-3): List of Apparatus Manufacture and Countries used in this study

Name Apparatus	Manufacture Company	Country
Digital Camera	Japan	Sony
Mini VIDAS	bioMérieux	France
Light Microscope	Olympus	Japan
Transmission Electron Microscope	JEOL Ltd.	Japan
Refrigerator	LG	Korea
Cobas analyzar	Roche	Germany

3.1.4 Experimental Design

The study population consists of 60 individuals of both sexes divided into four groups (15 Participants) aged between 20 and 60 years: Control group, Type 2 Diabetes Mellitus, Celiac Disease, and Obesity. Blood Samples were collected for cumulative and random sugar testing, as well as an anti-Tissue Transglutaminase. After the samples were obtained, they were stained with several stains in the tissue laboratory at the University of Maysan, College of Science, Hematoxylin and Eosin PAP and PAS.

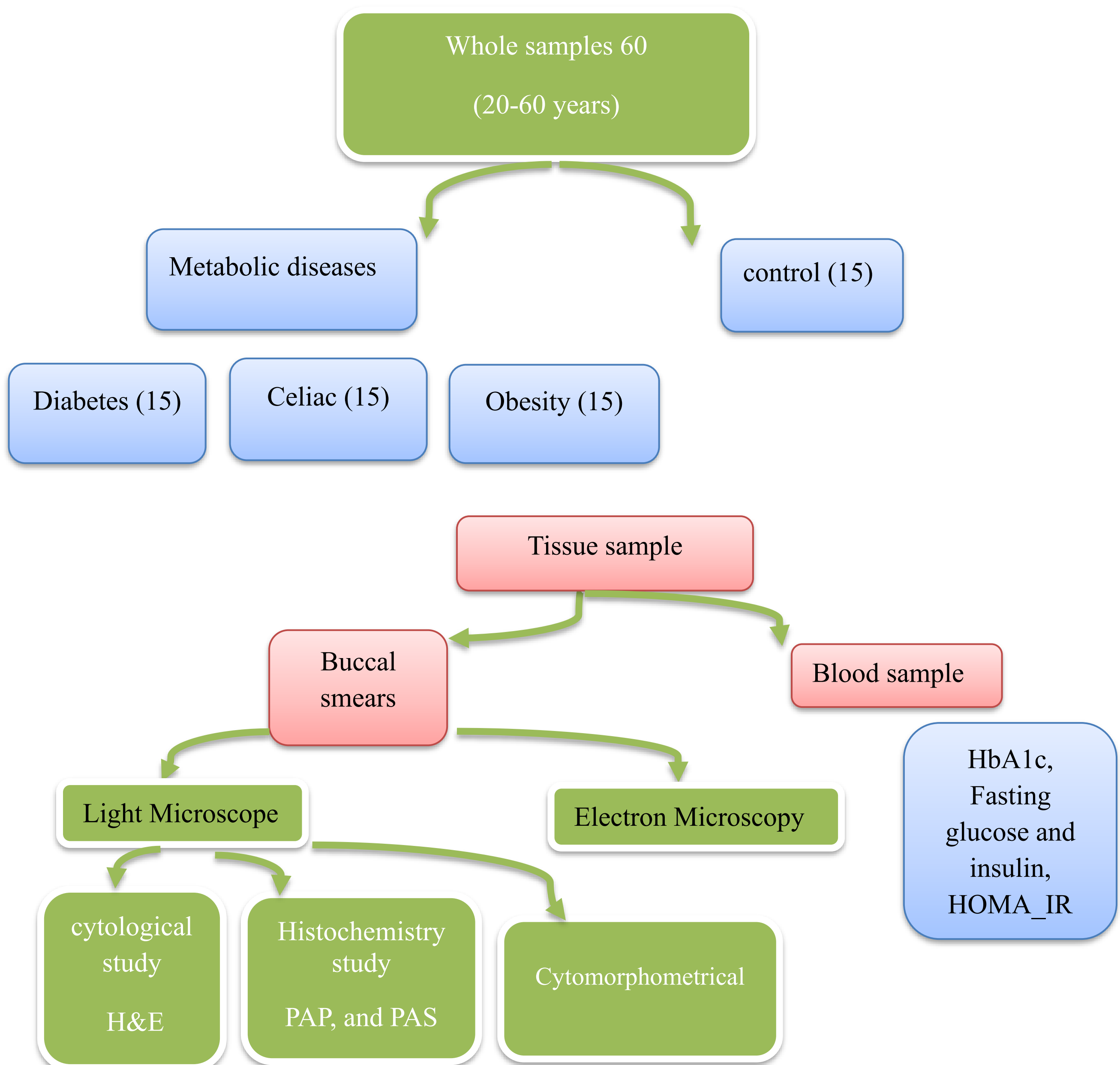


Figure (3.1) Experimental Design

3.2.Sample Collection:

The patients diagnosed with metabolic diseases including type 2 diabetes mellitus, obesity and celiac disease were selected after clinical and laboratory confirmation at the Diabetes and Endocrinology Center, Al-Hakim Teaching Hospital, and private laboratories in Maysan Province. After obtaining informed consent, the patients were instructed to rinse their mouth thoroughly with water and dry the oral mucosa using sterile gauze to remove cellular debris. Then, using a special cytological brush, a buccal smear was taken from the oral cavity, especially the cheek area, and then the collected material was spread in the middle of the glass slide marked with the names of the patients and left to dry in the air for the purpose of fixation by drying and staining with the following dyes: Papanicolaou stain (PAP), Hematoxylin & Eosin stain (H&E) and Periodic acid Schiff (PAS) stain.

3.2.1 Blood Sample

Blood samples were drawn from patients after fasting for at least eight hours. 5ml of venous blood was obtained by drawing venous blood from the arm using a G23 needle from patients with metabolic diseases and from healthy individuals. The samples were placed in tubes containing gel and EDTA and left at room temperature (22-25 °C)for a 15 of an hour until they stabilized, then the tubes with gel were placed in a centrifuge to separate at 3000 rpm for 10 minutes at room temperature, then the serum was collected, and the samples were stored in a freezing at a suitable temperature until biochemical analysis.

3.2.2 Excluded Criteria

Individuals who were suffering from systemic diseases related to metabolic processes, such as diabetes, celiac disease, obesity, hypertension, other chronic diseases as well as malignant diseases, and smokers between the ages of 20 and 60 all of them excluded from the control group.

3.2.3. Inclusion Criteria

The criteria for the patients included in this study included age, body mass index, and systemic diseases related to metabolic processes such as obesity, type 2 diabetes, and celiac disease.

3.3. Methods

3.3.1. Cytological Smear Technique

Using a special cytological brush, a buccal smear was taken from the oral cavity, specifically the cheek area, of people with metabolic diseases and compared to healthy people of both sexes and in age groups ranging from 20 to 60 years. It was quickly spread on a clean glass slide marked with the names of the people in the study and dried in the air for the purpose of fixation. After that, it was treated with a series of cytological dyes in different ways according to the type of dye used. After the samples were stained, a glass cover was placed on them and fixed on the glass slide by placing DPX for a clearer view when examining them using a light microscope to study the differences between normal cells and diseased cells by determining the cytomorphological characteristics of all samples and to count cells, the microscope stage was moved from left to right, then down, then across to avoid duplicating cells from different fields at ×40.

3.3.2. Measurement of Body mass index

Body Mass Index (BMI) is a common way to estimate weight relative to height. It is used as an indirect indicator of body fat percentage, as it is often related to total body fat. The higher the BMI, the greater the body fat percentage, This index is calculated using a simple equation based on a person's weight in kilograms and their height in meters squared The formula is BMI = Weight (kg) / $(\text{Height in meters})^2$ This index is used to classify individuals into different categories, such

as normal weight, overweight, and obesity, according to established criteria (WHO, 2020).

3.4. Cytological

3.4.1. Fixation

Fixation is an essential step in preparing biological samples for microscopic examinations, samples were fixed immediately after taking them to preserve the structural properties of the sample by fixing them in air, i.e. leaving them to dry for 15 minutes (Dalquen , 2022).

3.4.2. Staining

Standard staining Procedure were applied:

3.4.2.1. Staining Procedure of Hematoxylin & Eosin Staining

Hematoxylin and eosin stains are one of the most widely used stains due to their relative simplicity and their ability to clearly stain many different tissues. Basically, when using hematoxylin, it gives a blue-purple color to the nuclei of cells, while the cytoplasm of cells is colored pink, red, or orange when using eosin stain (Bancroft & Layton, 2018).

1. The samples were fixed on the slides by letting them dry air.
2. Place the slides in distilled water for a minute.
3. Stain the slides with hematoxylin for 5 minutes.
4. Wash the slides in running water for 1-2 minutes.
5. Place the slides in eosin stain for one minute.
6. Wash the slides with running water for 1-2 minutes.
7. Then, place the slides in ascending concentrations of ethanol starting from 70%_95%_100% for each concentration, three immersions.
8. Then place the slides in xylene for a minute.

9. Place the cover slip over the sample and secure it with DPX

-The nucleus is stained with hematoxylin because the dye is basic and reacts with the DNA of the nucleus, giving it a purple color. As for the acidic eosin, it reacts with the basic cytoplasm, giving the cytoplasm a pink color.

3.4.2.2. Staining Procedure of Periodic Acid Schiff Stain

PAS stain is used to visualize carbohydrates such as glycogen, mucins such as glycoproteins, glycolipids, and mucins in tissues (Neumann *et al*., 2023).

1. The samples were fixed on the slides by letting them dry air.
2. Place the slides in distilled water for a minute.
3. Place the slides in Periodic acid for 5 minutes.
4. Wash the slides in distilled water three times, immersing them three times.
5. Stain the slides in Schiff's reagent for 5 minutes.
6. Wash it in running water for 5 minutes.
7. Then stain the slides with hematoxylin for half a minute.
8. Wash the slides with running water for 1-2 minutes.
9. Use ascending concentrations of ethanol starting from 70%_95%_100% for each concentration of one minute.
10. clear in xylene for a minute.
11. Place the slip glass over the sample and secure it with DPX.

    -When using PAS stain, this dye interacts with cells depending on the percentage of carbohydrates in them, as it stains the nucleus violet and the cytoplasm pink, while the cell membranes are pinker than the cytoplasm.

3.4.2.3. Staining Procedure of Papanicolaou Stain

Pap stain is a common technique for examining cells (such as epithelial cells) and is particularly useful for examining cervical cells in Pap smear tests. This stain is used to identify cell changes and diagnose cancerous tumors or abnormal changes in cells (Raju, 2016 ; Dalquen , 2022) .

1. The Samples were fixed on slides by spraying them with absolute acetone.
2. Place samples in ethanol 96 % (fixing) for 10 minutes
3. Rehydrate the slides by placing them in descending concentrations of ethanol (96% - 80% - 60% - 50% - distilled water) for 2 minutes at each concentration.
4. Stain the slides with hematoxylin (nuclei staining) for 10 minutes.
5. Rines in distilled water for 30 seconds
6. dehydrate the slides by placing them in Ascending concentrations of ethanol (50% _60% _80% _96%) for 2 minutes at each concentration.
7. Stain the slides with orange G (cytoplasm staining) for 4 minutes.
8. Place the slides in 96% ethanol quick dip.
9. Stain the slides with eosin 50 for 5 minutes.
10. Place the slides in 96% ethanol quick dip
11. clear in xylene quick dip
12. Place the cover glass over the sample and secure it with DPX.

Results

The nuclei should appear blue/black

Cytoplasm (non-keratinizing squamous cells) blue/green

3.5 Cytomorphology Study

An eyepiece micr-oculometer was used to obtain measurements of nucleus diameter, cytoplasm diameter, and the nucleus to cytoplasm ratio for Cytomorphometric evaluation after the buccal smear were stained with PAS, hematoxylin and eosin and then viewed under a compound light microscope.

3.6 Serological Test:

3.6.1 Measurements of Cumulative Blood Sugar (HbA1c)

Specific kit for measuring human HbA1c concentrations in blood was supplied by Roche, Germany.

3.6.2 Measurements of Fasting Glucose (FG)

Specific Kit for measuring human fasting glucose concentrations in serum was supplied by Roche, Germany.

3.6.3 Measurements of Insulin

Specific kit for measuring human insulin concentration in serum was supplied by Roche, Germany.

3.7 Ultrastructural Study

Samples were collected from the study subjects in Maysan province and sent to Iran for examination under transmission electron microscope to determine the changes that occur in the mitochondria.

3.8 Examination of the Slides

After the staining process was completed correctly and following the basic staining standards, the slides were examined under a light microscope using a magnification of 100×, 400× and 1000× to examine the cells.

3.9 Statistical Analysis

The data were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) Version 30 (IBM Co., Armonk., NY., USA). The statistically analysis was performed by the one-way-Analysis-Of-Variance (ANOVA), followed by Least Significant Difference (LSD) The significance level was determined at $p \leq 0.05$ to determine the relationship between the calculated cell count and diseases related to metabolic processes (Al-Rawi & Khalaf Allah, 2000).

Chapter Four

Results

4.1 Cytomorphometrical Study

4.1.1 Results of the Cell count in the Buccal Smear.

The results of the study conducted on patients with metabolic diseases and healthy people revealed the presence of a variation in the cell count from the oral cavity and taken from the buccal smear. The results showed the presence of statistically significant decrease ($P \leq 0.05$),where there are large difference was observed in the cell count, as the average cell count for the control group were 1445 ± 481.38, which is the a large cell count can be observed in the slides under examination, and the group can be identified through microscopic examination before counting, due to the large cell count observed in the section (figure 1), the diabetic group were 335 ± 115.96 (figure 2), the celiac group were 477 ± 133.64 (figure 3), and the obesity group were 299 ± 122.88 (figure 4) ,The examination results showed a small cell count observed under the microscope and a difference from the appearance of the cells in the control group, where the small number appeared clearly before counting, as shown in Table (4.1) and figure (4.1)

Table (4.1) Average Cell counts from buccal smear in different study groups.

Control group NO.	Diabetic group NO.	Celiac group NO.	Obesity group NO.
1445 ± 481.38[a]	335 ± 115.96[b]	477 ± 133.64[cb]	299 ± 122.88[db]

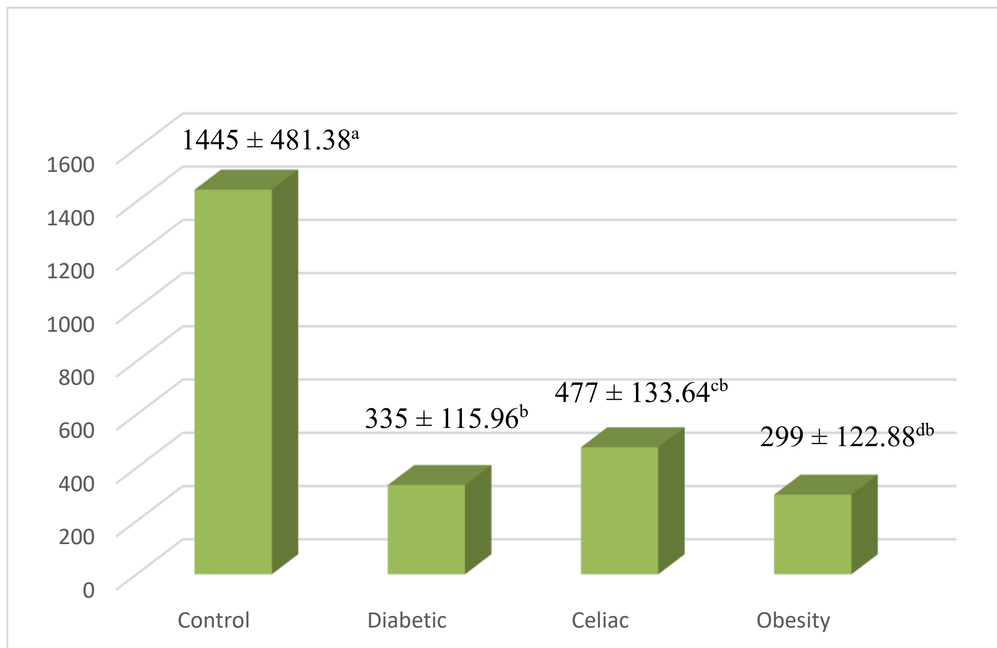


Figure (4.1): Cell count from buccal smear in different groups.

- The values represent mean ± SD
- Different small letters represent significant differences ($P \leq 0.05$) between the groups.
- Similar small letters represent no significant differences between groups

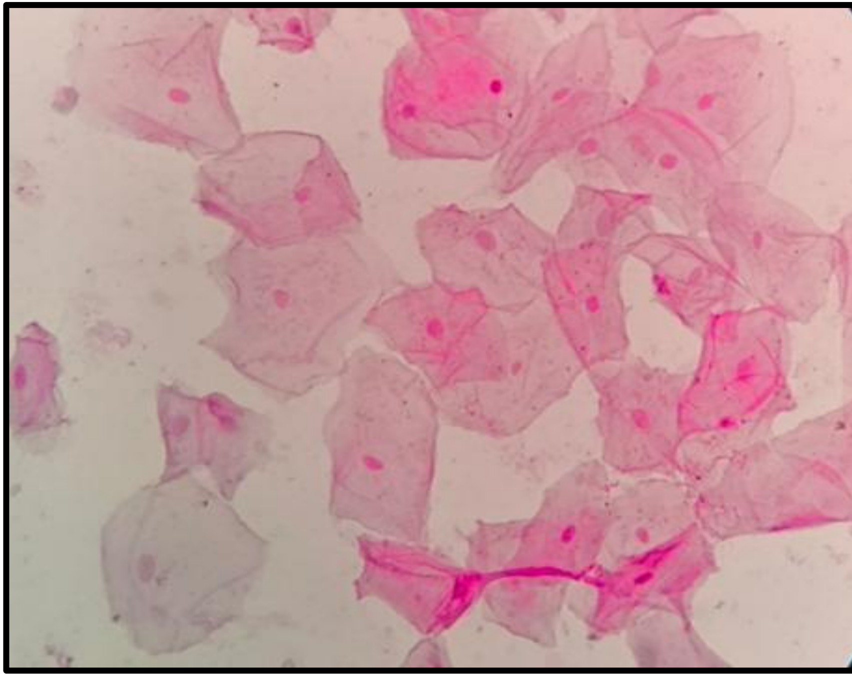


Figure (4.2) A buccal smear of control group showed accumulative the cells in the slide (PAS stain 400x)

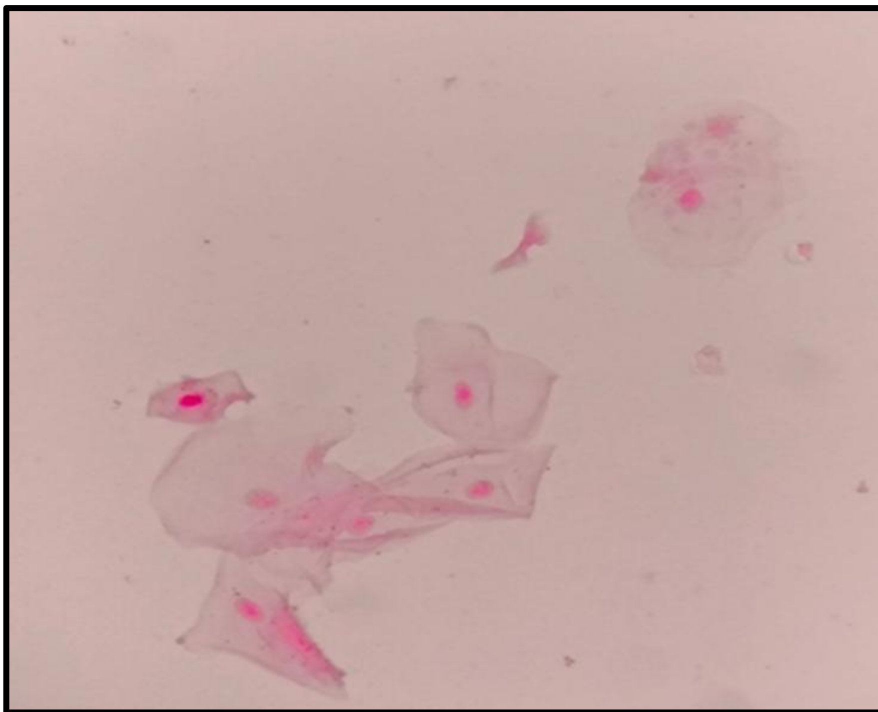


Figure (4.3) A buccal smear of diabetic group showed separate and scanty cells (PAS stain 400x)

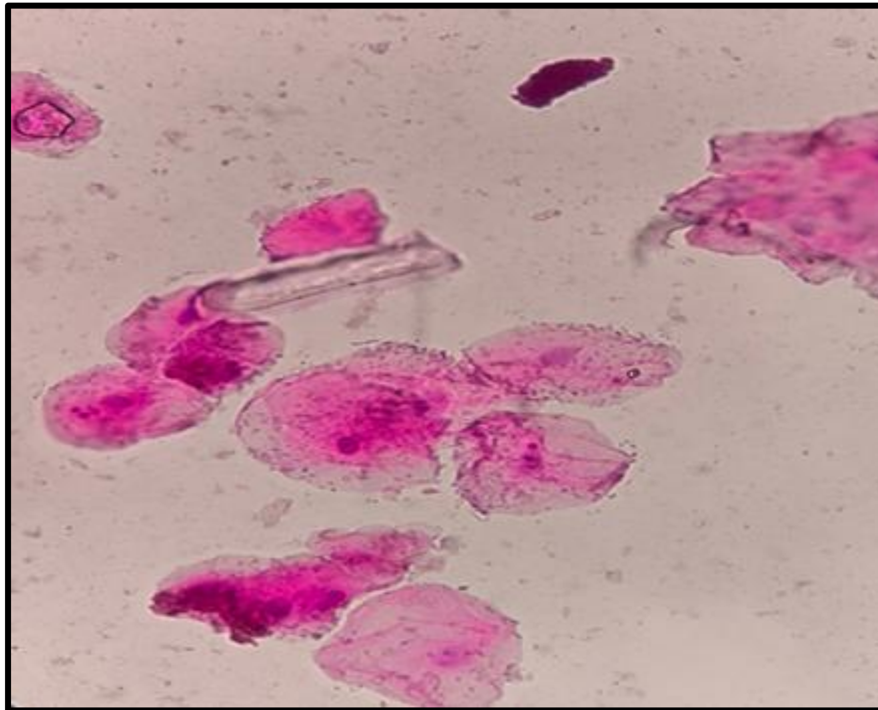


Figure (4.4) A buccal smear of celiac group showed separate and scanty cells (PAS stain 400x)

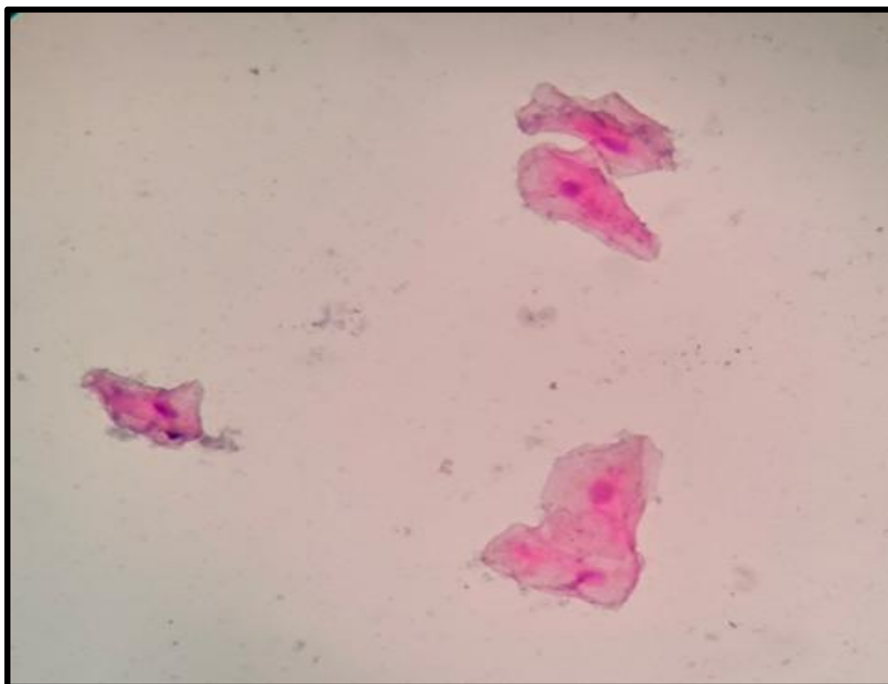


Figure (4.5) A buccal smear of obesity group showed separate and scanty cells (PAS stain 400x)

4.1.2 Results the Cells Count According to the Age

Age related analysis of buccal cell counts revealed a decline in cell number with increasing age across all groups.In the control group, the highest mean cell count was observed in the (20–30) year age group (1916 ± 456), followed by a gradual decrease with increasing age, where it was found in the (31–40) year group (1380 ± 381), in the age group (41–50) years (1409 ± 379), reaching the lowest value in the (51–60) year age group (1076 ± 107).In the diabetic group, cell counts decreased progressively with age, ranging from (250 ± 62.5) in the (20–30) year to (180 ± 10.31) in the age (31–40) years, (182 ± 80) in the age group (41–50) years, and (130 ± 65) in the age (51_60) .As for the celiac group, the average cell counts in the age group (20 _ 30) was (482±159), in the age group (31 _ 40) it was (522±104), and in the age group (41_ 50), it was (334±136), while in the last age group, (51_ 60), the average cell count was (295±25). Finally, in the obesity group, the average cell counts in the age group (20_ 30) was (220 ± 86), and the age group (31_ 40) was (217± 102), the age group (41 _ 50) had an average number of (208 ± 65.74), while in the last age group, represented by (51 _60), it was (203±78.65). As shown in Table (4.2) and figure (4.6)

Table (4.2): Cell counts according to the age in different groups.

Age	Control group	Diabetic group	Celiac group	Obesity group
20_30y	1916±456[a]	250±62.5[b]	482±159[cb]	220 ± 86[db]
31_40y	1380 ± 381[a]	180±10.31[b]	522±104[c]	217± 102[db]
41_50y	1409±379[a]	182±80[b]	334±136[cb]	208 ± 65.74[db]
51_60y	1076±107[a]	130±65[b]	295±25[cb]	203±78.65[db]

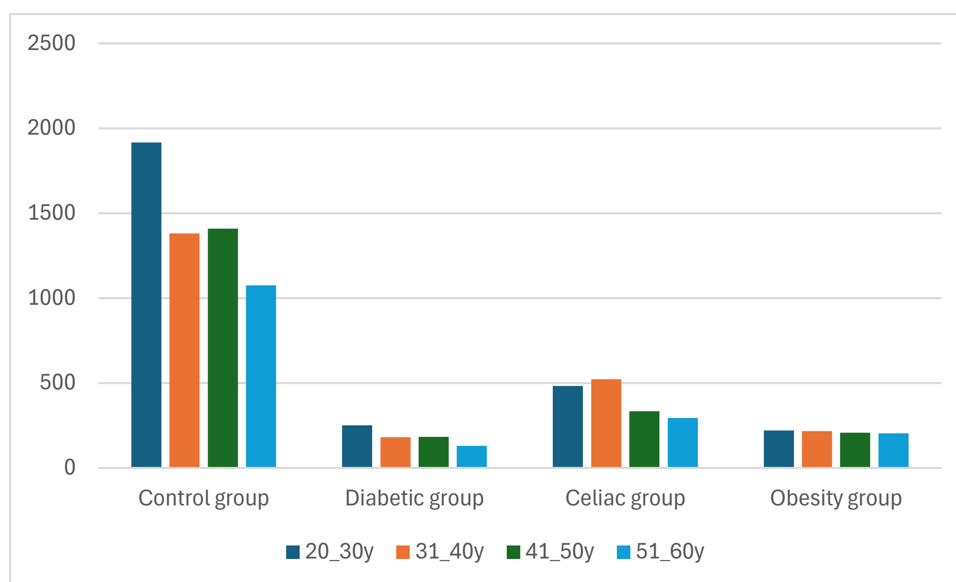


Figure (4.6): Cell counts according to age in different study groups.

- The values represent mean ± SD
- Different small letters represent significant differences (P ≤0.05) between the groups.
- Similar small letters represent no significant differences between groups

4.1.3 Results of Cytomorphology Study

The results of the current study showed no statistical significance differences in nuclear diameter in the cells taken by buccal smear for the four groups in (P ≤0.05), the control group by (8.34 ± 0.33) µm, in the obesity group by (8.73 ± 0.88)µm, followed by the diabetes group by (8.45 ± 0.61) µm, and finally as the lowest values were in the celiac group was (8.31 ± 0.50) µm. In contrast significant differences (P ≤0.05) for the cytoplasm diameter a decrease in the average cytoplasm diameter between the disease groups, while the control group exhibited largest mean (45.62 ± 1.38) µm , and in the obesity group the lowest values were by (38.3 ± 5.30) µm, then the diabetes group was (40.02 ± 8.04) µm, and in the celiac group the average cytoplasm diameter was (39.3 ± 3.92) µm Also the results observed a decrease in the ratio of Nucleus to Cytoplasm(N/C), as the average ratio of nucleus to cytoplasm in the control group was (22.11 ± 0.25), in the obesity group it was (30.24 ± 5.32), in the diabetes group it was

(28.27 ± 7.01), and in the celiac group it was (27.30 ± 4.16). These results indicate Cytomorphology alterations in the buccal epithelial cells associated with metabolic diseases , table (4.3), figures (4.7 - 4.8 - 4.9).

Table (4.3) Cytomorphometric Comparison of different groups.

groups	Nuclear Diameter µm (ND)	Cytoplasm diameter µm (CYD)	Nuclear/Cytoplasmic ratio µm
Control	8.34 ± 0.33[a]	45.62 ± 1.38[a]	22.11 ± 0.25[a]
Diabetic	8.45 ± 0.61[a]	40.02 ± 8.04[b]	28.27 ± 7.01[b]
Celiac	8.31 ± 0.50[a]	39.3 ± 3.92[cb]	27.30 ± 4.16[cb]
Obesity	8.73 ± 0.88[a]	38.3 ± 5.30[db]	30.24 ± 5.32[db]

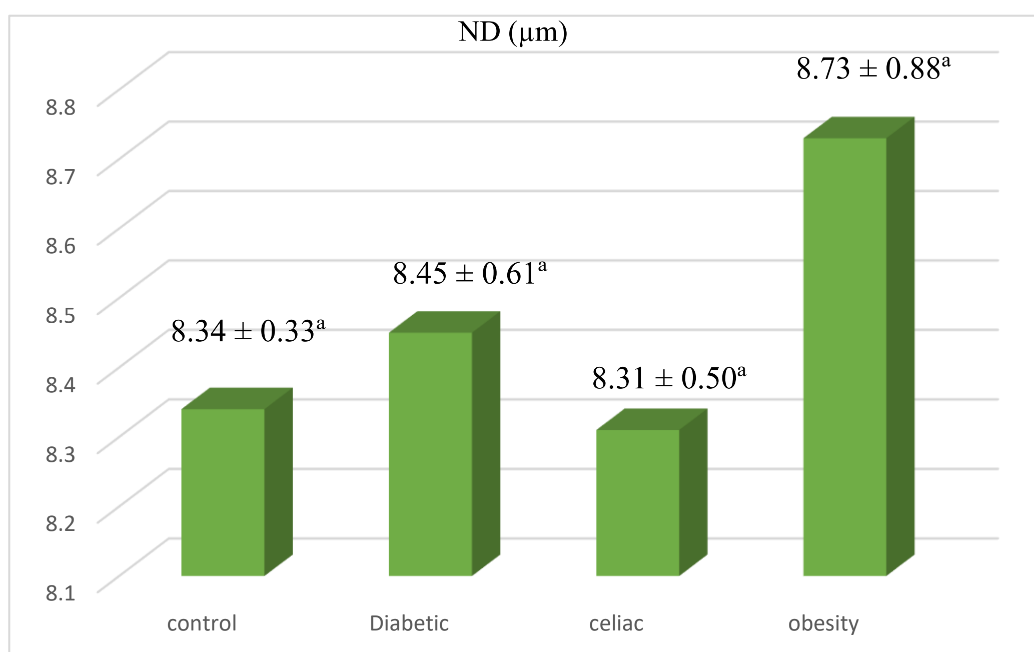


Figure (4.7) Diameter of Nuclear in buccal smear different study groups.

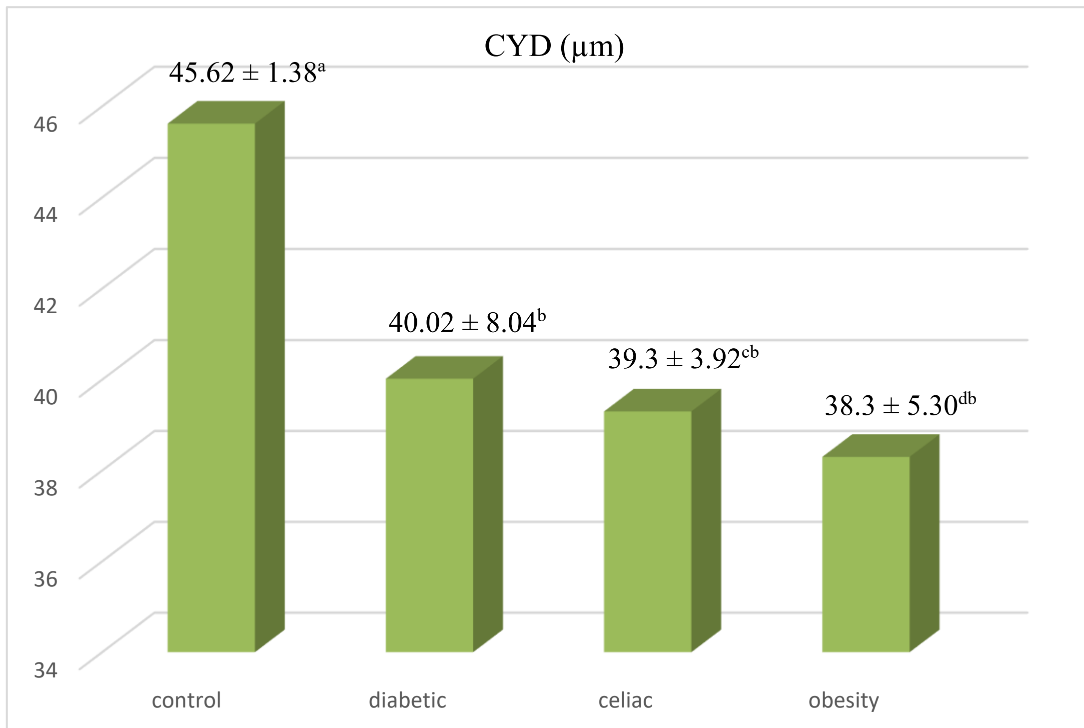
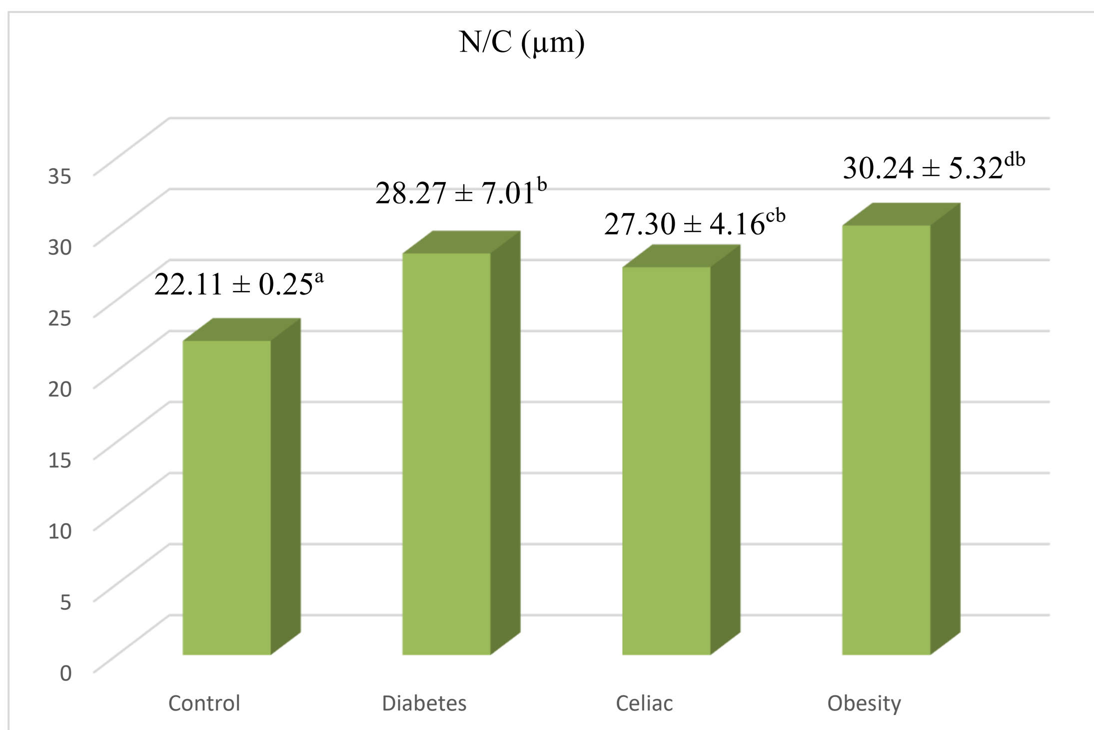


Figure (4.8) Cytoplasm Diameter in different groups.



Figure(4.9) Nuclear/Cytoplasmic ratio in different groups.

- The values represent mean ± SD
- Different small letters represent significant differences (P ≤0.05) between the groups.
- Similar small letters represent no significant differences between groups

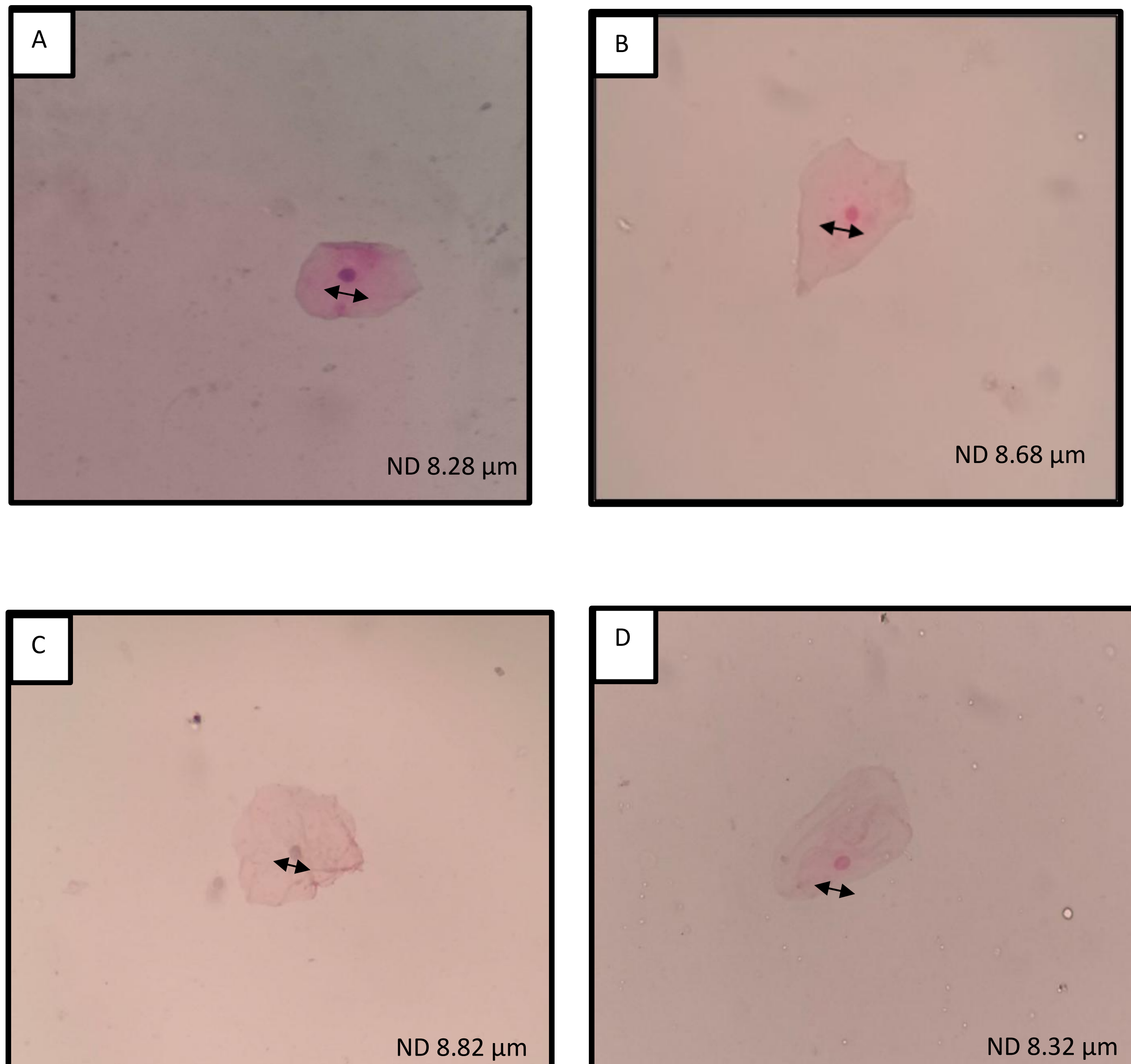


Figure (4.10) Buccal smear of different groups showing nuclear diameter (A) control group, B) diabetic group, (C) celiac group and (D) obesity group (H&E, ×100).

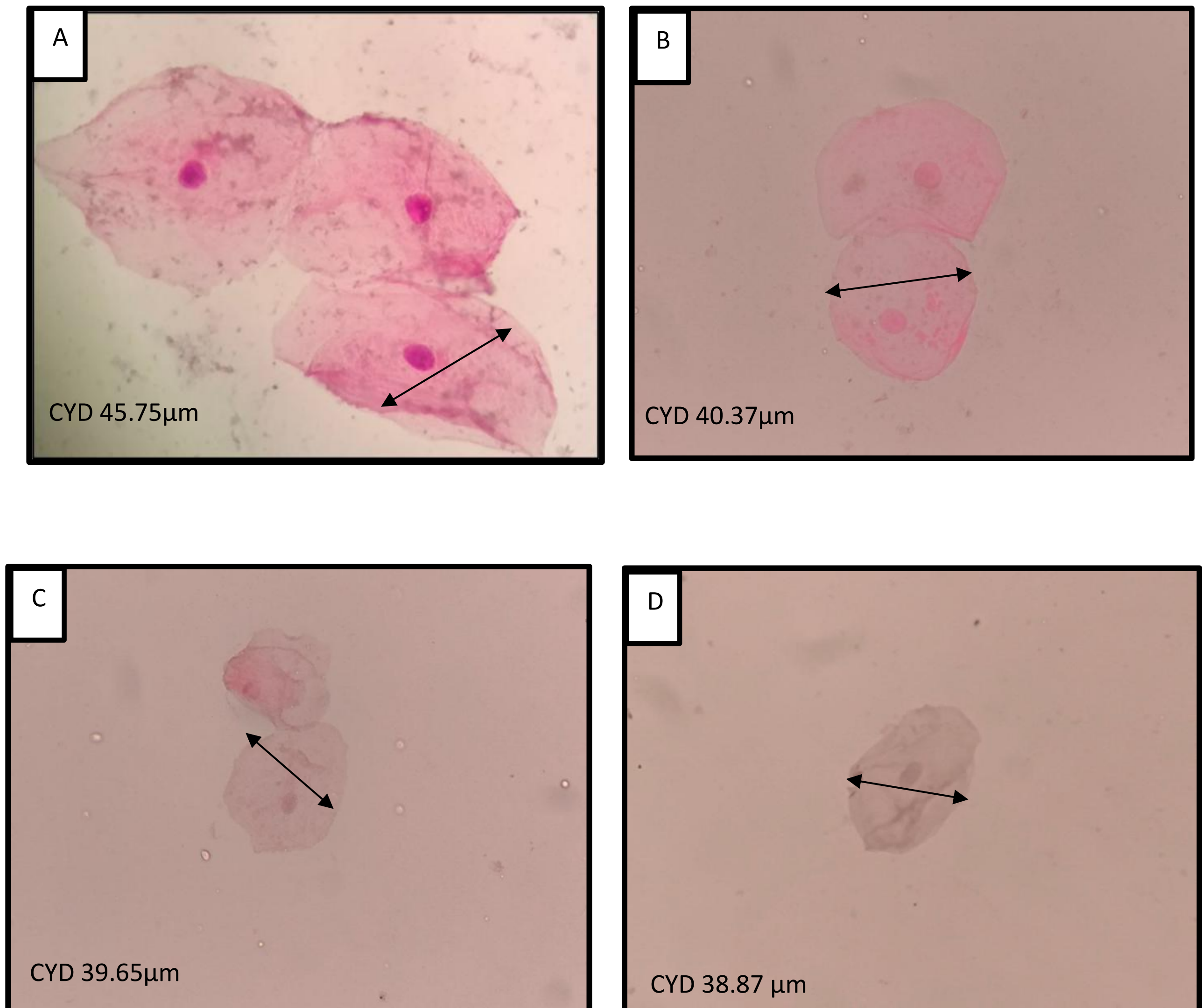


Figure (4.11) Buccal smear showing Cytoplasmic diameter in different groups (A) control group, (B) diabetic group, (C) celiac group and (D) obesity group (H&E, ×400).

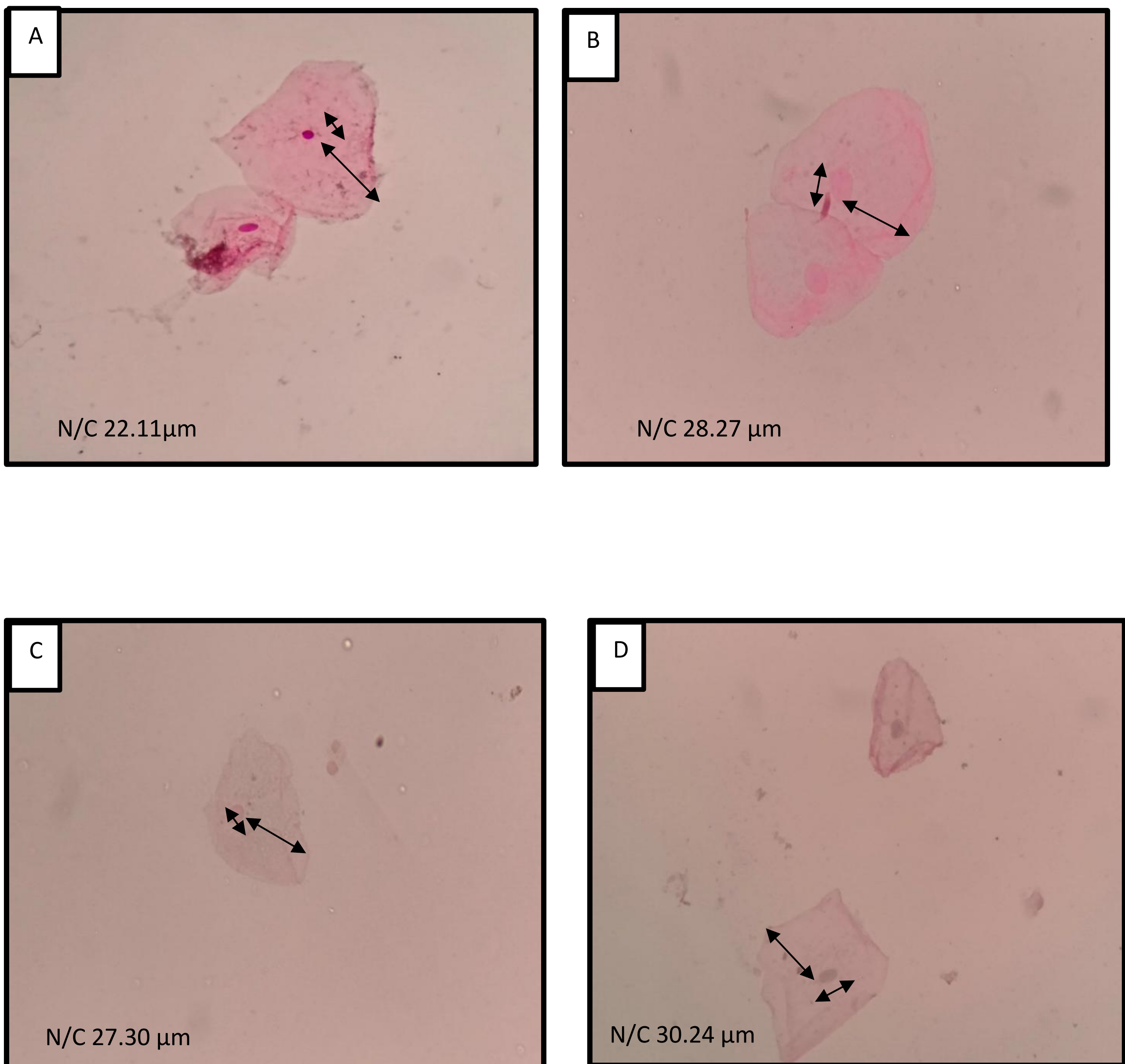


Figure (4.12) Buccal smear showing Nuclear/Cytoplasmic ratio in different groups (A) control group, (B) diabetic group, (C) celiac group and (D) obesity group (H&E ×400).

4.2 Results of Morphological Changes of Nucleus

The current results showed distinct variations in nuclear morphological changes, including karyolysis, Pyknosis and karyorrhexis, were the most variable morphological changes among the four groups, while the morphological changes of Binucleation and micronucleation exhibited lower variations among the different groups.as shown in table (4.4)

Table (4.4) Mean ± SD of nuclear cell morphological Changes in different groups.

morphological changes	Control group Mean ± SD%	Diabetic group Mean ± SD %	Celiac group Mean ± SD %	Obesity group Mean ± SD %
karyolysis	1.51 ± 0.59^{a}	5.50 ± 3.23^{bc}	4.12 ± 2.51^{c}	8.41 ± 5.81^{d}
Pyknosis	3.09 ± 1.58^{a}	5.87 ± 4.64^{ab}	7.37 ± 3.99^{bc}	9.45 ± 6.16^{c}
karyorrhexis	2.22 ± 0.96^{a}	6.91 ± 5.41^{b}	4.87 ± 1.88^{cb}	5.76 ± 3.98^{db}
Binucleation	0.87 ± 0.55^{a}	2.09 ± 1.29^{b}	2.70 ± 1.14^{cb}	1.28 ± 0.66^{d}
micronucleation	1 ± 0.67^{a}	1.55 ± 1.01^{b}	2.59 ± 1.47^{cb}	3.90 ± 3.66^{d}

- The values represent mean ± SD
- Different small letters represent significant differences (P ≤0.05) between the groups.
- Similar small letters represent no significant differences between groups

4.2.1 Karyolysis

The results revealed a significant difference in the karyolytic of the diabetic (5.50 ± 3.23), celiac (4.12 ± 2.51) and obese group (8.41 ± 5.81) when compared with the control group (1.51 ± 0.59), while a significant difference was found among the diabetic and obese group, and there were no significant differences among the diabetic and celiac group. Finally, there was a significant difference between the celiac and obese group.as shown in figure (4.13).

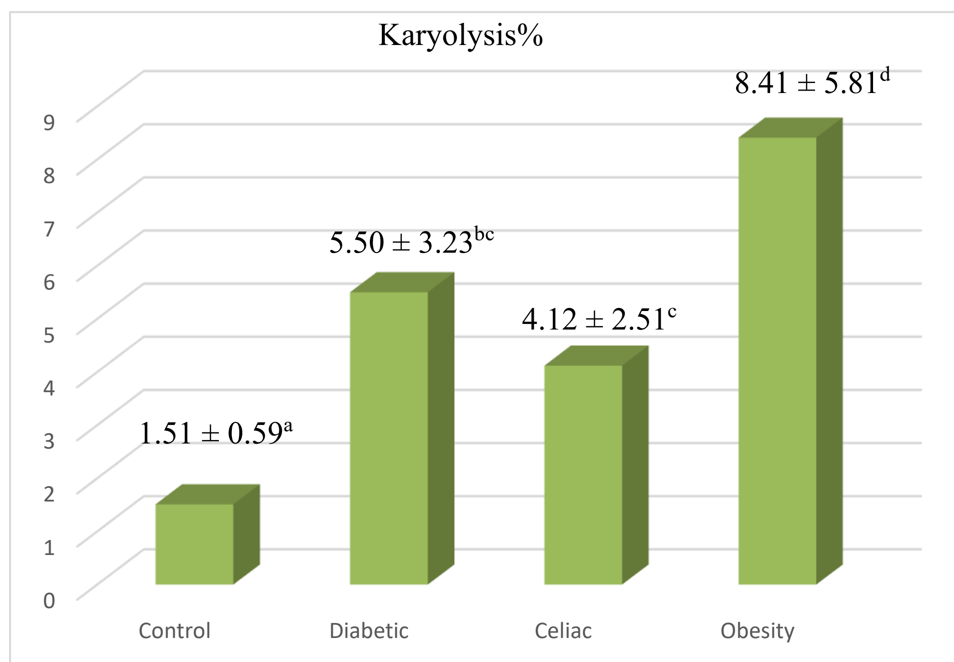


Figure (4.13) Demonstrates the percentage of karyolysis in different groups.

4.2.2 Pyknosis

The results showed a no significant differences in the percentage of Pyknosis, in the diabetic group (5.87 ± 4.64) compared to the control group (3.09 ± 1.58) and a significant differences in both the celiac (7.37 ±3.99) and obese groups (9.45 ± 6.16) compared to the control group, while there was a no significant differences between the diabetic and celiac groups and a significant differences between the diabetic and obese groups. There were no significant differences between the celiac group and the obese group. figure (4.14).

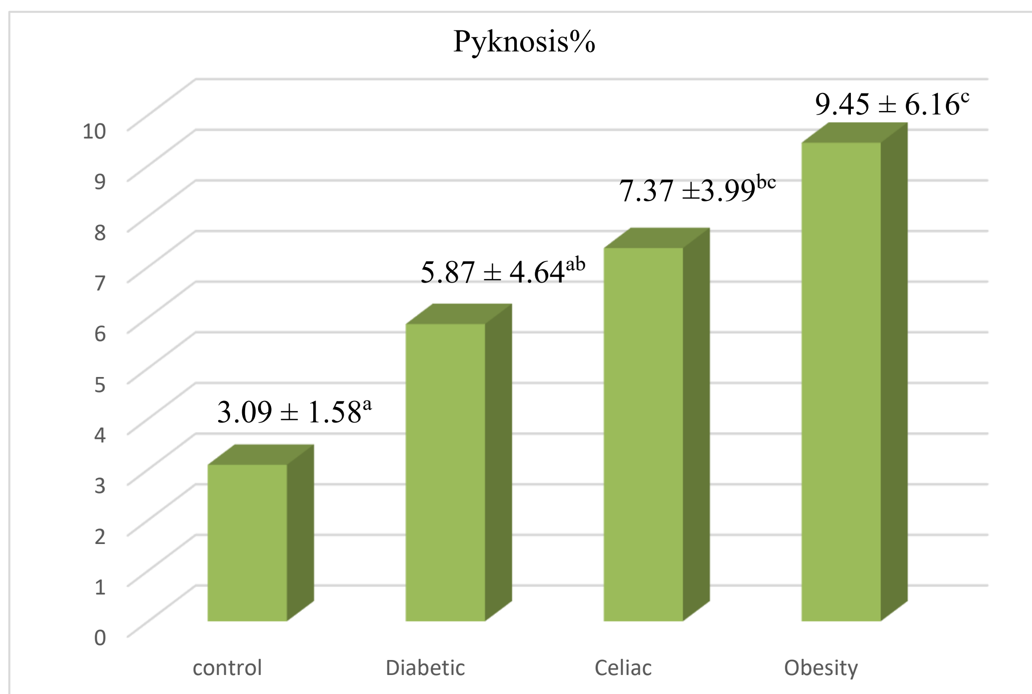


Figure (4.14) Demonstrates the percentage of Pyknosis in different groups.

4.2.3 Karyorrhexis

The results revealed a significant increase in karyorrhexis in diabetic group (5.87 ± 4.64) and significant increase in celiac (7.37 ± 3.99) and obesity (9.45 ± 6.16) as compared with control group ($p \leq 0.05$).No statistically significant was observed between the diabetic and celiac groups or between the celiac and obesity groups Figure (4.15).

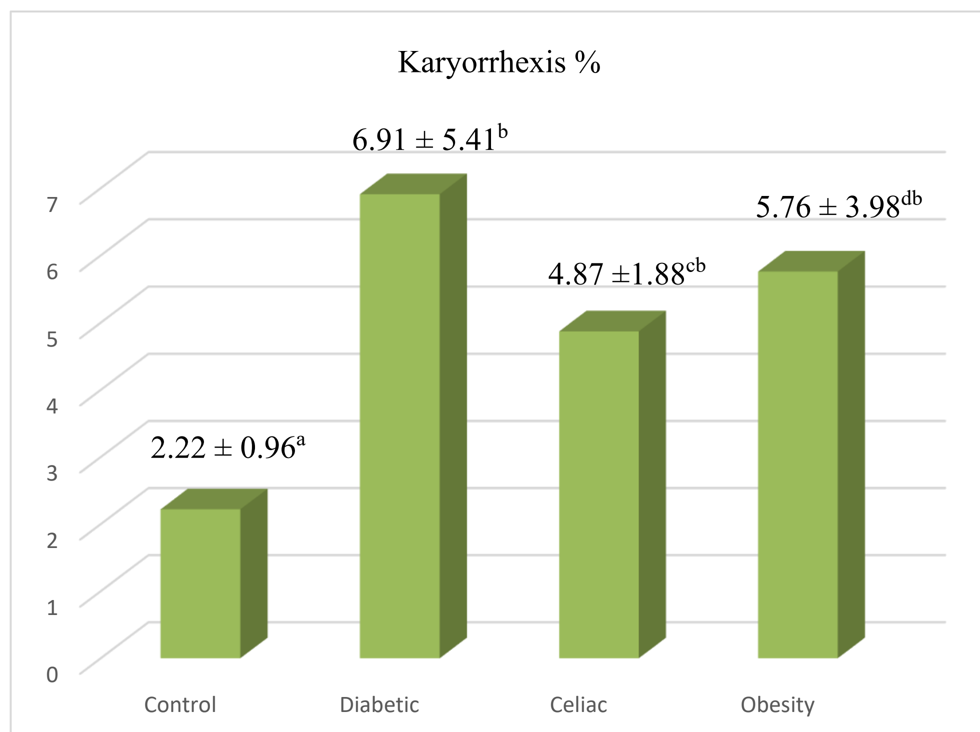


Figure (4.15) Demonstrates the percentage of Karyorrhexis in different groups.

4.2.4 Binucleation

A significant increase in binucleated cells was observed in diabetic (2.09 ± 1.29), celiac (2.70 ± 1.14) and obesity (1.28 ± 0.66) compared with the control group (0.87 ± 0.55) ($p \leq 0.05$). However, there was no significant difference between the diabetes group and the celiac group, while there was a significant difference when comparing the diabetes group with the obesity group. In addition, a significant difference was found between the celiac group and the obesity group. as shown in figure (4.16).

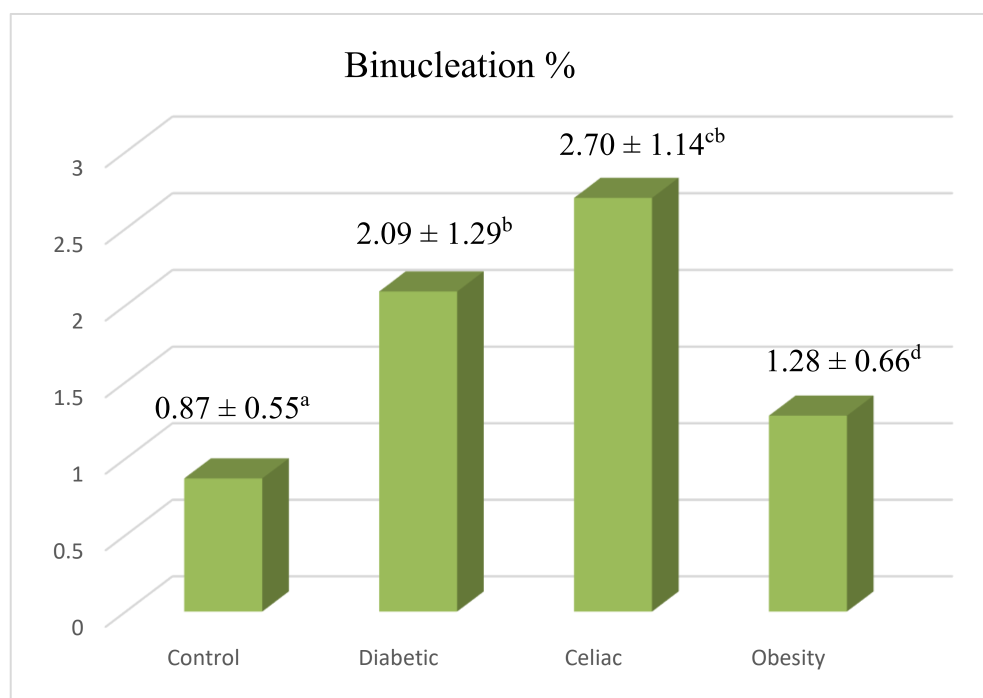


Figure (4.16) Demonstrates the percentage of Binucleation in different groups.

4.2.5 Micronucleation

The results showed a significant increase in micronucleated cells in diabetic (1.55 ± 1.01), celiac (2.59 ± 1.47) and obesity groups (3.90 ± 3.66) when compared with control group (1.00 ± 0.67) in ($p \leq 0.05$). However, there was no significant difference between the diabetes group and the celiac group, while there was a significant difference when comparing the diabetes group with the obesity group. In addition, a significant difference was found between the celiac group and the obesity group figure (4.17)

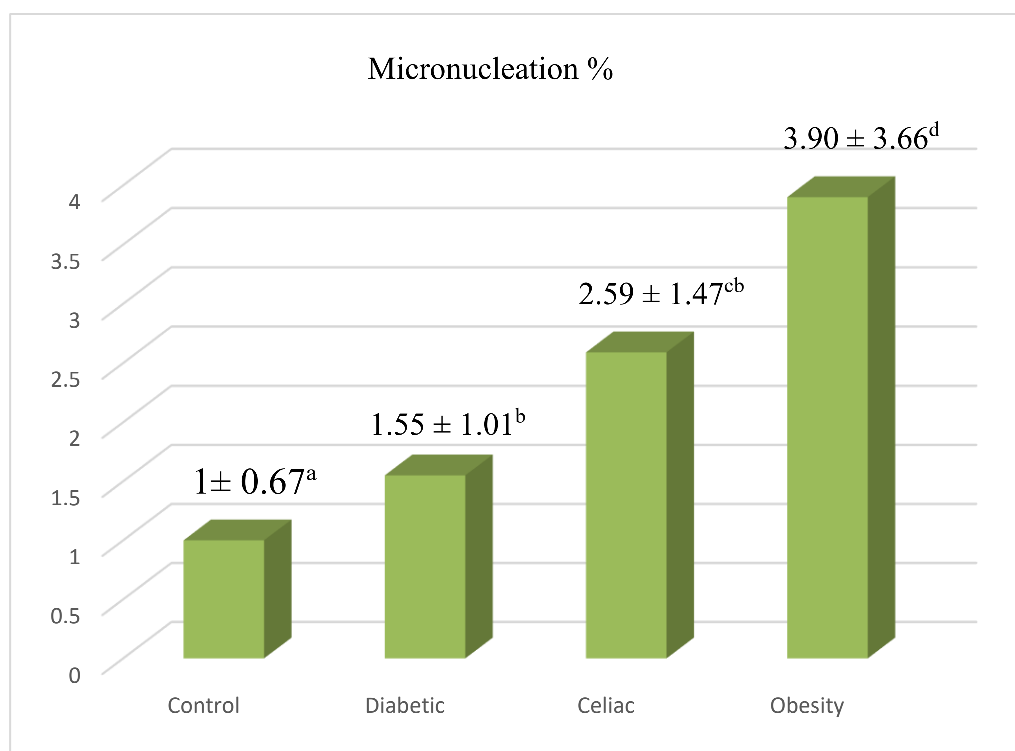


Figure (4.17) Demonstrates the percentage of Micronucleation in different groups.

- The values represent mean ± SD
- Different small letters represent significant differences (P ≤0.05) between the groups.
- Similar small letters represent no significant differences between groups

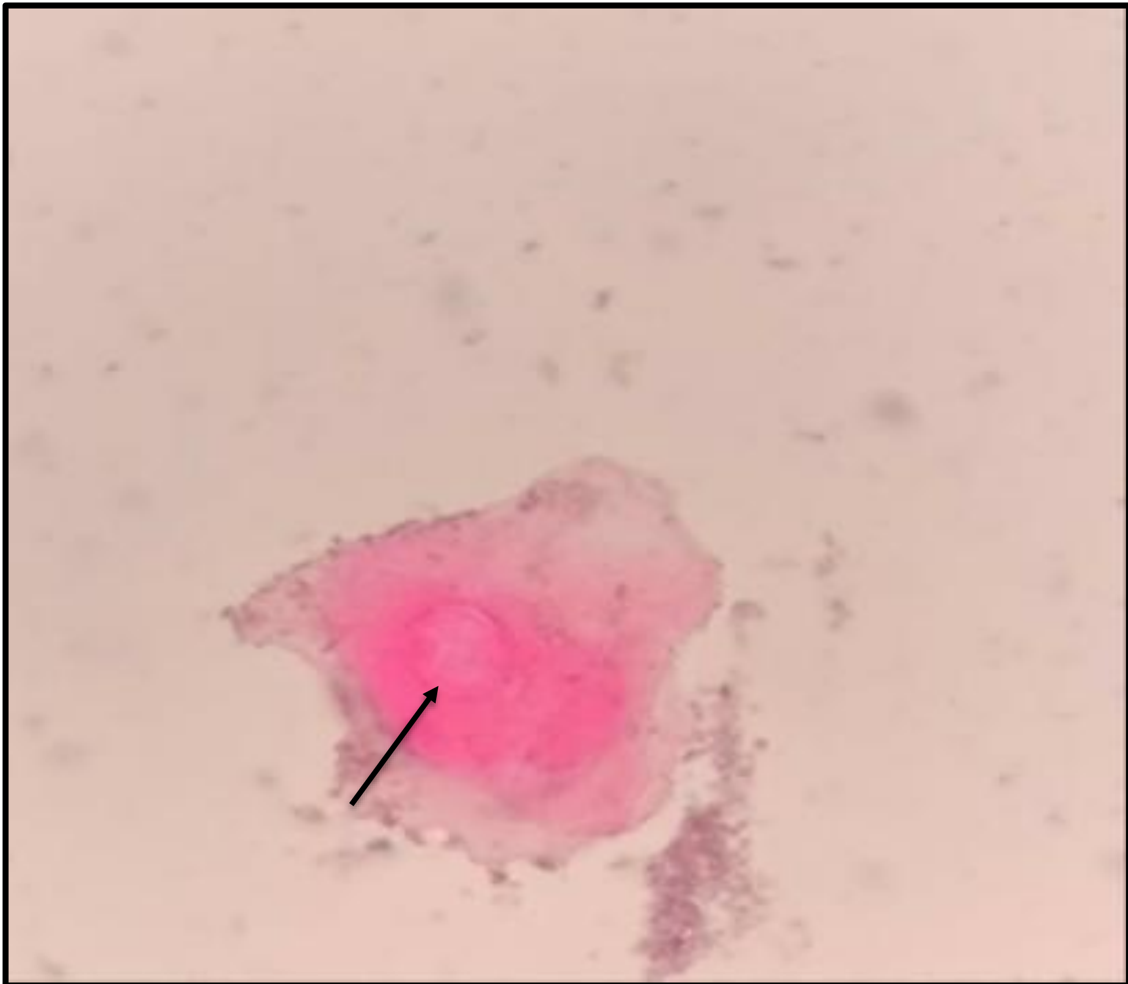
4.2.6 Relationship between Nuclear morphological changes and Cell Count

When comparing the morphological changes with the cell count for the four different groups, the results showed that the control group, which exhibited the lowest percentages of morphological changes, had the highest cell count (1445 ±481.38). As for the diabetic group, which had high morphological changes compared to the control group, the cell count in it was (335 ± 115.96). The celiac group had the least morphological changes among the four groups. In contrast, the cell count in it was (477 ± 133.64), higher than the diabetic and obesity group. While the obesity group was the highest among the other groups in terms of

morphological changes, it had the lowest cell count among the four different groups by an amount of (299 ± 122.88) table (4.5).

Table (4.5): Mean ±SD of cell morphological Changes in different groups as compared with cell count.

morphological changes	Control group Mean ± SD%	Diabetic group Mean ± SD %	Celiac group Mean ± SD %	Obesity group Mean ± SD %
karyolytic	1.51 ± 0.59[a]	5.50 ± 3.23[bc]	4.12 ± 2.51[c]	8.41 ± 5.81[d]
Pyknotic	3.09 ± 1.58[a]	5.87 ± 4.64[ab]	7.37 ±3.99[bc]	9.45 ± 6.16[c]
karyorrhexis	2.22 ± 0.96[a]	6.91 ± 5.41[b]	4.87 ±1.88[cb]	5.76 ± 3.98[db]
Binucleation	0.87 ± 0.55[a]	2.09 ± 1.29[b]	2.70 ± 1.14[cb]	1.28 ± 0.66[d]
micronucleation	1± 0.67[a]	1.55 ± 1.01[b]	2.59 ± 1.47[cb]	3.90 ± 3.66[d]
Cell Count	1445 ±481.38[a]	335 ± 115.96[b]	477 ± 133.64[cb]	299 ± 122.88[db]



Figure(4.18) Karyolysis cells in the obesity group ⟶ showing nuclear lysis ((PAS stain, ×400).

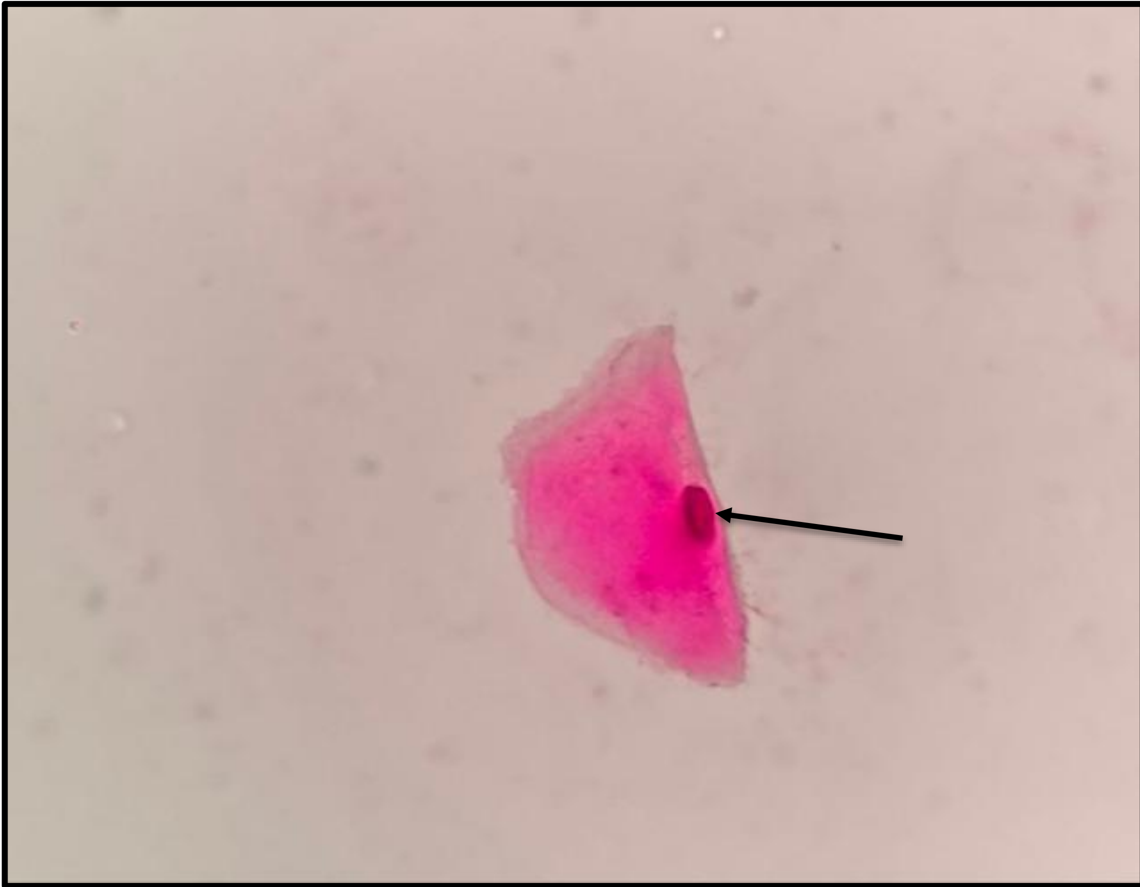


Figure (4.19) pyknosis cells in the diabetic group ⟶ showing nuclear condensation (PAS stain, ×400).

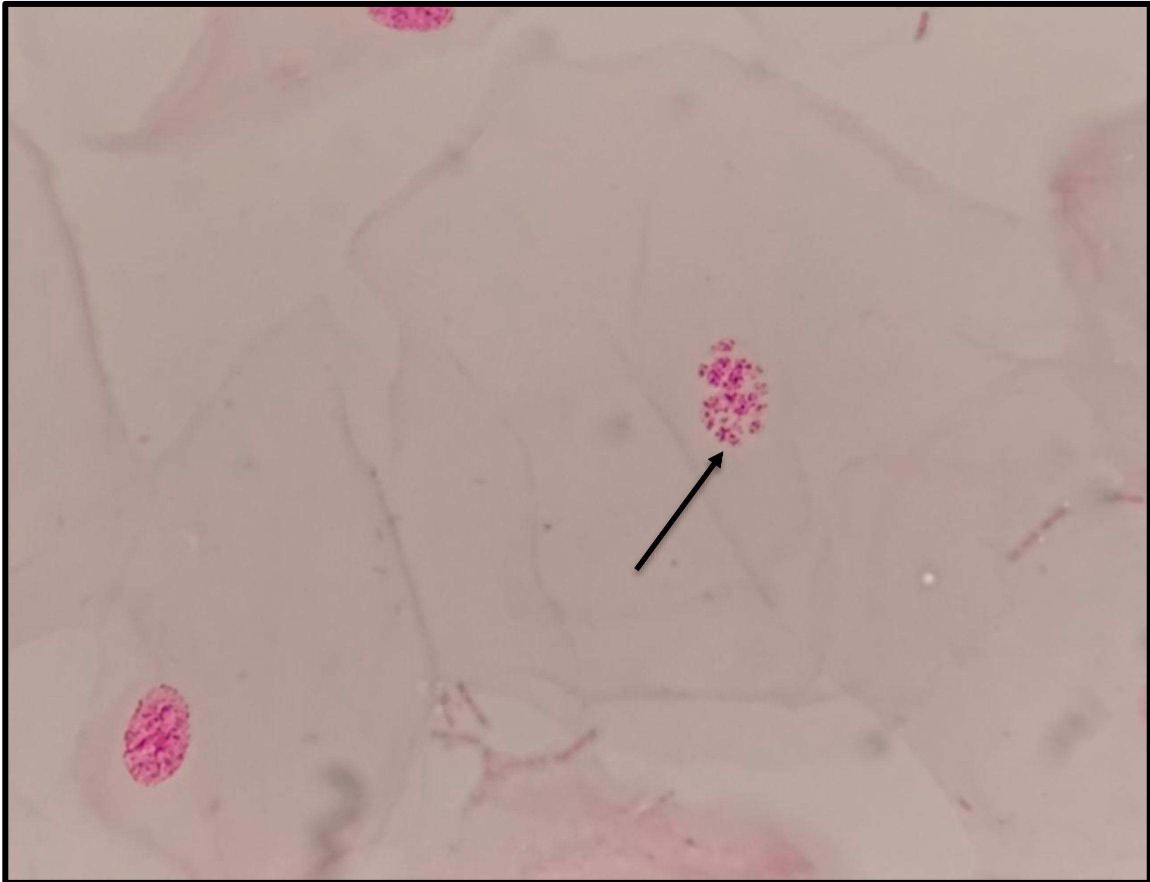


Figure (4.20) karyorrhexis Cells from the celiac group showing ⟶ nuclear fragmentation (PAS stain, ×1000).

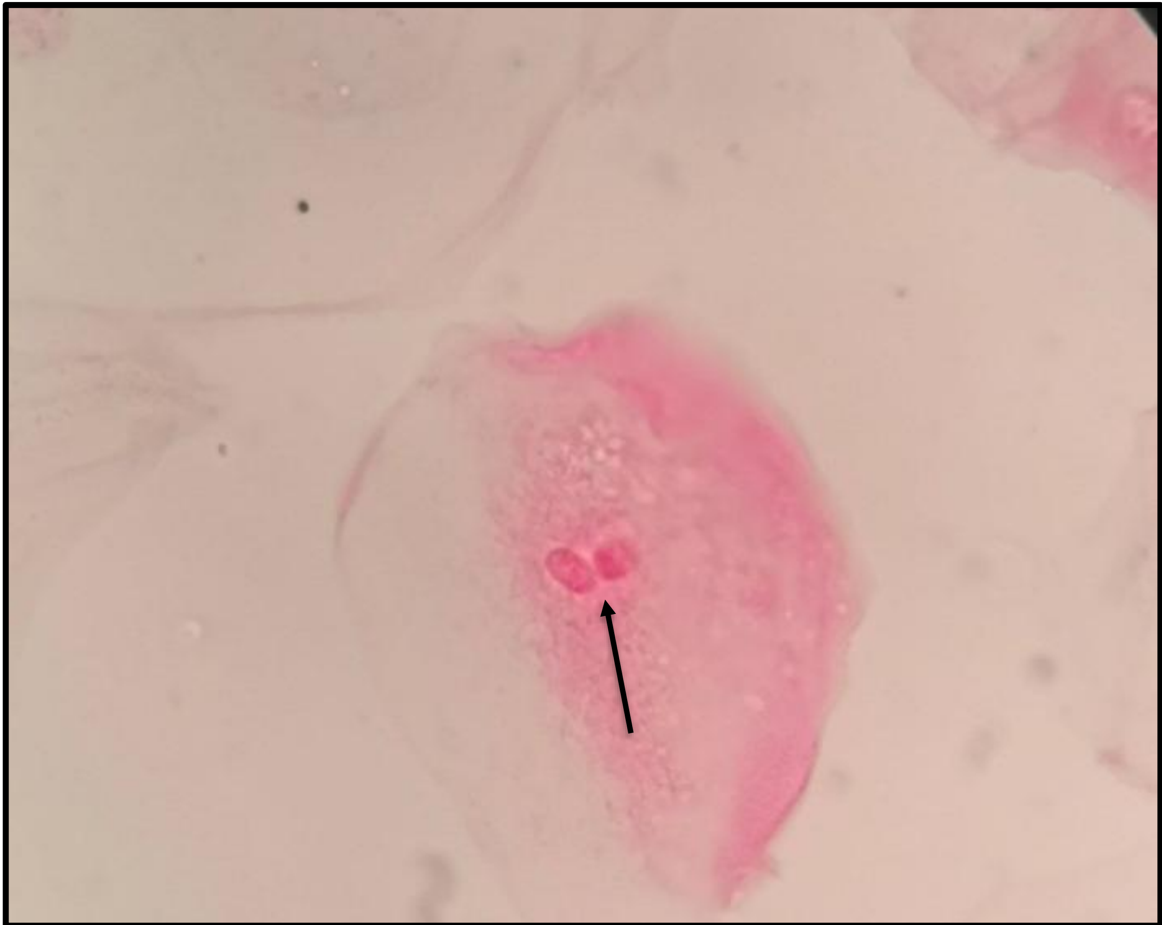


Figure (4.21) Binucleated cells from the celiac group ⟶ showing two distinct nuclei (PAS stain, ×1000).)

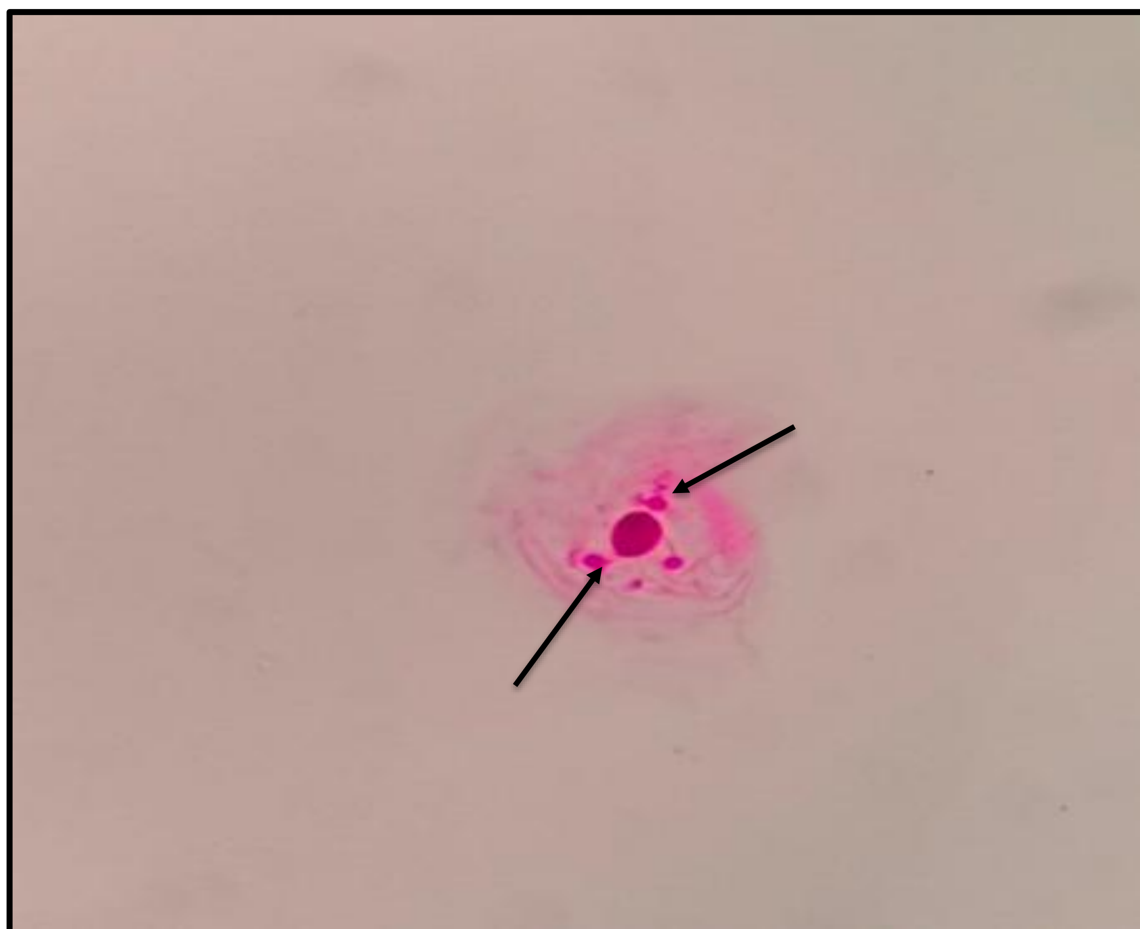


Figure (4.22) Micronucleated cells in the obesity group ⟶ showing small bodies around the nucleus (PAS stain, ×400).

4.3 Results of Biochemical Markers

Table (4.6) The level of Biochemical markers in different groups.

parameters	Control group	Diabetic group	Celiac group	Obesity group
Fasting glucose	88.58± 7.08^{a}	177.73 ± 65.82^{b}	90.38 ± 4.19^{c}	93.44 ± 10.19^{d}
Fasting insulin	8.33 ± 0.89^{a}	27.68 ± 16.51^{b}	16.73 ± 11.17^{c}	26.83 ±12.37^{db}
HOMA_IR	1.86 ± 0.23^{a}	12.75 ± 7.09^{b}	3.74 ± 2.66^{cd}	6.44 ± 3.43^{d}

4.3.1 Fasting Blood Sugar FBS

The significant elevation in fasting glucose levels in diabetic (177.73 ± 65.82 mg/dL) patients as compared with control group (88.58± 7.08 mg/dL). In contrast, blood glucose levels in both the diabetic and obese groups were slightly higher than in the control group, but remained within the normal physiological range despite the statistical difference. while there was a significant difference when comparing the diabetes group with the celiac group and the obesity group, as well as a significant difference when comparing the celiac group with the obesity group figure (4.23)

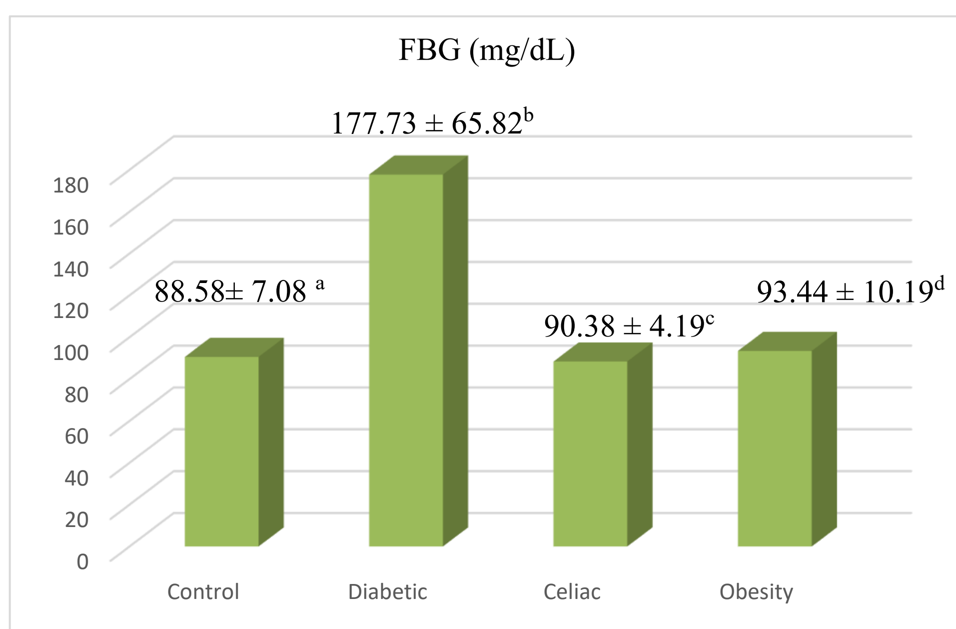


Figure (4.23) Fasting blood sugar levels in different groups.

4.3.2 Fasting Insulin

The results revealed a significant increase in fasting insulin levels at the significance level of (P ≤0.05) when comparing the control group (8.33 ± 0.89 µU/ml) with each of the diabetes group (27.68 ± 16.51 µU/ml), the celiac group (16.73 ± 11.17 µU/ml), and the obesity group (26.83 ± 12.37 µU/ml), while there was also a significant decrease between the diabetes group and the celiac group, and a no significant increase when comparing the diabetes group with the obesity

group. In addition, there was a significant increase between the obesity group and the celiac group as shown in figure (4.24).

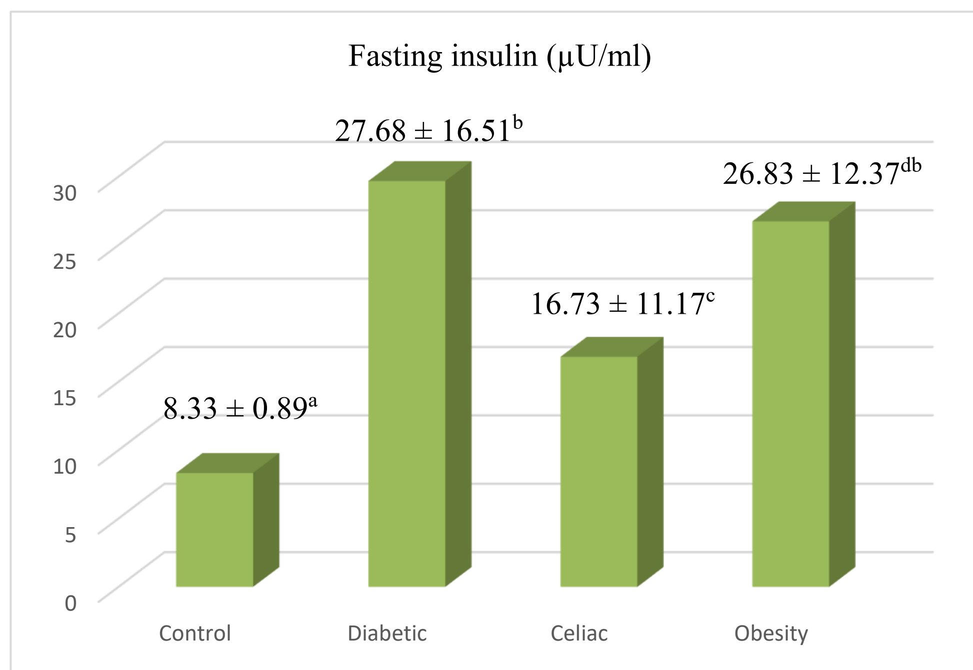


Figure (4.24) Fasting insulin levels in different groups.

4.3.3 Homeostasis Model Assessment of Insulin Resistance (HOMA_IR)

The results showed a significant increase in the significance level of ($P \leq 0.05$) between the control group (1.86 ± 0.23), the diabetes group (12.75 ± 7.09), and the obesity group (6.44 ± 3.43), while there was no significant increase when comparing the control group with the celiac group (3.74 ± 2.66). On the other hand, when comparing the diabetes group with the celiac group and the obesity group, there was a significant decrease, and the increase was no significant when comparing the celiac group with the obesity group figure (4.25).

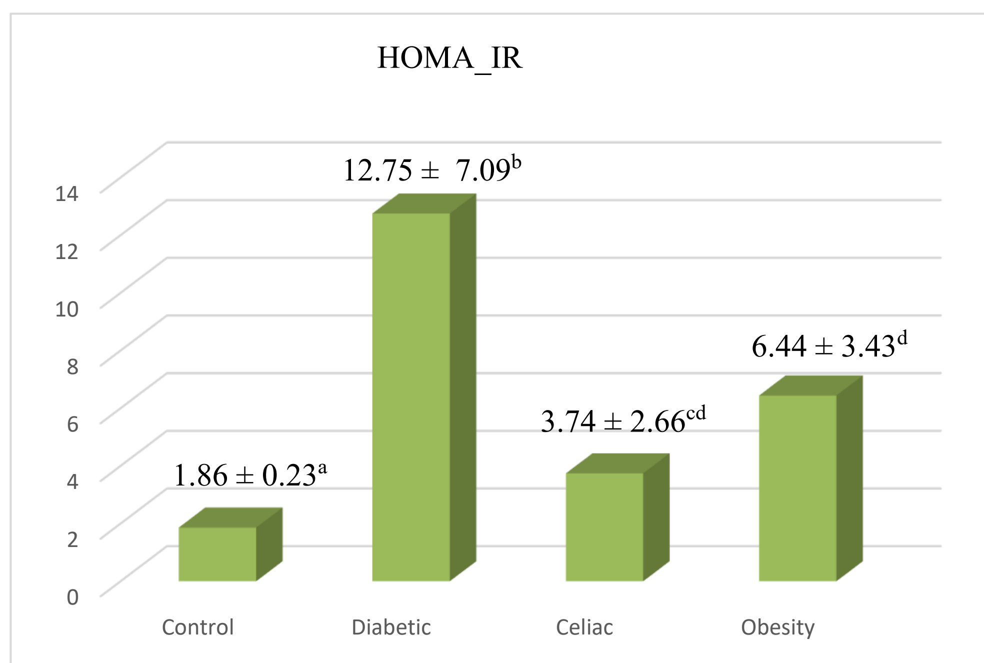


Figure (4.25) HOMA_IR levels in different groups.

- The values represent mean ± SD
- Different small letters represent significant differences ($P \leq 0.05$) between the groups.
- Similar small letters represent no significant differences between groups.

4.3.4 Relationship between Biochemical Markers and Cells Count

The results of the current study showed that the average fasting glucose in the control group was (88.58± 7.08 ml/dL), fasting insulin was (8.33 ± 0.89 µU/ml), and insulin resistance was (1.86 ± 0.23), while the average cell count was the highest among the groups, amounting to (1445 ± 481.38). In the type 2 diabetes group, there was an increase in the average fasting glucose of (177.73 ± 65.82 mg/dL), as well as an increase in the average fasting insulin of (27.68 ± 16.51 µU/ml), and an increase in the average insulin resistance of (12.75 ± 7.09), while there was a decrease in the cell count by (335 ± 115.96) compared to the control group.The celiac group, showed moderate increase fasting glucose (90.38 ± 4.19 mg/dL), and the average fasting insulin was (16.73 ± 11.17 µU/ml), while the average insulin resistance was (3.74 ± 2.66), and the average cell count was (477 ± 133.64) compared to the control group.The obesity group, the average fasting

glucose was slightly higher, by (93.44 ± 10.19 mg/dL), while the average fasting insulin was also increased by (26.83 ± 12.37 uU/ml),, and the average Insulin resistance was the lowest among the other groups, at (6.44 ± 3.43), while the average cell count was significantly lower, at (299 ± 122.88), compared to the other groups table (4.7) and figure (4.26).

Table (4.7) Relationship between Biochemical markers and cells count in different groups.

parameters	Control group	Diabetic group	Celiac group	Obesity group
Fasting glucose	88.58± 7.08[a]	177.73 ± 65.82[b]	90.38 ± 4.19[c]	93.44 ± 10.19[d]
Fasting insulin	8.33 ± 0.89[a]	27.68 ± 16.51[b]	16.73 ± 11.17[c]	26.83 ±12.37[db]
HOMA_IR	1.86 ± 0.23[a]	12.75 ± 7.09[b]	3.74 ± 2.66[cd]	6.44 ± 3.43[d]
Cell Count	1445 ± 481.38[a]	335 ± 115.96[b]	477 ± 133.64[cb]	299 ± 122.88[db]

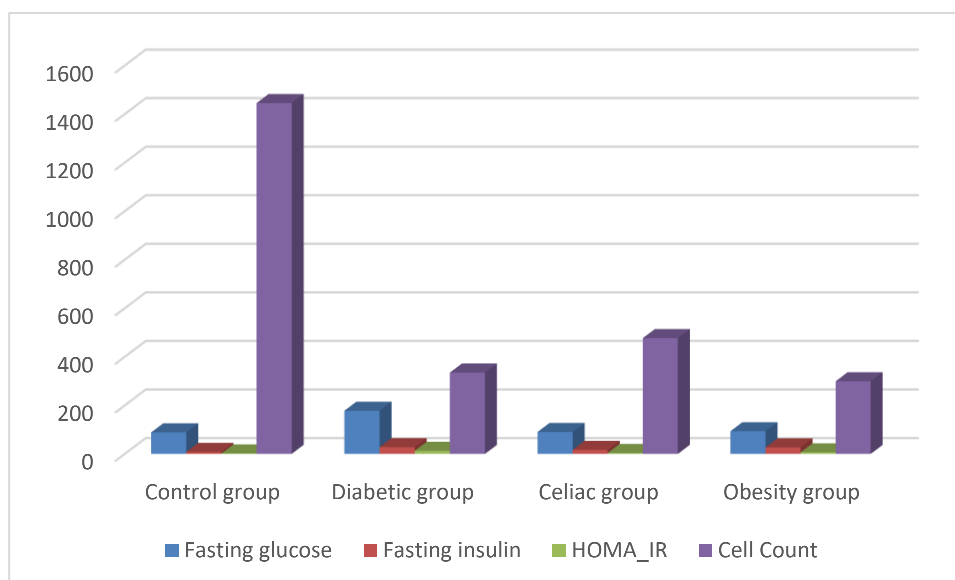


Figure (4.26): Relationship between Biochemical markers and cells count in different groups.

4.3.5 Comparison of the Biochemical Markers and HbA1c between Control and Diabetic Groups.

The results of the current study showed that the average cumulative sugar values were (4.21 ± 0.34) in the control group, the average fasting glucose values were (88.58± 7.0 mg/dL), fasting insulin was (8.33 ± 0.89 uU/ml), and insulin resistance was (1.86 ± 0.23) compared to the diabetic group, where the cumulative sugar values were high by (9.29 ± 2.52) , and the average fasting glucose was high by (177.73 ± 65.82 mg/dL), fasting insulin was (27.68 ± 16.51uU/ml), and insulin resistance was (12.75 ± 7.09), These results confirm poor long-term glycemic control and severe insulin resistance in diabetic patients table (4.8) and figure (4.27)

Table (4.8): Illustrates Biochemical markers and HbA1c between control and diabetic groups .

parameters	control group	Diabetic group
Fasting glucose	88.58± 7.08 [a]	177.73 ± 65.82[b]
Fasting insulin	8.33 ± 0.89 [a]	27.68 ± 16.51 [b]
HOMA_IR	1.86 ± 0.23 [a]	12.75 ± 7.09 [b]
HbA1c	4.21 ± 0.34 [a]	9.29 ± 2.52 [b]

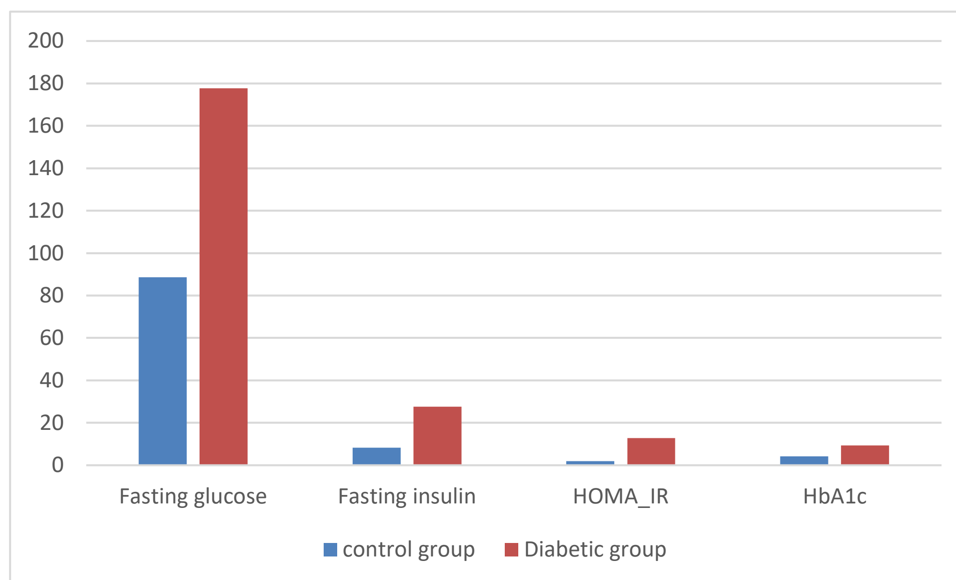


Figure (4.27) Illustrates Biochemical markers and HbA1c between control and diabetic groups .

4.3.6 Comparison of the Biochemical Markers and (tTG-IgA) between Control and Celiac Groups.

The present results demonstrated that the control group exhibited normal levels of tissue transglutaminase (1.76 ± 0.28 U/ml), along with normal fasting blood glucose (88.58 ± 7.08 mg/dL), fasting insulin (8.33 ± 0.89 µU/ml), and insulin resistance index (1.86 ± 0.23).In contrast, the celiac disease group showed a marked elevation in tissue transglutaminase levels, reaching (18.4 ± 3.78 U/ml). This increase was accompanied by metabolic alterations, including a slight rise in fasting blood glucose (90.38 ± 4.19 mg/dL), a pronounced increase in fasting insulin levels (16.73 ± 11.17 µU/ml), and a higher insulin resistance value (3.74) table (4.9) and figure (4.28).

Table (4.9) Illustrates Biochemical markers and tTG-IgA between control and celiac groups .

parameters	Control group	Celiac group
Fasting glucose	88.58± 7.08[a]	90.38 ± 4.19[b]
Fasting insulin	8.33 ± 0.89 [a]	16.73 ± 11.17[b]
HOMA_IR	1.86 ± 0.23 [a]	3.74 ± 2.66 [b]
tTG-IgA	1.76 ±0.28 [a]	18.4 ± 3.78 [b]

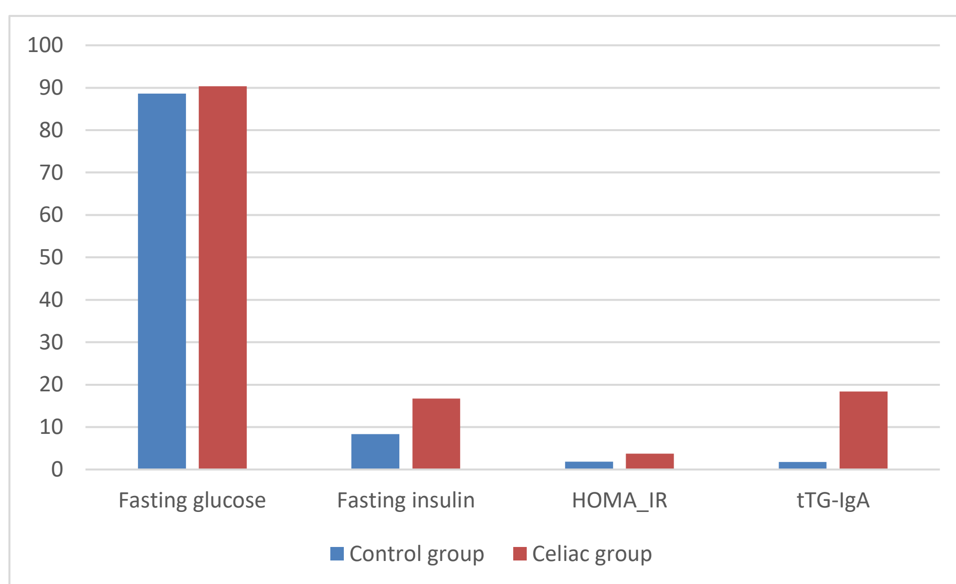


Figure (4.28) Illustrates Biochemical markers and tTG-IgA between control and celiac groups.

4.3.7 Comparison of the Biochemical Markers and BMI between Control and Obesity Groups.

The results of the current study for the control and obesity groups showed that fasting glucose in the control group was (88.58± 7.08 mg/dL) , while in the obese group it was slightly high at (93.44 ± 10.19 mg/dL). Fasting insulin in the control group was (8.33 ± 0.89 µU/ml) and in the obese group it was high at (26.83 ±

12.37 µU/ml). Insulin resistance was (1.86 ± 0.23) in the control group, while it was high for the obese group at (6.44 ± 3.43) compared to the control group. Thus, we note that the normal levels of glucose, insulin and insulin resistance correspond to a body mass index of (22.09 ± 2.37 Kg/m^2) in the control group. In the obese group, the increase in fasting glucose, insulin and insulin resistance corresponds to an increase in the body mass index at (32.41± 2.46 Kg/m^2). as shown in table (4.10) and figure (4.29)

Table (4.10) Illustrates Biochemical markers and BMI between control and obesity groups.

parameters	Control group	Obesity group
Fasting glucose	88.58± 7.08^{a}	93.44 ± 10.19^{b}
Fasting insulin	8.33 ± 0.89^{a}	26.83 ± 12.37^{b}
HOMA_IR	1.86 ± 0.23^{a}	6.44 ± 3.43^{b}
BMI	22.09 ± 2.37^{a}	32.41± 2.46^{b}

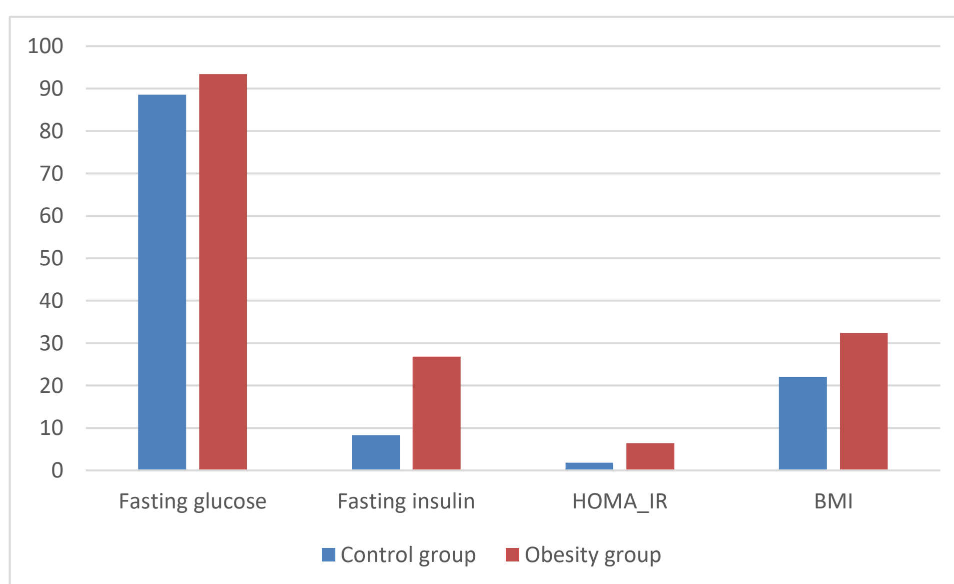


Figure (4.29) Illustrates Biochemical markers and BMI between control and obesity groups.

4.3.8 Relationship between Nuclear Morphological Changes and HOMA_IR in Different Groups.

The results of the study showed that the Morphological changes in the nuclease control group with a significant decrease in insulin resistance compared to the other groups, as the insulin resistance was 1.86, in contrast Morphological changes in the diabetes group were high, as well as insulin resistance by 12.75 compared to the control group. As for the celiac group, the Morphological changes and insulin resistance were recorded to be lower than in the diabetes and obesity group as the insulin resistance was 3.74 and the obesity group was the group with the highest increase in Morphological changes with insulin resistance by 6.44, table (4.11).

Table (4.11) Illustrates insulin resistance and nuclear Morphological changes in different groups.

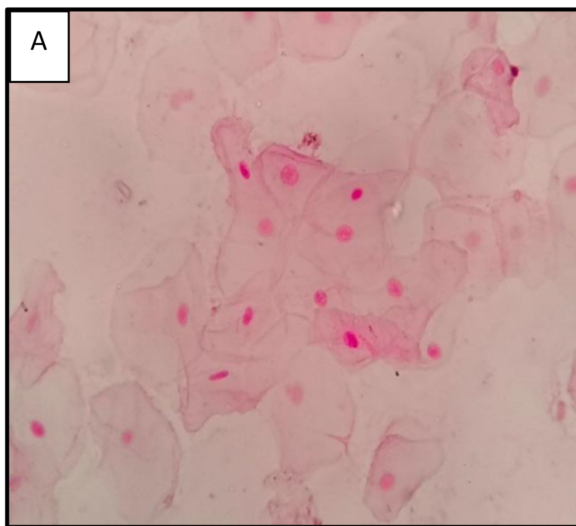
changes	Control group		Diabetic group		Celiac group		Obesity group	
	Mean	HOMA_IR	Mean	HOMA_IR	Mean	HOMA_IR	Mean	HOMA_IR
karyolytic	1.5[a]	1.86 [a]	5.50[a]	12.75[b]	4.12[a]	3.74[b]	8.41[a]	6.44[b]
pyknotic	3.09 [a]	1.86[b]	5.87[a]	12.75[b]	7.37[a]	3.74[b]	9.45[a]	6.44[b]
karyorrhexis	2.22 [a]	1.86[b]	6.91[a]	12.75[b]	4.87[a]	3.74[b]	5.76[a]	6.44[b]
binucleation	0.87 [a]	1.86[b]	2.09[a]	12.75[b]	2.70[a]	3.74[b]	1.28[a]	6.44[b]
micronucleation	1[a]	1.86[b]	1.55[a]	12.75[b]	2.59[a]	3.74[b]	3.90[a]	6.44[b]

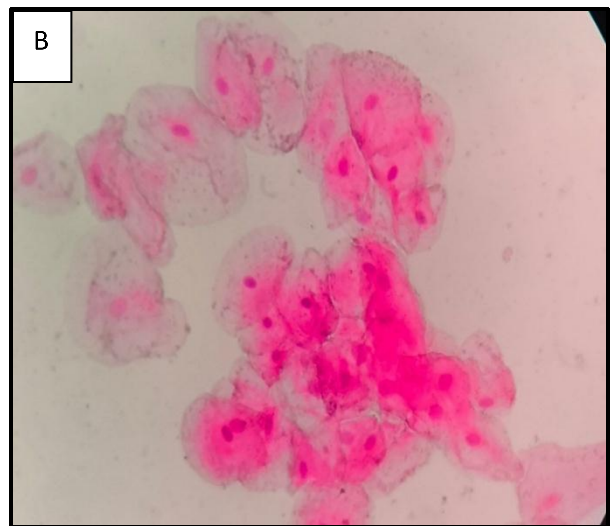
- Different small letters represent significant differences ($P \leq 0.05$) between the groups.
- Similar small letters represent no significant differences between groups.

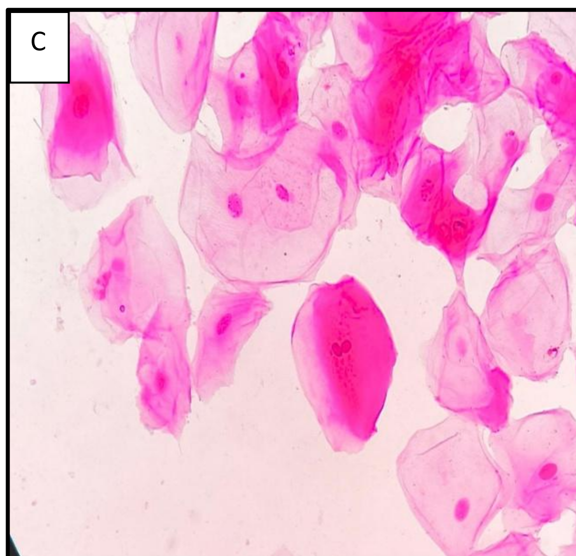
4.4 Results of Histochemistry

4.4.1Periodic Acid Schiff (PAS)

The results of the PAS staining for the four different groups showed variations in staining intensity, likely due to the accumulation of carbohydrates within the cells. In the control group, the cells weaken stain. In the diabetes group, the cells moderate stain, with varying degrees of intensity. In the celiac disease group, the staining ranged from pink to dark purple, while in the obesity group, the cells stained consistently dark purple. Figure (4.30)







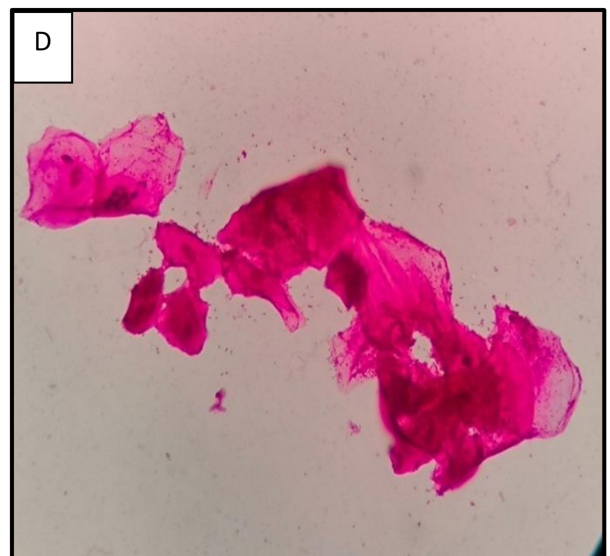
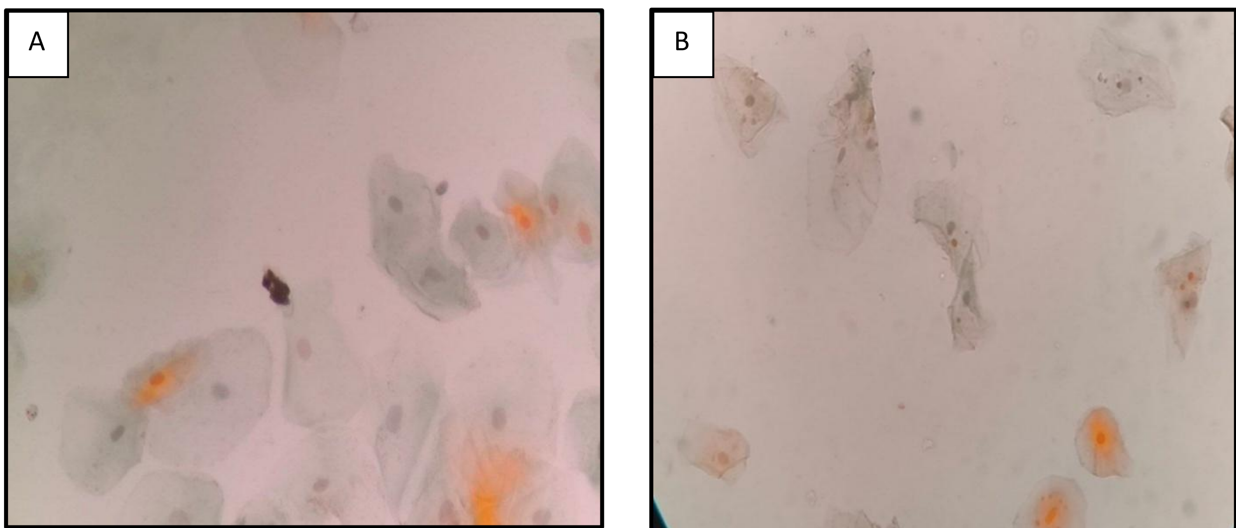


Figure (4.30) Buccal smear of the control group, A showed weak PAS staining intensity, B showed moderate PAS staining intensity in diabetic group, C showed moderate PAS staining intensity in celiac group and D showed high PAS staining intensity in obesity group (PAS stain, ×400).

4.4.2 Papanicolaou (PAP)

The results of the Papanicolaou staining for the four different groups showed a difference in the intensity of cell staining. In the control group, the cells were stained green blue, which is the expected color for cells taken from the cheek, as this tissue is non-keratinized stratified squamous epithelium. In the diabetes group, the cells also stained green blue, but with a lower intensity than in the control group. In the celiac group, the percentage of cells stained orange more than blue to green. Finally, in the obesity group, the cells stained more orange than green blue. as shown in Figure (4.31)



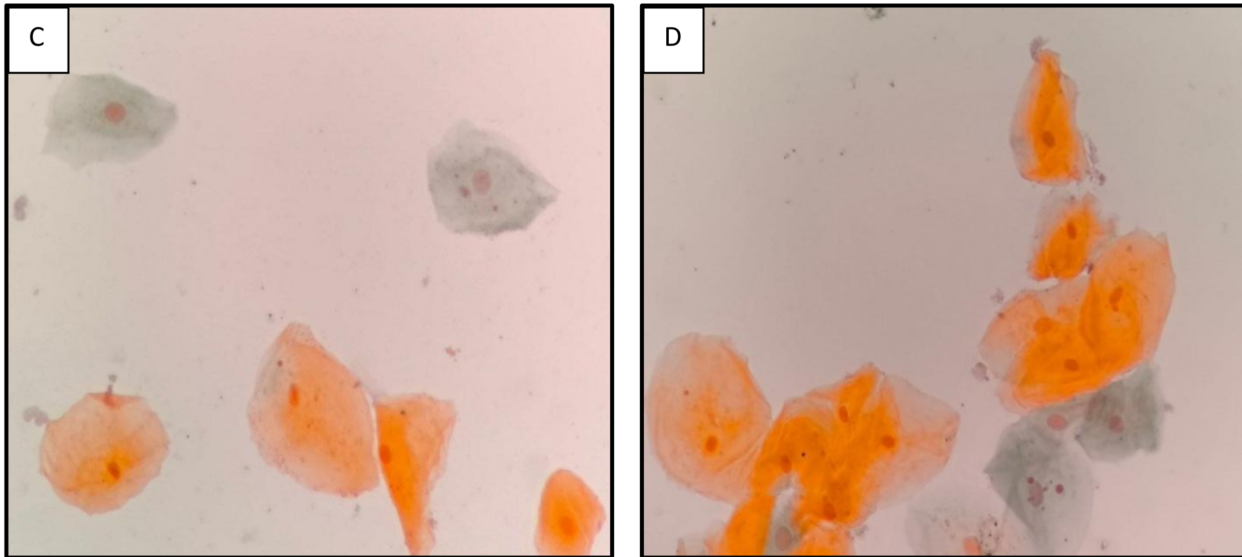


Figure (4.31) Buccal smear of the control group, (A) PAP staining intensity and the cells were stained blue to green, (B) showed PAP staining intensity and the cells were stained from blue to green in diabetic group, (C) showed PAP staining intensity and the cells were stained orange more than blue to green in celiac group and (D) showed PAP staining intensity and the cells were stained orange more than blue to green in obesity group (PAP stain, ×400).

4.5. Results of Transmission Electron Microscopic

_After staining with lead citrate and uranyl acetate (size bar = 500 nm), ultrastructural analysis of subsarcolemmal and intermyofibrillar mitochondria in control group showed distinct, homogeneous cristae. The findings showed that the mitochondria in control group operate normally using the JEOL JEM-1400 Flash with a STEM detector as illustrated in Figure (4.32)

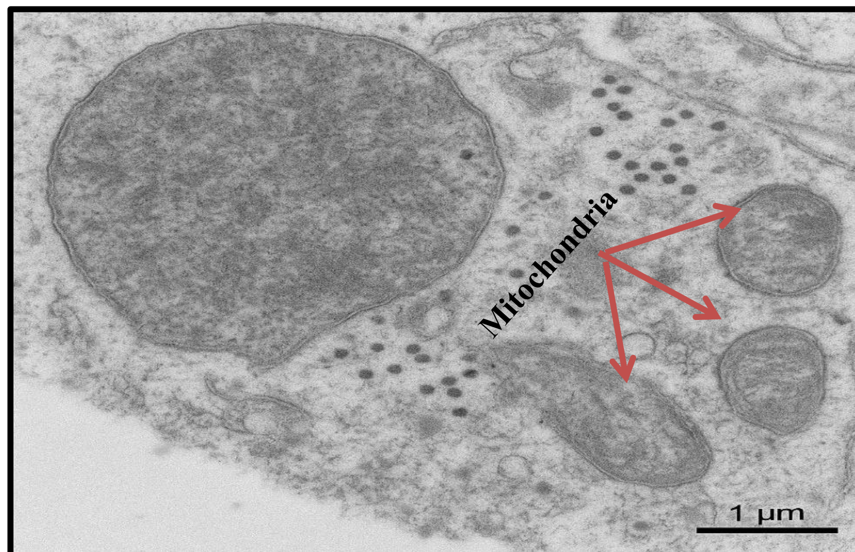


Figure (4.32) Transmission electron microscopy images, post-stained with lead citrate and uranyl acetate, revealed mitochondria with well-preserved, homogeneous cristae. The mitochondria appeared structurally intact and exhibited a normal distribution within the cytoplasm.

_Ultrastructural characteristics of buccal smear in patients with metabolic diseases (type2 diabetic, celiac and obesity) showed a few abnormalities, including hypertrophic epithelial cells, damaged outer mitochondrial membranes, and absence of mitochondrial cristae .There was a noticeable disorder and decrease in the quantity of mitochondrial cristae (scale bar = 500 nm). Using JEOL JEM-1400 Flash equipped with a STEM detector, the findings demonstrated that people with metabolic disorders had malfunctioning, degraded mitochondria. as shown in Figure (4.33)

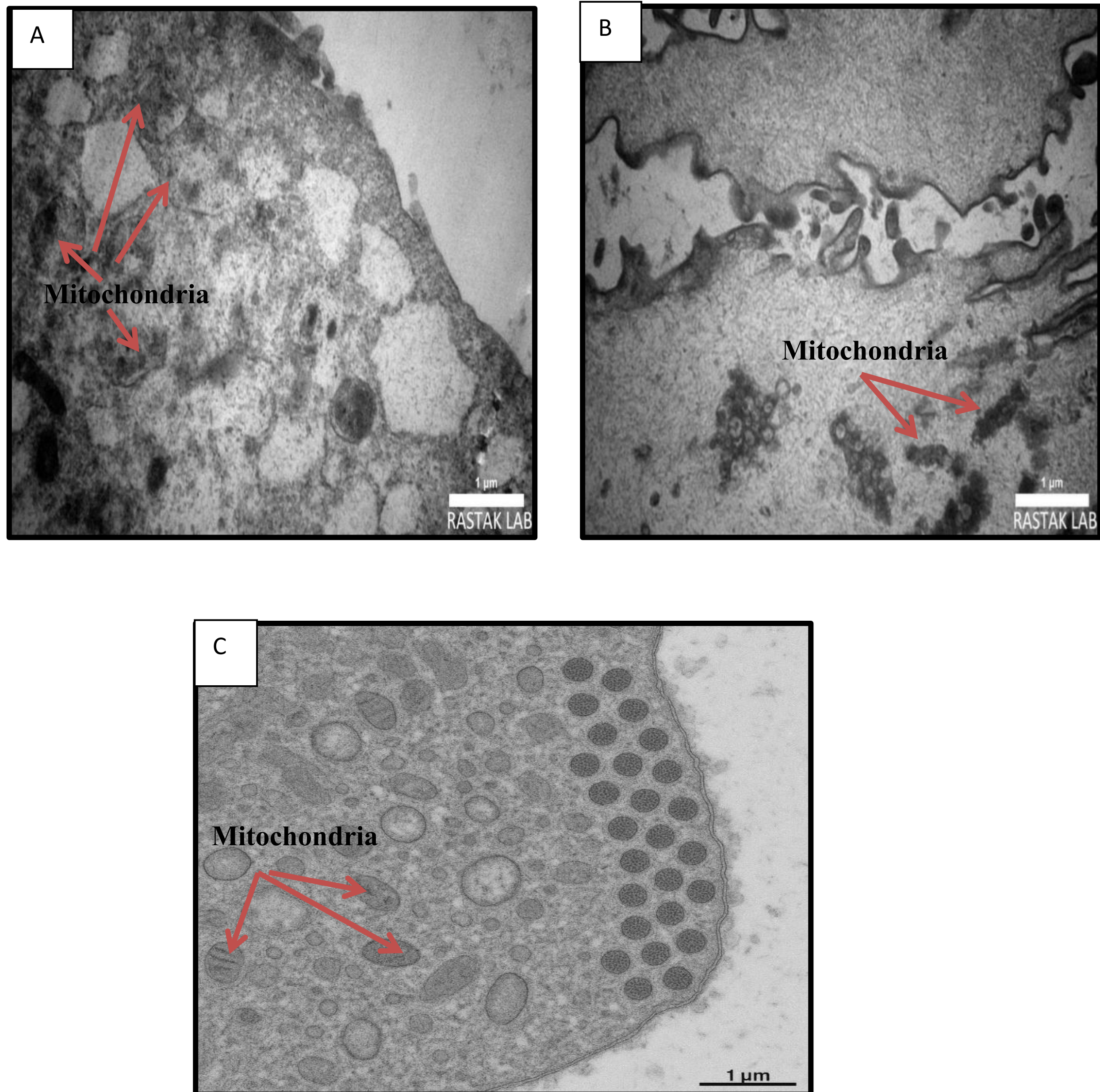


Figure (4.33) Transmission electron micrograph of buccal epithelial cells mitochondria showing degenerative changes in diabetic (A), celiac (B) and obesity (C) groups. Method of staining: Uranyl acetate/ Lead citrate, Bar:1µm.

Chapter Five

Discussion

5.1 Discussion Cytomorphometrical Study

5.1.1 Cell Counts

The results of the current study showed clear significant differences in the cell count taken through the buccal smear of the four groups, as the stained slides were analyzed with each of the stains of H&E, PAS and PAP stains. The control group recorded the highest mean cell count, which reflects the normal activity of cell division and continuous renewal of the oral epithelium. This agrees with what was stated in the study of Rappaport (1986) who reported that cell renewal of the oral epithelium is carried out with high efficiency. As for the second group, represented by the type 2 diabetes group, it recorded a significant decrease in the cell count, which indicates that continuous hyperglycemia may affect the cell cycle and slow down and hinder cell division, which was indicated by (Suvarna *et al*.,2012). Regarding the third group, the celiac group, it showed a decrease in the average cell count, this was because of gluten effect on the mitotic activity in the cells of the oral cavity. The findings align with those of Lähteenoja *et al*., (2000), who administered gliadin, a gluten derivative, via injection into the submucosal region of the oral cavity. This approach resulted in a notable polarization of lymphocyte numbers, without a corresponding rise in Ki_67, a nuclear protein that serves as a marker for cell proliferation and is expressed exclusively in dividing cells. This indicates that the cellular response that occurs after exposure of cells to gluten peptides in the mouth does not include dividing activity, and therefore cell renewal is slow or incomplete, which affects the growth of the oral epithelium and in the obese group, there was a significant decrease in the cell count, as the average number reached 299, this is due to poor perfusion, as scientific evidence indicates the existence of a vicious cycle of progressive dysfunction of the microvasculature, which plays a crucial role in insulin resistance (Vincent *et al.,* 2004). It has been proven that the function of the blood vessels is negatively affected by obesity, which leads to a chronic state

of vasculitis, which in turn leads to increased levels of proinflammatory cytokines, especially tumor necrosis factor alpha, as the latter works to inhibit the recruitment of microcapillaries in the skin and reduces insulin sensitivity, thus leading to poor perfusion in peripheral tissues and insulin resistance Additionally, obesity leads to increased levels of fat mass and a prolonged elevation of free fatty acids in the blood. This, in turn, causes vascular damage, impairs microcapillary recruitment, and ultimately leads to impaired cellular circulation and regeneration, this may be the scientific explanation for the decreased cells in buccal smear samples in obese individuals (Verma & Hussain,2017). As this study is consistent with what was stated in the study by Tandon *et al.,* (2022)

According to the results of our research, the cell counts decrease as the age group increases. In individuals with metabolic disorders, age plays a significant role in determining the efficiency of cell regeneration. Because the impact of disease on cellular function increases with age, and our tissues' ability to repair declines, older adults are more susceptible to cellular and tissue problems than younger adults. According to López-Otín *et al.*, (2013), senescent cells, which are cells resulting from disrupted division, accumulate and begin releasing inflammatory substances that damage surrounding tissues with age. This condition is exacerbated by metabolic diseases, which impede cell regeneration and promote chronic inflammation. In diabetes individual’s Chronic hyperglycemia has been shown to increase oxidative stress and free radical generation, which hinders DNA repair and regular cell cycles (Brownlee, 2005). Compared to younger individuals, older adults have weakened defenses against oxidative stress, which exacerbates the disease's effects on cell regeneration, especially in tissues like the skin, blood vessels, and mouth lining. This is consistent with (Cicmil *et al*., 2018 ; Ogura & Fukatsu, 2019) those who have demonstrated a negative effect of glucose on cell differentiation and renewal in the oral epithelium.

In celiac disease, damage from gluten intake can affect cell turnover and renewal in the intestinal mucosa, causing an immune response that may extend beyond the intestine, affecting cells in the oral cavities and causing slow or decreased cell turnover and decrease in numbers of the cells (Green & Cellier, 2007). Inflammatory mediators, including TNF-α and IL-6, secreted by adipose tissue in obesity, have been shown to affect cell division and inhibit cell regeneration in muscle, pancreatic, and liver cells the effects of obesity become more pronounced at the cellular level with age, due to decreased immune system effectiveness and insulin sensitivity. The oral epithelium may be affected as a result (Grigor & Hotamilgil, 2011).

5.1.2 Cytomorphology Study

The observed increase in average nucleus diameter in the diabetic group can be explained by the fact that the nucleus contains genomic DNA, histones, and several proteins. Therefore, the diameter of the nucleus can change depending on its DNA and proteins. There is usually a double amount of protein compared to DNA in the nucleus (Bibbo & David, 2008). On the other hand, hyperglycemia causes a compensatory increase in the amount of insulin secreted by pancreatic beta cells, which in turn leads to increased protein synthesis. These factors may explain the increased nucleus diameter observed in diabetic patients (Heddle *et al.,*1983).

Regarding the decreased cytoplasmic diameter, the explanation for this may be due to dehydration caused by increased blood glucose, which leads to frequent urination and thirst. This hinders the transport of glucose across cell membranes, leading to increased osmotic pressure, which causes water to be transported from inside the cells to outside them. This explains the decreased cytoplasmic diameter in diabetic patients (Rani & Yadav, 2015). These factors are also the reason behind the increased ratio of nucleus to cytoplasmic diameter in the diabetic group. These

results are consistent with Jajarm *et al*., (2008) who did not observe significant differences in nucleus size between groups, however, this is not consistent with Alberti *et al.,* (2003) who found a significant increase in nucleus diameter in diabetic patients, and Prasad *et al*., (2010) who indicated that increased ND was associated with the severity of diabetes as measured by HbA1c.

In a study conducted by cervino *et al.,* (2018), which studied both changes in saliva and tissue changes at the cellular level, the results of this study showed that gum infection is inversely proportional to the rate of saliva flow resulting from malnutrition. We conclude from this that it is possible that saliva deficiency has a role in the changes that occur at the cellular level. This leads to dry mouth, which in turn causes hypoxia, which leads to the appearance of aphthous ulcers resulting from inflammation due to increased activity of cytokines, including interleukin-2 (IL-2), which affects the growth of the oral epithelium, and where it was found that its level is increased in patients with celiac disease as a result of chronic inflammation resulting from its overactivity, causing atrophy affects the oral tissue (Sanchez-Solares *et al*., 2021) , the patients with celiac disease suffer from folic acid and B12 deficiency as well as calcium deficiency and all these factors may be the cause of the changes in the diameter of the nuclei and cytoplasm (Postek *et al*., 2009 ; Bao *et al*., 2018). Also, a significant decrease in the intercellular junctional protein was observed in intercellular junctional protein in patients with celiac disease, which indicates damage to the oral mucosa (Lähteenoja *et al*., 2000). These reasons as the cause of the changes in nucleus diameter and cytoplasm diameter in this study, as there were not enough studies referring to the Cytomorphometric changes of the oral cavity in patients with celiac disease.

Obesity is a condition associated with metabolic and inflammatory regulatory disorders at the cellular level. Obesity causes an increase in the accumulation of fat in fat cells, leading to excessive production of free radicals and oxidative stress. This in turn causes damage to the cells' DNA and disruptions in its repair. This leads to the accumulation of DNA damage in the cells (Rhodus, 1990; Kantovitz *et al*., 2006).

On the other hand, DNA damage in fatty cells and the pancreas contributes to the emergence of problems, including insulin resistance and chronic inflammation (Vergoni *et al*., 2016). Inflammation, in turn, increases the production of cytokines that work to gradually narrow blood vessels, causing tissue damage due to poor perfusion resulting from the decrease in saliva flow, which is a result of malnutrition, as confirmed by a study (Johansson *et al.,* 1992 ; Tandon *et al*., 2022), this may be reflected in the tissues of the oral cavity, as obesity may lead to functional malnutrition, such as a deficiency of essential vitamins and minerals (Flink *et al.,* 2008) .These factors may be the reason for the slight increase in nucleus diameter as well as the decrease in cytoplasmic diameter and for the lack of sufficient studies on obesity and its effect on the cell, especially nucleus diameter and cytoplasmic diameter, except for one study that included people with diabetes and obesity together, the study of Tandon *et al*., (2022), which was consistent with our current study.

5.2 Morphological Changes of Nucleus

The results indicated the appearance of morphological changes in the nucleus, indicating programmed death, such as the presence of micronucleation and a binucleation, the appearance of which is due to several factors, such as oxidative stress, which plays a role in destroying DNA by releasing free radicals that cause an increase in the levels of ROS, causing oxidative damage to DNA and thus causing breaks in the DNA, so the micronucleus appears. In addition,

binucleation results from cell fusion or abnormal mitosis, which is the result of errors that occur during division, such as cytoplasmic division, chromosomal instability, and cellular stress (Abukhalil *et al*.,2021; Abdalwahab *et al*., 2025).

The occurrence of morphological changes in the buccal smear in patients with type 2 diabetes is the result of several factors, represented by age, genetics, diet and lifestyle, as well as cellular factors represented by a lack of nutritional supplies. In addition, high blood sugar, the result of advanced glycation, which is considered the main cause of type 2 diabetes, contributes to cell aging, as aging cells show a decrease in proliferation and a decrease in average lifespan. Therefore, aging cells show morphological changes represented by karyolysis, pyknosis, karyorrhexis, as well as binucleation and micronucleation.

Sahay *et al*., (2017) found that morphological changes in oral epithelial cells could be assessed using exfoliative cytology. These changes were associated with hyperglycemia and various factors related to age, environment, lifestyle, etc.

The results of the study indicated an increase in the indicators of programmed cell death in the celiac group, this may be attributed to the activity of the TTG enzyme, which participates in the process of programmed cell death in the intestinal cells, which is considered an early damage to them. Because the oral and intestinal dorsal cells are similar in structure and function, only the immune response and histological abnormalities spread to the oral cells, leading to histological changes indicating early cell death, represented by karyolytic, pyknotic, karyorrhexis, binucleation, and micronucleation.

Farrace *et al*., (2001) found that TTG leads to the stimulation of programmed cell death in the intestinal cells, as it is considered an intracellular enzyme involved in diseases characterized by the stimulation of programmed cell death.

The marked hyperglycemia observed in obese individuals, and the cellular and nuclear stress associated with obesity and the cytokines resulting from

inflammation play a role in stimulating morphological changes at the level of oral cells. The process of nuclear abnormalities occurring during the process of nuclear division also plays a major role in the emergence of these changes, represented by karyolysis, pyknosis, karyorrhexis, binucleation, and micronucleation, all of which are considered mechanisms of programmed cell death, as programmed cell death is associated with the removal of cells that are likely to be malignant, as well as hyperplasia and disease progression. The process of programmed cell death also contributes to preventing the development of aneuploidy and other genetic abnormalities. LaPorte *et al.,* (2008) proposed that cytokine interactions with the extracellular receptor modulate intracellular signaling events at the proximal membrane.

5.3 Biochemical Markers

Insulin resistance is represented by the lack of cells response to insulin due to the weakness of insulin receptors in those cells. In the case of type 2 diabetes, chronic high blood sugar levels are caused by weak insulin secretion or action. As a result of this chronic elevation, the pancreatic beta cells try to secrete more insulin to get rid of excess glucose, thus weakening the function of beta cells in secreting insulin, causing insulin resistance resulting from glucose toxicity. In a study by Del Prato (2009), it was indicated that the weakness of insulin secretion may, over time, cause a gradual decline in the function of pancreatic beta cells, and thus the occurrence of insulin resistance resulting from a dysfunction in beta function.

In a study by Krebs & Roden (2005) the type 2 diabetes patients' insulin resistance can be attributed to mitochondrial malfunction, which results in a lack of lipolysis inhibition. The increase in fatty acid releases that results from fatty acid impairment of insulin sensitivity in the muscle and liver may lead to insulin resistance in individuals with type 2 diabetes. Consequently, inflammatory

cytokines linked to an increase in fat mass are released by fat cells. In the case of obesity, because of a high body mass index and an increase in visceral fat, and due to the weak response of tissues to the action of insulin, this leads to a deficiency in the process of inhibiting lipolysis, which leads to the release of more free fatty acids, causing insensitivity to insulin in both the liver and skeletal muscles. Consequently, a state of inflammation occurs resulting from the increased secretion of cytokines from the fat mass, causing insulin resistance. Barazzoni and his group demonstrated (2018) that altered adipose tissue function plays a key role in causing the disease once fat accumulates, and that insulin sensitivity is linked to several changes, including redox balance, oxidative stress, and inflammation.

People with celiac disease may suffer from insulin resistance or the beginning of insulin resistance, because a gluten-free diet is rich in sugars, proteins, saturated fats, and complex carbohydrates. The higher glycemic index of gluten-free foods compared to foods containing gluten may be the reason for the emergence of insulin resistance in celiac patients. The study is agreed with a study conducted by Norsa and colleagues (2013), which found that 3.5% of 20 children following a gluten-free diet suffered from resistance, while Zanini and colleagues (2013), indicated that insulin resistance remained stable from the beginning of the study, which he conducted on youth and adults, and even after follow-up. Changes in mitotic activity play an important role in metabolic diseases, as they affect tissue regeneration. A defect in insulin secretion causes insulin resistance, which in turn affects cell growth and division, and affects tissues. Thus, morphological changes and changes in cell numbers can be used to predict insulin resistance. Results of this study agreed with Huang *et al*., (2016) were found that high insulin concentration contributes to reducing division, increasing cellular stress, and stimulating programmed cell death.

5.4 Histochemistry

Buccal smears showed differences in cell color between the diseased and control groups. Carbohydrate accumulation is a functional change in the metabolic activity of epithelial cells. There are several possible causes for the accumulation of carbohydrates, especially glycogen and keratin, in diseases related to metabolic processes, including a defect in glycogen phosphorylation due to a deficiency in glucose-6-phosphate dehydrogenase enzymes. Insulin resistance also plays a role, as cells are unable to introduce glucose, which accumulates in the blood and in other cells in the form of excess glycogen. Other possible causes are chronic inflammation that affects cell renewal and turnover, as well as mineral and nutrient deficiencies, which play a role in carbohydrate metabolism. Sharif *et al.,* (2017), Hallikerimath *et al*., (2011), Agrawal *et al*., (2016) reported that glycogen accumulation is associated with hyperplasia, DeFronzo *et al*., (2015) reported that insulin resistance plays a role in the accumulation of carbohydrates in cells.

5.5 Transmission Electron Microscopic

Clear structural alterations were found when mitochondrial cells taken from diabetes individuals were analyzed. These included the formation of vacuoles or changed mitochondrial morphology, partial or whole loss of the inner crest (cristae), and mitochondrial swelling. These alterations show that the cell's ability to produce energy and respire is compromised due to mitochondrial stress brought on by persistently high glucose levels, the findings show that while inner crest loss indicates a decline in mitochondrial respiration function, which lowers the cell's capacity to withstand the chronic oxidative stress linked to diabetes, mitochondrial swelling is a cellular response to make up for the decreased ATP production brought on by impaired inner crest efficiency (Kim & Roy, 2020 ; Wang *et al*., 2025).Oxidative stress brought on by the immune system's response to gluten causes changes in the mitochondria of those who have celiac disease,

including alterations in the density, shape, and swelling of the mitochondrial crest. Reduced energy production, and heightened vulnerability to programmed cell death are the results of this deficiency. Defects in the mitochondria's DNA are also thought to be the cause of the dysfunction (Malik & Czajka, 2013; Orlando *et al*., 2019).

Mitochondria were somewhat enlarged in obese people, and the internal crest's density and form were slightly reduced. Given the detrimental impacts of obesity, such as elevated blood sugar and fat levels that manifest as an increase in body mass, these alterations might mark the start of a structural and functional malfunction in the mitochondria. These effects therefore impact the mouth in addition to the fatty tissue, Reduced inner crista density indicates compromised mitochondrial respiration, whereas mitochondrial enlargement represents the cell's attempt to make up for the decreased ATP generation brought on by inner crista malfunction. These modifications could cause oral cells to experience more oxidative stress, which would impair their capacity to react to long-term metabolic stress. Since mitochondrial dysfunction lowers a cell's efficiency in energy synthesis and glucose metabolism, it contributes to the aberrant metabolic state associated with obesity. These alterations are directly connected to insulin resistance (de Mello *et al*., 2018 ; Woo *et al*., 2019 ; Xia *et al*., 2024).

Chapter Six

Conclusions

&

Recommendations

6.1 Conclusions

1. This study indicated the possibility of using buccal epithelial cell count to determine whether an individual is suffering from a metabolic disease through analysis of buccal smear.
2. It cell count method is considered a safe, non-surgical, inexpensive method, and rapid compared to other diagnostic methods.
3. Cell count analysis provides useful information regarding the frequency of nuclear morphological alterations associated with cellular stress and degeneration.
4. The study highlights the potential value of cell count analysis as a predictive tool for insulin resistance. Although no statistically significant differences in insulin resistance were observed among the metabolic disease groups, all groups showed a clear decrease in all cell counts.
5. Increased oxidative stress and decreased cellular activity result from a disturbance of the mitochondria's energy-producing function, as evidenced by the identified mitochondrial changes in metabolic disorders. These alterations point to the crucial part mitochondria play in the development of metabolic diseases and raise the possibility that they may be used as an early biological marker.

6.2 Recommendations

1. Routine incorporates oral exfoliative cytology tests on a regular basis into clinical evaluations for metabolic disease patients to identify cellular alterations early.
2. Further studies should include larger sample sizes and long time periods to better evaluate cellular changes in relation to the type, severity and duration of metabolic diseases.
3. Using a transmission electron microscope to observe changes in the other cellular organelles and to evaluate their functional integrity and the extent to which they are affected by disease complications.
4. Experimental studies using laboratory animals and taking a tissue biopsy from the oral cavity to count the cells that undergo the stages of meiosis and programmed death and studying them more clearly.

References

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الخلاصة

هدفت الدراسة الحالية، باستخدام علم الخلايا التقشري، إلى دراسة التغيرات الفموية على المستوى الخلوي والجزيئي وعلاقتها بالأمراض الأيضية وتطورها. قُيّمت أعداد الخلايا في مسحات الخد، ودُرست تغيرات البنية الشكلية النووية والتغيرات الخلوية الشكلية ، وارتباطها بتغيرات مستويات الأنسولين، وسكر الدم الصائم، والسكر التراكمي، ومؤشر كتلة الجسم، ومضادات ترانسغلوتاميناز الأنسجة، ومقاومة الأنسولين. جُمعت العينات خلال الفترة من ديسمبر 2024 إلى مايو 2025 من مركز السكري والغدد الصماء، ومستشفى الحكيم التعليمي، والمختبرات الخاصة. بلغت العينة الكاملة (60) عينة من كلا الجنسين، تتراوح أعمارهم بين (20 - 60) سنة. تم صبغ العينات باستخدام أصباغ H&E و PAS وPAP، وقُسّمت إلى أربع مجموعات رئيسية (15 لكل مجموعة):

المجموعة الضابطة (الأصحاء)، مجموعة مرضى السكري من النوع الثاني، مجموعة حساسية الحنطة و مجموعة السمنة (مؤشر كتلة الجسم أكبر من 30 كغم/م^2).

أظهرت النتائج انخفاضًا معنويًا ($p \leq 0.05$) في عدد الخلايا عند مقارنة المجموعات المختلفة بالمجموعة الضابطة. كما لوحظ تغير في عدد الخلايا مع التقدم في السن، بالإضافة إلى وجود علاقة بين عدد الخلايا والمؤشرات البيوكيميائية.

أظهرت نتائج الدراسة الخلوية الشكلية عدم وجود زيادة معنوية ($p \leq 0.05$) في قطر النواة في المجموعات المختلفة عند مقارنتها بالمجموعة الضابطة، بينما أظهر قطر السيتوبلازم ونسبة النواة إلى السيتوبلازم زيادة معنوية ($p \leq 0.05$) في المجموعات المختلفة عند مقارنتها بالمجموعة الضابطة.

أما بالنسبة لنتائج دراسة تغيرات البنية الشكلية النووية، فهناك زيادة معنوية ($p \leq 0.05$) عند مقارنة نتائج المجموعات المختلفة بالمجموعة الضابطة، كما توجد علاقة بين هذه التغيرات والمعايير الكيميائية الحيوية.

وفيما يتعلق بالمعايير الكيميائية الحيوية، فقد كانت هناك زيادة معنوية ($p \leq 0.05$) في المجموعات المختلفة مقارنة بالمجموعة الضابطة. كما كشفت النتائج عن وجود اختلاف في شدة تلطيخ الخلايا في المجموعات المختلفة مقارنة بالمجموعة الضابطة.

يمكن مناقشة الجانب الخلوي من خلال تقييم تأثير مؤشر كتلة الدهون، ومستوى الجلوكوز، ومقاومة الأنسولين، ومضادات ترانسغلوتاميناز الأنسجة على دورة الخلية، وتجديد الأنسجة، والتغيرات البنية الشكلية الخلوية والنووية لدى كل من مجموعات مرضى السكري، وحساسية الحنطة، والسمنة.

بناءً على هذه الدراسة، نستنتج أن عدّ الخلايا الفموي تقنية منخفضة التكلفة، وغير جراحية، وآمنة، ويمكن استخدامها في التشخيص الأولي للأمراض الأيضية. كما أنها تساعد في التنبؤ بمقاومة الأنسولين وتقييم التغيرات في البنية الشكلية للنواة. وقد أظهرت جميع المجموعات المشاركة في الدراسة انخفاضًا ملحوظًا في عدد الخلايا.

الخلاصة

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

التحليل الخلوي التقشيري المقارن للغشاء المخاطي الفموي لدى المرضى المصابين بالأمراض الأيضية في محافظة ميسان

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

صدّيقة حسن جاوي

بكالوريوس كلية العلوم / علوم حياة (2022)

بإشراف

أ . د . علي خلف علي

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