# "NUCLEAR MORPHOMETRIC AND MORPHOLOGICAL STUDY OF EXFOLIATED BUCCAL MUCOSAL CELLS IN TYPE- 2 DIABETES MELLITUS PATIENTS."


**By**

**Dr. WAHENGBAM JYOTSNA DEVI**


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UNDER THE GUIDANCE OF


**Dr. B.R.YELIKAR** M.D,
PROFESSOR & HEAD
DEPARTMENT OF PATHOLOGY

&

Co –Guidance of

**Dr. S. S. DEVARMANI** M.D,
Professor Department of Medicine,

**B.L.D.E. UNIVERSITY'S SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE**

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Date:
Place: Vijayapur

**Dr. WAHENGBAM JYOTSNA DEVI**
Post graduate student
Department of Pathology
BLDEU Shri B.M.Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

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Date:

Place: Vijayapur.

**Dr. B.R.YELIKAR** M.D,

Professor and H.O.D,

Department of Pathology

BLDEU Shri. B.M.Patil Medical College, Hospital and Research Centre, Vijayapur, Karnataka.

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Date:

Place: Vijayapur.

**Dr. S.S.DEVARMANI** M.D,

Professor,

Department of Medicine

BLDEU Shri. B.M.Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

**B.L.D.E UNIVERSITY'S**

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## ENDORSEMENT BY THE HEAD OF DEPARMENT

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Date:

Place: Vijayapur.

**Dr. B.R.YELIKAR** M.D,

Professor and H.O.D,

Department of Pathology,

BLDEU Shri.B.M.Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

**B.L.D.E UNIVERSITY'S**

**SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPUR**

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Date:

Place: Vijayapur.

**Dr. S. P. GUGGARIGOUDAR**
Principal,
BLDEU Shri.B.M.Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

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Date: **Dr. WAHENGBAM JYOTSNA DEVI**

Place: VIJAYAPUR

# ABSTRACT

**BACKGROUND**

Type 2 Diabetes Mellitus (DM) is a heterogeneous group of disorders characterized by variable degrees of insulin resistance and impaired insulin secretion. It causes secondary pathophysiological changes in various organs including the oral cavity. There are very few studies about the oral epithelial cell changes in Type 2 DM patients using advanced tools like MIPS (Magnified Image Processing System). Accordingly this study is planned.

**OBJECTIVE:**

To study the nuclear morphometry and morphology of exfoliated buccal mucosal cells in normal subjects and in Type 2 DM patients by Magnified Image Processing System (MIPS).

**METHODS:**

A cross sectional hospital based study was conducted on 50 diagnosed Type 2 Diabetes Mellitus patients attending outpatient 'Diabetic clinic' and age and sex matched 50 control group who were healthy individuals with no risk of diabetes from 1st November, 2015 to 30th June, 2017 keeping in view the inclusion and exclusion criterias.

The diabetic patients were selected according to the American Diabetic Association criteria. The Fasting plasma glucose levels (FPG), Two-hour plasma glucose levels (PP) and $HbA_{1c}$ levels for the study groups were evaluated. After an informed consent, smears were collected from the clinically normal appearing sites of the buccal mucosa and from different sites to reduce the effect of localized infections.

All the smears were made using a sterile wooden spatula with gentle scraping motion and immediately fixed in 95% ethyl alcohol for about 30 minutes and later stained with Papanicolaou (PAP) staining method in the laboratory.

The well prepared smears were then screened under the light microscope for nuclear and cytomorphometric changes.

The cytomorphometric evaluation of the selected PAP stained smears were performed by MIPS (Magnified Image Processing System) calibrated with ZEN Software analysis DIZIMIZER Image analyzer 0.4.3 version.

An average of 20 clearly defined cells were selected manually and the parameters evaluated were Nuclear Area (NA), Cytoplasmic Area (CA) and Nuclear Cytoplasmic ratio (NCR).

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square ($\chi^2$)/ Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means of analysis variables between two independent groups was tested by unpaired t test. The difference of the means of analysis variables between more than two independent groups was tested by ANOVA and F test of testing of equality of Variance. Normal linear regression analysis was employed to assess the adjusted effect of determinants of continuous study variable. If the p-value was $< 0.05$, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0. and Microsoft office.

**RESULTS:**

The average age of presentation of Type 2 Diabetes mellitus was 54.9±11.1 years. The cytomorphometric parameters, NA and NCR were significantly increased in Type 2 DM patients when compared to non-diabetic healthy group with NA (93898.9±41727.6 $\mu m^2$ vs 58126.3±13410.5 $\mu m^2$) and ($p\text{-value} < 0.001$) and NCR (0.2±0.2 vs 0.1±0.1) and ($p\text{-value} < 0.001$).

However, the CA ratio showed a decline in diabetic group when compared to non-diabetic (455099.9±122960.8 $\mu m^2$ vs 630153.4±349161.9 $\mu m^2$) and p-value = 0.001).

A significant statistical correlation was found between $HbA_{1c}$ values and both average NA and NCR in the diabetic group.

**CONCLUSION**

The cytomorphometric evaluation of the exfoliative buccal mucosal cells using NA, CA and NCR alterations can serve as an adjunct and a reliable non-invasive technique for screening of Type 2 diabetes and evaluation of the glycemic control in known diabetics.

These findings may help to substantiate the findings of similar studies.

**KEY WORDS:** Exfoliated buccal mucosa, type 2 diabetes mellitus, cytomorphometry, papanicoloau stain.

# LIST OF ABBREVATIONS USED

| | |
|---|---|
| WHO | World Health Organization |
| ADA | American Diabetic Association |
| IDF | International Diabetic Federation |
| DM | Diabetes mellitus |
| NA | Nuclear area |
| CA | Cytoplasmic Area |
| NCR | Nuclear Cytoplasmic Ratio |
| MIPS | Magnified Image Processing System |
| FBS | Fasting Blood Sugar |
| PPBS | Post Prandial Blood Sugar |
| $HbA_{1c}$ | Glycosylated hemoglobin |
| MODY | Maturity Onset Diabetes of the Young |
| GAD | Glutamic acid decarboxylase autoantibodies |
| IGT | Impaired Glucose Tolerance |
| IFG | Impaired Fasting Glucose |
| OGTT | Oral Glucose Tolerance Test |
| AGEs | Advanced Glycation End Products |
| GLUT4 | Glucose Transporter Type 4 |

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# INTRODUCTION

Diabetes mellitus encompasses a diverse group of metabolic disorders with the characteristic prominent feature of hyperglycemia.

It is a rapidly expanding metabolic disease which has the capacity to weaken the health services and has an immense impact in the socio economic development globally.[1] In the year 2009 World Health Organization (WHO) estimated 171 million people with Diabetes mellitus (DM), a figure implicated to increase to 366 million by 2030.[2]

It has further been projected that approximately 79.4 million Indians will be affected by diabetes mellitus by the year 2030.[3]

The WHO defines diabetes as a metabolic disorder with multiple aetiolgy characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, action or both. The effects of which lead to long term damage, dysfunction and failure of various organ systems in the body.[4]

Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. Based on the pathogenic process leading to hyperglycemia, as opposed to previous criteria such as age of onset or type of therapy implicated. They include Type 1 Diabetes mellitus (Type1 DM), Type 2 Diabetes mellitus (Type2 DM), other specific types, and gestational diabetes. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production.[3,4]

The metabolic dysregulation associated with DM causes varied secondary pathophysiologic changes in multiple organ system including the oral cavity.[5]

The oral problems of DM are periodontal diseases, and many others which include oral dryness, taste alterations, increase in susceptibility to buccal infections such as candidiasis, increase in teeth decay and salivary gland impairment which leads to alterations in the buccal epithelial cells.[6,7]

These concomitant oral manifestations and the importance of dental care in diabetic patients provides an ability to look through the profound effect of metabolic derangement of the diabetes state. There are several methods to evaluate the oral mucosa with DM which includes biopsy as incisional or excisional but it is an aggressive procedure. [8-10]

In view of providing a greater patient comfort a new method to complement or compare the results which is less aggressive, simplified in nature and cost effective is the use of exfoliative cytology with advanced tools like MIPS (Magnified image processing system) calibrated with ZEN software analysis.

This can analyze images of the of the buccal mucosa cells morphometrically by using the DIZIMIZER Image analyzer 0.4.3 version for evaluation of the quantitative and qualitative changes in the epithelial cells of the oral mucosa in diabetes.

Hence, this study was conducted to determine the cytomorphometric changes using exfoliative cytology in oral epithelial cells from patients with Type 2 DM and normal subjects.

# OBJECTIVE OF THE STUDY

To study the nuclear morphometry and morphology of exfoliated buccal mucosal cells in Type-2 Diabetes mellitus patients and in normal subjects.

# REVIEW OF LITERATURE

## DIABETES MELLITUS:

Diabetes is a constellation of clinical syndromes sharing the common feature of hyperglycemia due to absolute or relative insulin deficiency. The chronic hyperglycemia and t metabolic derangements are associated with the long-term impairment, dysfunction and failure of various organ systems especially the kidneys, eyes, nerves and blood vessels. Diabetes often goes undiagnosed because many of its symptoms seem seemingly harmless. The major classical findings of DM are polyuria, polydipsia, polyphagia, weight loss and fatigue. The symptoms are not severe or may be absent, concomitant hyperglycemia sufficient to cause the pathological and functional changes may be present for long before a diagnosis is made. So an early diagnosis plays an important aspect for health care.[11]

CRITERIA FOR DIAGNOSIS FOR DIABETES: TABLE 1 [12,13]

(Source: According to the American Diabetic Association (ADA) 2014)

- Symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/L (200mg/dl) [a] *or*
- Fasting plasma glucose ≥7.0mmol/L (126mg/dl) [b] *or*
- $A_{1c}$ > 6.5% [c] *or*
- Two- hour plasma glucose ≥ 11.1 mmol/L(200 mg/dl) during an oral glucose tolerance test.[d]

---

[a] Random is defined as without regard to time since the last meal.

[b] Fasting is defined as no caloric intake for at least 8 hours.

[c] The test should be performed in laboratory certified according to $A_{1c}$ standards of the Diabetes Control and Complications Trial.

[d] The test should be performed using glucose load containing the equivalent of 75g anhydrous glucose dissolved in water, not recommended for routine clinical use.

## EPIDEMIOLOGY OF DIABETES:

Diabetes is one of the most common non communicable diseases in the world with a rapidly rising prevalence globally, where sadly India is nowhere behind. The current status of diabetes in India is grim as it is evolving as a potential epidemic, being referred the “diabetic capital of the world”. The WHO estimated that the number of persons with DM about 170 million and expected to rise to 366 million by 2030, mostly in developing countries.

The worldwide impact of diabetes is immense and around 85-95% of all cases are Type 2 diabetes. This increasing trend of type 2 DM in Indian context is due to the rapid urbanization leading to environmental and lifestyle changes. By the year 2030, approximately 46% of India’s population will be residing in urban areas. Based on the International Diabetic Federation (IDF), it is estimated that around 41 million Indians are diabetic and projected to rise to 70 million by the year 2025.[14] (Figure 1). Also based on a recent data it is estimated that nearly 79.4 million Indians will develop DM by 2030.[3]

It is considered as Indians are more prone to develop diabetes, the so called “Asian Indian Phenotype” which refers to certain unique clinical and biochemical abnormalities in Indians i.e., higher waist circumference despite low BMI, lower adiponectin and higher C-reactive protein levels.[15]

However, there exist a wide disparity in the prevalence of diabetes between urban and rural areas. It is estimated that the prevalence of diabetes in urban India was 12% in 2002 as compared to rural India which had a prevalence of 6.4%.[16]

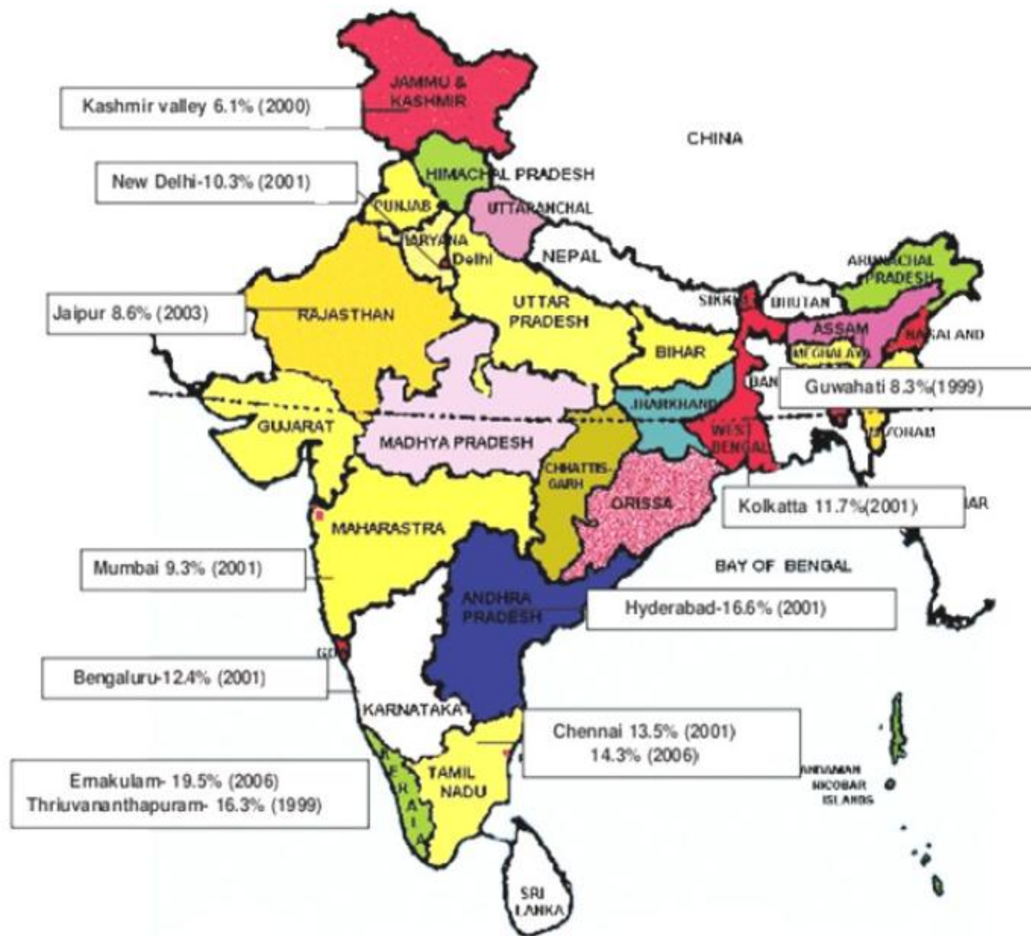



Figure 1: A region wise distribution of diabetes in India.[14]

Also the prevalence of Impaired Glucose Tolerance (IGT) is thought to be around 8.7% per cent in urban areas and 7.9 percent in rural areas. It is also estimated that around 34 per cent of IGT individuals eventually develop type 2 diabetes and hence India is facing a healthcare crisis.[17]

In the recent times studies have also noted a rapid conversion of the IGT to diabetes in the southern states of India, where the estimated prevalence of diabetes among the adults is around 20% in the urban populations and 10% in the rural populations.[18,19]

ETIOLOGICAL CLASSIFICATION OF DIABETES: [12,20] (Table 2)

I. Type 1 diabetes ( β- cell destruction, usually leading to absolute insulin deficiency)
- A. Immune mediated
- B. Idiopathic

---

II. Type 2 diabetes (ranges predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

---

III. Other specific types

A. Genetic defects of β-cell function
1. MODY 3(Chromosome 12, HNF-1α)
2. MODY 1(Chromosome 20, HNF-4α)
3. MODY 2( Chromosome 7, glucokinase)
4. Other very rare forms of MODY (eg., MODY4: Chromosome 13, insulin promoter factor-1; MODY 6: chromosome 2, NeuroD1; MODY 7: Chromosome 9, carboxyl ester lipase)
5. Transient neonatal diabetes (most commonly ZAC/HYAMI imprinting defect on 6q24)
6. Permanent neonatal diabetes (most commonly KNCJ11 gene encoding Kir6.2 subunit of β-cell $K_{ATP}$ channel)
7. Mitochondrial DNA
8. Others

B. Genetic defects in insulin action
1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendelhall syndrome
4. Lipoatrophic diabetes
5. Others

C. Diseases of the exocrine pancreas
1. Pancreatitis
2. Trauma/pancreatectomy
3. Neoplasia
4. Cystic fibrosis
5. Hematochromatosis
6. Fibrocalculous pancreatopathy
7. Others

D. Endocrinopathies

E. Drug or chemical induced
1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. β-Adrenergic agonists
8. Thiazides
9. Dilantin
10. γ-Interferon
11. Others

F. Infections
1. Congenital rubella
2. Cytomegalovirus
3. Others

G. Uncommon forms of immune-mediated diabetes
1. Stiff-man syndrome
2. Anti- insulin receptor antibodies
3. Others

H. Other genetic syndromes sometimes associated with diabetes
1. Down syndrome
2. Klinefelter syndrome
3. Turner syndrome
4. Wolfram syndrome
5. Friedreich ataxia
6. Huntington chorea
7. Laurence-Moon-Biedl syndrome
8. Myotonic dystrophy
9. Porphyria
10. Prader-Willi syndrome
11. Others

---

IV. Gestational diabetes mellitus

Patients with any form of diabetes may require insulin treatment at some stage of their disease.
Such use of insulin does not, of itself, classify the patient.

## TYPE 1 DIABETES MELLITUS:

Type 1 diabetes accounts for 5-10% of all cases of diabetes, which was previously referred as insulin dependent diabetes or juvenile onset diabetes is associated with cell mediated autoimmune destruction of the β-cells of the pancreas. However, insulin is required for the survival and to avoid the complications of diabetic ketoacidosis and coma. Immune destruction markers of the β-cell can be detected in 80-90% of these patients like islet cell autoantibodies, autoantibodies to insulin, autoantibodies to anti-Glutamic Acid Decarboxylase Autoantibodies (GAD).

### Idiopathic diabetes mellitus:

Some patients of type 1 diabetes are very much prone to develop ketoacidosis due to permanent insulinopenia but are not associated with autoimmunity. It most commonly affects individuals of Asian and African origin and it is strongly inherited. These patients develop episodic ketoacidosis which can be life threatening but there is no evidence of autoimmune destruction of the β-cells nor it is HLA associated. Nevertheless, there is a need of absolute insulin replacement therapy.[20]

## TYPE 2 DIABETES MELLITUS:

Around 90-95% of the diabetic patients have type 2 diabetes, previously regarded as non-insulin dependent diabetes, or adult-onset diabetes are considered in individuals with insulin resistance and relative insulin deficiency rather than absolute. The specific etiology is unknown however there is no associated autoimmune β-cell destruction. Majority of the patients with this form of diabetes are overweight and are obese, which causes some degree of insulin resistance. In non-obese persons by the traditional weight criteria it is associated with the increased percentage abdominal body fat distribution. Ketoacidosis is rare and is associated with concomitant stress of another illness such as infection.

Hyperglycemia in this form of diabetes develops gradually and in initial stages it is not severe enough for the patient to recognize the classic symptoms which goes undiagnosed for many years. The risk of developing of this form of diabetes increases with age, obesity and sedentary life style, however age is not a defining criteria anymore. The frequency is more especially in women with prior Gestational diabetes mellitus (GDM) and in individual with hypertension, dyslipidemia but its incidence varies with varied racial/ethnic subgroups.[21]

**GESTATIONAL DIABETES MELLITUS (GDM)**

Gestational diabetes is defined as any degree of glucose intolerance with onset or first recognition during pregnancy giving rise to variable degree of hyperglycemia. The definition is applied whether or not the condition persisted after delivery and does not strictly exclude the possibility of an unrecognized glucose intolerance which may have antedated or begun concomitantly with the pregnancy.

The International Association of the Diabetes and Pregnancy Study Groups, representatives of various obstetrical and diabetes organizations including the American Diabetic Association, recommended that high-risk women who are found to have diabetes at their initial prenatal visits, using standard criteria are labelled as overt, not gestational, diabetes.[22]

**OTHER SPECIFIC TYPES OF DIABETES:**

**Genetic defects of the β-cells**

The monogenetic defects in the β-cells function are associated with certain forms of diabetes. These are associated with an early onset (approximately around 25 years) which are referred as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin

action. Their inheritance pattern is autosomal dominant and are linked with certain abnormalities at the six genetic loci on different chromosomes.

The mutation at chromosome 12 in a hepatic transcription factor called hepatocyte nuclear factor (HNF)- 1α is the most common. A second form is the mutation in the glucokinase gene on Chromosome 7p resulting in defective glucokinase molecule. Glucokinase converts the glucose to glucose-6-phosphate which stimulates the β-cells to secrete insulin and it also serves as the glucose sensor for the β-cells.

These defects lead to the increased plasma glucose levels which are necessary for the normal levels of insulin secretion. The less common transcription factors mutation are HNF-4α, HNF-1β, insulin promoter factor (IPF)-1 and NeuroD1.

The neonatal diabetes is associated with the mutation of and defect in encoding the Kir6.2 subunit of the β-cell $K_{ATP}$ channel.[23]

**Genetic Defects in Insulin Action**

Some genetically determined abnormalities of insulin action are associated with certain rare causes of diabetes. The underlying metabolic defect are linked with the mutations of insulin receptors leading to hyperinsulinemia and modest hyperglycemia to severe diabetes.

The clinical presentation are variable with some patients presenting with acanthosis nigricans, women may be virilized and presented with enlarged cystic ovaries.

Two main pediatric syndromes worth mentioning are the Leprechaunism and the Rabson-Mendenhall syndromes which are associated with mutations in the insulin receptor gene resulting in insulin receptor functions alterations and extreme degree of insulin resistance.

These alterations in the insulin receptor and functions cannot be demonstrated in patients with insulin-resistant lipotrophic diabetes, as a result these lesions are assumed that they reside in the post receptor signal transduction pathways.[24]

**Diseases of exocrine pancreas:**

The diffuse injury of the pancreas by any mechanism can cause diabetes. Some of the acquired causes are pancreatitis, trauma, infections, pancreatectomy and pancreatic carcinomas. Apart from the carcinoma, the extensive injury of the pancreas is a must for diabetes to occur for example, adenocarcinoma of the pancreas involving only a part of it has been found to associated with diabetes which implies the mechanism of destruction rather than the simple reduction in the β-cell mass.
Extensive injury associated with cystic fibrosis and hemochromatosis are associated with β-cell destruction and decrease insulin secretion. Fibrocalculous pancreatopathy is associated with pancreatic calcification which are detected by X-ray examination.[25]

**Endocrinopathies:**

The insulin action is antagonized by various hormones example growth hormone, cortisol, glucagon, epinephrine with their respective syndromes associated with its excess secretions i.e. acromegaly, Cushing’s syndrome, glucagonoma, pheochromocytoma causes diabetes.

These occurs most commonly in those individual with underlying defects in insulin secretion and the associated hyperglycemia eventually resolve with the correction of the hormone excess.[25]

**Diabetes induced by Drugs or Chemicals:**

Insulin secretion is impaired by certain drugs and may precipitate diabetes in those individuals with insulin resistance. In such situations the typing is unclear as the sequence and the relative value of β-cell dysfunction and insulin resistance is unknown.

Some of the toxins worth mentioning are Vacor (a rat poison) and intravenous pentamidine which leads to permanent destruction of the β-cells. Nicotinic acid and glucocorticoids are also associated with impairment of insulin action. Recent studies have shown that the usage of α-inteferon have been reported to develop diabetes with the formation of islet-cell antibodies and insulin deficiency.[25]

**Infections:**

The viruses associated with β-cell destruction are coxsackievirus B, cytomegalovirus, adenovirus and mumps. It has also been found that diabetes occurs in patients with congenital rubella, although these patients show HLA and immune markers characteristic of type 1 diabetes.[25]

**Uncommon forms of Immune-Mediated Diabetes:**

In this context two conditions worth mentioning are Stiff-man syndrome which is an autoimmune defect characterized by axial muscle stiffness with painful spasms. These patients have high titers of GAD autoantibodies and one-third develop diabetes. Anti- insulin receptor antibodies causes diabetes by binding to insulin receptor, thereby blocking the insulin to bind to target tissues. These anti-insulin receptor antibodies are found in patients with systemic lupus erythematosus and in some autoimmune diseases.[26]

**Other genetic syndromes which are associated with diabetes:**

Certain types of genetic syndromes are associated with diabetes which include the chromosomal abnormalities of Down Syndrome, Klinefelter syndrome, and Turner syndrome. Another autosomal recessive disorder called Wolfram syndrome is associated with insulin deficient diabetes and are found to have absence of β-cells at autopsy studies.[26]

## PATHOGENESIS OF TYPE 1 DIABETES:

Type 1 diabetes mellitus is a chronic autoimmune disorder associated with selective destruction of insulin producing pancreatic β- cells. The end stage of β-cells destruction represents the onset of the clinical disease. The several features which characterize type 1 diabetes as an autoimmune disease:

1. The presence of immune-competent and accessory cells in infiltrated pancreatic islets.
2. The susceptibility to the disease association with the class II (immune response) genes of the major histocompatibility complex (MHC; human leucocyte antigens HLA)
3. The specific islet cell autoantibodies
4. The CD+ T- cell mediated immunoregulation alterations
5. The interleukins producing monokines and TH1 cells in the disease process
6. Response to immunotherapy
7. Frequent occurrence of other organ specific autoimmune diseases in the affected individuals or in the family members.

The marked heterogeneity of the pancreatic lesions in the type 1 DM makes it difficult to follow the pathogenesis of the selective β-cell destructions. A mixture of

pseudo-atrophic islets with cells producing glycogen (**Alpha cells**), somatostatin (**Delta cells**) and pancreatic polypeptide (**PP cells**), normal islets and islets containing both β-cells and infiltrating lymphocytes and monocytes are seen at the onset of the disease process. The infiltration of lymphocytes are seen only in islet containing residual β-cells reflecting the chronicity of the  of type 1 DM by its heterogeneity. However, in the absence of immunosuppression β-cells are found to be rapidly destroyed in transplanted pancreas from identical twins into their long term diabetic twin mates. In this context there is development of massive insulinitis associated with T cell infiltration indicating anamnestic autoimmune reaction.[27]

In the study conducted by Gill and Haskin, it was concluded that the absolute prerequisite for the development of type 1 DM in animal models is the activation of the islet cell antigen specific CD4+ T cells.[28]

Mandrup-Poulsen *et al*[29] study showed that Interleukin-1(IL-1) and tumor necrosis factor (TNFα), the two cytokines mainly produced by the macrophages are responsible to induce structural changes of the β-cells and thereby suppresses their insulin releasing capacity.

So the underlying autoimmune destruction of the β-cells gives rise to the development of insulin deficient state resulting in the metabolic derangements seen in type 1DM.[30] (Figure 2)

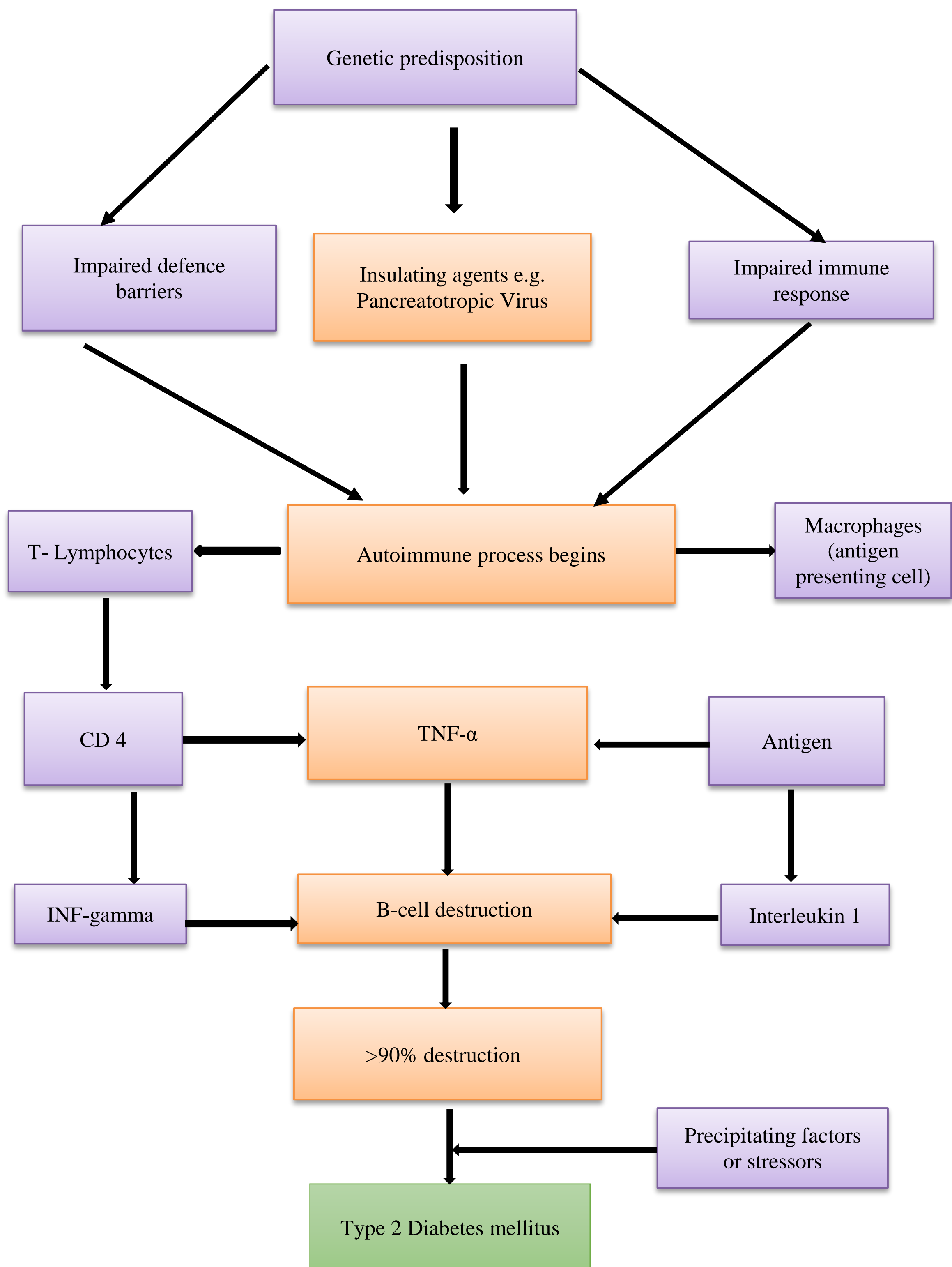



**Figure: 2 Pathogenesis of Type 1 diabetes mellitus.[30]**

## PA'THOGENESIS OF TYPE 2 DIABETES MELLITUS

Type 2 diabetes is a complex disease that involves an interplay of genetic and environmental factors and a pro-inflammatory state. The combination of impaired insulin secretion and peripheral resistance to insulin action contribute to the evolvement of type 2 DM.

Despite the intricately regulated and dynamic interactions between issue sensitivity to insulin(especially in the liver) and insulin secretion and a wide fluctuations in the demand and supply under normal physiological state the plasma glucose levels are maintained within a narrow range.

It is suggested that the two main pathological defects in type 2DM are impaired insulin secretion through a dysfunction of the pancreatic β-cells and impaired insulin action through insulin resistance.[31,32]

A decrease in the glucose responsiveness seen before the clinical manifestation of the disease is due to impaired insulin secretion. A decrease in the glucose responsive early-phase insulin secretion and a decrease in additional insulin secretion after the meals causing post prandial hyperglycemia induces impaired glucose tolerance (IGT).

Obese individuals show a decrease in early phase secretory response which is an important factor giving rise to the pathophysiological changes during the onset of the disease in all ethnic groups. There is a progressive impairment in the insulin secretion leading to glucose and lipotoxicity states and when in long term basis leads to decrease in the pancreatic cell mass. In the early stages of the disease development there is an increase in the post prandial blood glucose due to an increase in the insulin resistance and decrease in the early phase secretion, the progressive deterioration of

the pancreatic cell function eventually causes a permanent state of hyperglycemia.[33,34] (Figure 3)

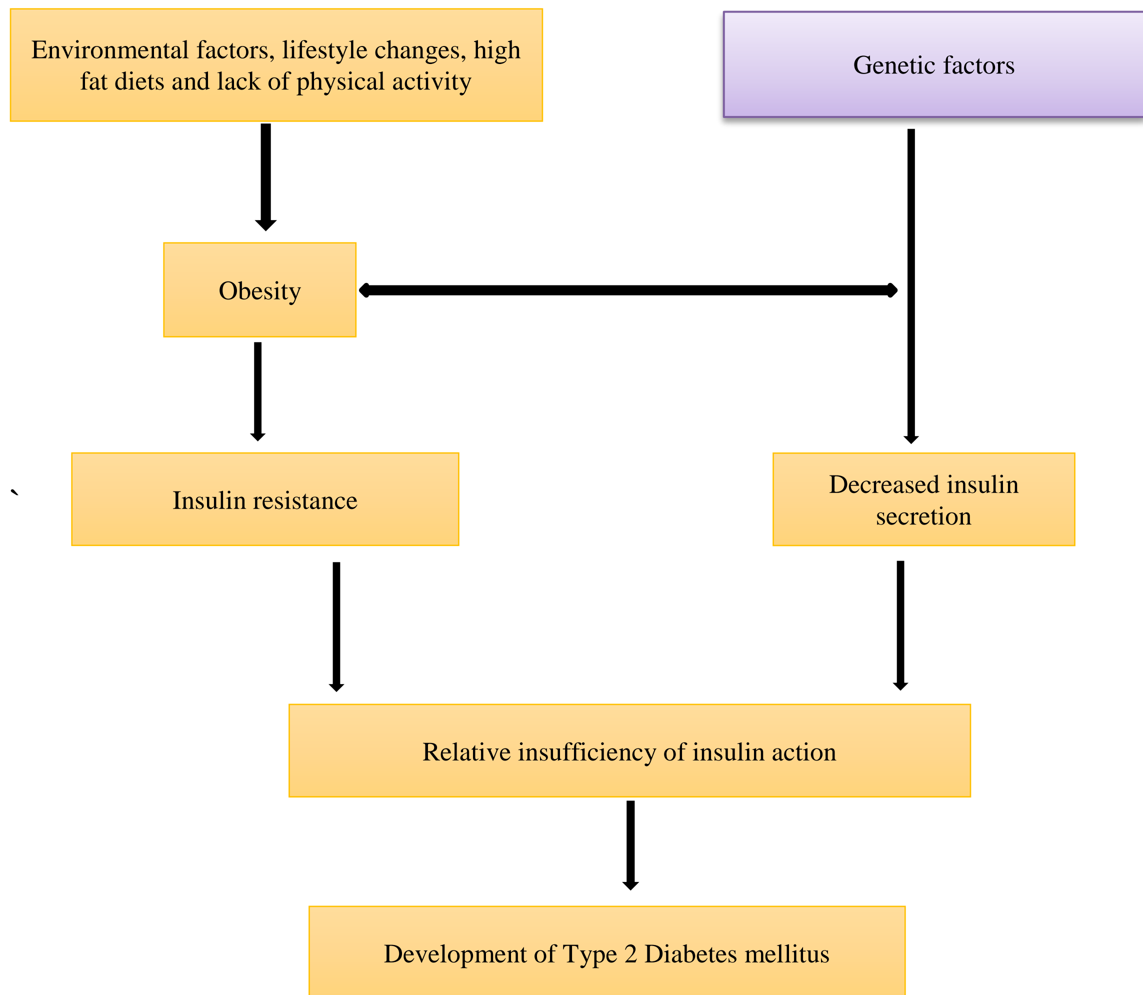



**Figure 3: Pathogenesis of Type 2 Diabetes mellitus** [33]

**Insulin resistance**

The two main organs involve in this process is the liver and the muscles. The preexisting insulin resistance even before the onset of the disease is the condition in which there is an insufficiency in the insulin action proportional to its blood concentration. The GLUT2 gene expressed by the pancreatic beta cells and the liver,

and the GLUT4 gene expressed by the skeletal muscle and adipocytes, are the major genes for the genetic susceptibility to the development of type 2 DM.[35]

Certain genetic polymorphisms involving the insulin receptor and insulin receptor substrate (IRS)-1 genes not only affect the insulin signaling but also the polymorphism of other genes such as the adrenergic receptor genes and the uncoupling protein gene, associated with visceral obesity and further promoting insulin resistance. Recent studies suggested the role of adipokines, TNF, leptin, resistin and free fatty acids in insulin resistance also, while adiponectic improves the resistance.[36.37]

Some of the clinical tests to evaluate the extent of insulin resistance are homeostasis model assessment for insulin resistance (homa-ir), insulin sensitivity test (loading test), steady-state plasma glucose, minimal model analysis and insulin clamp technique.[38]

## COMPLICATIONS ASSOCIATED WITH DIABETES MELLITUS

The morbidity and long term complications associated with either type of diabetes are mainly due to the damage in the large and medium sized muscular arteries (diabetic macro-angiopathy) and small arteries (diabetic micro-angiopathy) due to long standing hyperglycemia state. The macro-angiopathies are associated with accelerated atherosclerosis leading to increased risk of myocardial infarction, stroke and peripheral vascular diseases. On the other hand the resultant effects of micro-angiopathies are most profoundly seen in retina, kidneys and peripheral nerves giving rise to diabetic retinopathy, nephropathy and neuropathy respectively that altogether constitute the leading cause of death.[39]

A long standing hyperglycemia can also pave the way for various periodontal diseases in type 2 DM leading to various oral manifestations and changes in the buccal mucosa and periodontal disease has been recognized as the "sixth complication" of diabetes.[40]

Studies have found that around 4% of the adult patients who receive buccal treatment are diabetics and a majority are unaware that they are diabetic and/or have periodontal diseases.[41,42]

The prevalence of these oral manifestations among diabetics has a wide variation. However, Carda *et al*[43] study suggested that 100% of diabetics presented with periodontal diseases as compared to 50% in non-diabetics.

Several studies have found a prevalence of 9.8% in type 1DM, non-diabetic individual 1.6% and in type2 DM the risk is 3 times higher.[44]

**Pathogenesis**

The association of DM and changes in the oral cavity have been the subject of keen interest in both the medical and dental literatures. Multiple mechanisms both at the cellular and metabolic levels play a role in the outcome of these changes in type2 DM patients.

These mechanisms can be grouped together into the following aetiopathogenic categories. All these mechanisms leads to increased susceptibility to infection, wound healing impairment capabilities and vascular injury following chronic hyperglycemia.[45]

The deleterious effects of persistent hyperglycemia can be due to at least four distinct mechanisms, although the primacy of one over the other is unclear, however

the increased glucose flux through the various intracellular metabolic pathways are thought to contribute to end-organ damage.

- Non-enzymatic glycation reactions (advanced glycation end products- AGEs)
- Activation of protein kinase C- diacylglycerol pathway.
- Sorbitol-myoinositol osmolarity changes
- Oxidative –redox stress. [46]

**Formation of advanced glycation end products (AGEs)**

The AGEs are formed due to the non-enzymatic reactions between intracellular glucose derived dicarbonyl precursors (glyoxal, methylglyoxal, and 3-deoxyglucosone) with the amino groups of intracellular and extracellular proteins and their formation capacity is greatly increased in hyperglycemia. These AGEs bind to the specific receptors(RAGE) expressed on inflammatory cells(macrophages and T-cells), endothelium and vascular smooth muscle, which adversely affect the collagen stability and vascular integrity. The deleterious effects of AGEs-RAGE on the vascular compartments include:

- Secretion of cytokines and growth factors, including transforming growth factor β (TGFβ), which leads to the deposition of basement membrane material and vascular endothelial growth factor (VEGF)
- Reactive oxygen species(ROS) generation in the endothelial cells
- Enhanced procoagulant activity on endothelial cells and macrophages
- Increased proliferation of vascular smooth muscle cells and synthesis of extracellular matrix.

All these changes predispose to vascular stress and endothelial injury and thus accelerating atherogenesis. Further the deleterious effects of ischemia and atherosclerosis in diabetes results in decreased cellular turnover leading to alterations in the epithelial cells of the oral mucosa.

**Activation of Protein Kinase C**

Hyperglycemia leads to the de novo synthesis of diacyl glycerol(DAG) and causes enhanced activation of intracellular protein kinase C (PKC) which leads to the production of VEGF, TGFβ, procoagulant protein plasminogen activator inhibitor-1 (PAI-1) by the endothelium, leading to diabetic micro-angiopathy.

**Oxidative stress and disorders in the polyol pathways**

The hyperglycemia state leads to the increased intracellular glucose which is metabolized by the enzyme aldose reductase to sorbitol, a polyol and eventually to fructose in which NADPH (reduced form of nicotinamide dinucleotide phosphate) as a co-factor is used. However, NADPH is also required by the enzyme glutathione reductase for the generation of GSH (reduced glutathione) which is an important antioxidant in the cell and any reduction in GSH increases the cellular susceptibility to ROS (oxidative stress).[47]

**Oral changes in type 2 diabetes mellitus**

The concomitant oral manifestations of type2 DM that impacts the dental care can have the ability to determine the profound effect of metabolic control of the hyperglycemic state. Some of the oral complications include, periodontitis, gingivitis, alterations in salivary glands (sialosis), xerostomia, taste impairment, susceptibility to buccal infections(candidiasis, mucormycosis, lichen planus), delayed wound healing, decay and halitosis.

The type 2DM induces immunological cell changes resulting in the enhancement of the proinflammatory cytokines from monocytes /polymorphs nuclear leucocytes and downregulation of the growth factors from macrophages, predisposing to chronic inflammation, aggressive tissue destruction and decreased tissue repair capacity. [48]

**Sialosis**

It is defined as an asymptomatic, non-inflammatory, nonspecific enlargement of the salivary glands due to metabolic causes frequently found in diabetic patients, most commonly seen in parotid glands. It is characterized by the bilateral enlargement of the parotid glands and is associated with the fatty infiltration of the interstitium and enlargement of the acinar cells. This leads to the compensatory enlargement of the parenchyma following a decrease in the plasma insulin levels and eventually leads to the decrease production of the saliva leading to xerostomia.[49]

**Xerostomia**

The function of the salivary gland is an important aspect in maintaining the oral and systemic health. It plays a vital role in the digestion, mastication, taste, deglutination and preservation of the mucosal epithelial tissues. The microvascular disorders, autonomic neuropathy, dehydration and loss of urinary electrolytes due to polyuria are responsible for the mechanisms underlying the decreased salivary secretion in diabetics. [49]

Xerostomia refers to the subjective sensation of oral dryness has been reported in diabetic patients. The decreased salivary secretion in diabetics is associated with the disorder, and various buccal alterations occur which include-

a. Increased in the concentration of glucose and mucin.
b. Impaired production and/ action of antimicrobial factors.
c. Absence of a metalloprotein called gustin, that contains zinc and is responsible for the constant maturation of the taste papillae.
d. Bad taste.
e. Buccal candidiasis.
f. Increased cell exfoliation after contact, due to decreased lubrication.
g. Increased proliferation of pathogenic microorganisms.
h. Coated tongue.
i. Halitosis.

Also chronic hyperglycemia can lead to tongue alterations (burning mouth), periodontal diseases, white spots due to demineralization of the teeth, delayed wound healing and increased tendency to infections.[44,50]

**Periodontium and Type 2 DM**

Periodontitis has now been regarded as the sixth complication of diabetes. The defective polymorph nuclear leukocyte function, the increased susceptibility to certain infections, increased collagenase activity, decreased synthesis and the maturation of collagen and extra cellular matrix all give rise to the causes of poor periodontal health in diabetics. The abnormal state of the collagen is attributed to the formation of AGEs in hyperglycemia states, which further crosslink the collagen making it less soluble making it less likely to be repaired or replaced. [40,45,51]

Takeda *et al* [51] study reported that the critical determinant for susceptibility to severe periodontitis in type 2DM patients is the inflammatory response associated with it.

Taylor *et al* [52] study suggested that an untreated chronic periodontitis inducing chronic inflammatory response may contribute to insulin resistance, and thereby worsening the glycemic control.

In another study done by Bandyopadhyay *et al*[53] concluded that is a significant association between the progression of the periodontal disease and diabetes control status.

### Traumatic ulcers and irritation fibromas

There is a high prevalence of traumatic ulcers and formation of irritation fibromas in diabetics. These are cause by the delayed wound healing, micro-angiopathy and the defection function of the polymorph nuclear leucocytes.[54]

## RISK FACTORS OF DIABETES MELLITUS

Diabetes encompasses a variable degree of multifactorial risk factors. Based on the WHO consensus they can be divided into modifiable or behavioural risks factors and metabolic or physiologic risks factors. The presence of any one of the risk factors places an individual in a high risk category of developing diabetes.[47] (Table 3)

TABLE: 3 RISK FACTORS OF DIABETES MELLITUS.

| MODIFIABLE RISKS | NON MODIFIABLE RISKS |
|---|---|
| Obesity(truncal and total)and overweight | Ethnicity |
| Sedentary life style | A positive family history of Type 2 DM |
| Previously identified glucose intolerance (IGT/or IFT) | Age |
| Metabolic syndrome:<br>Hypertension<br>Decreased HDL cholesterol<br>Increased triglycerides | Gender |
| Dietary factors | History of gestational diabetes |
| Intrauterine environment | Polycystic ovarian syndrome |
| Inflammation | |

MODIFIABLE RISK FACTORS:

Sedentary lifestyle:

Rapid urbanization is one of the major factors contributing to the lifestyle changes which further increase the risk for diabetes. These changes which are associated mainly with in dietary patterns, decreased physical activity due to improved transportation, high level of mental stress associated with modernization, all give rise to weight gain which further contribute to existing insulin inertia. Studies have shown that increased physical exercise increases the insulin sensitivity, corrects dyslipidemia and lowers of blood pressure and eventually decrease the cardiovascular disease. [56]

PREVIOUSLY IDENTIFIED GLUCOSE INTOLERANCE (IGF)

Impaired glucose regulation (IGT and IFG) refers to a metabolic state of intermediate between normal glucose homeostasis and diabetes. They represent different abnormalities of glucose regulation, one in the fasting state and one post-prandial. Oral glucose tolerance test is recommended if the fasting plasma glucose ≥ 6.1-6.9 mmol/l or 110-125 mg/dl. IFG and IGT are not clinical entities in their own right, but rather risk categories for development of diabetes and /or cardiovascular disease. [57]

OBESITY

It is defined as the excessive deposition of body fat or adipose tissue in relation to lean body mass. According to WHO overweight is defined as a body mass index (BMI) ≥ 30 kg/m$^2$ and a grade II obesity was associated with 7-12 fold increase in the incidence of type2 diabetes. Obese men (body weight 125% or more than the ideal according to the metropolitan life insurance standard) had a higher risk of developing diabetes 1.5 times higher than those with body weight less than 110% of ideal body weight. Further it has been documented that a waist circumference of ≥ 88cm in women or ≥ 92 cm in men are at more risk of developing diabetes.[58]

METABOLIC SYNDROME

Previously referred as Syndrome X, the Insulin Resistance Syndrome is characterized by the cluster of risk factors which include central (upper body) obesity, hypertension, dyslipidemia with or without hyperglycemia and is associated with a five-fold increase risk of diabetes mellitus and macrovascular disease. Based on the IDF, an individual to be labelled as having the metabolic syndrome he or she must have central obesity plus any two of the following four factors: [59] (Table 4)

TABLE: 4 COMPONENTS OF METABOLIC SYNDROME.

| | |
|---|---|
| Increased plasma triglycerides → | ≥ 150 mg/dl ( 1.7 mmol/l ) or specific treatment for this lipid abnormality. |
| Reduced HDL cholesterol → | < 40mg/dl (1.03 mmol/l) in males<br>< 50 mg/dl (1.29 mmol/l) in females<br>Or specific treatment for this lipid abnormality. |
| Raised blood pressure → | systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension. |
| Raised fasting plasma glucose → | FPG ≥ 100 mg/dl (5.6 mmol/l)<br>or previously diagnosed type 2 DM. If above 100mg/dl or 5.6mmol/l, a OGTT is recommended but it not necessary to define the presence of the syndrome. |

INFLAMMATION

Over the past several years inflammation has emerged to be an important factor in the pathogenesis if type 2 DM. The proinflammatory cytokines that are secreted in response to excess nutrients such as the free fatty acids(FFAs) and glucose which results in both insulin resistance and β- cell dysfunction. The excess FFAs within the macrophages and the β-cells leads to the activation of the inflammasome, a multiprotein cytoplasmic complex which further leads to the secretion of cytokine interleukin IL-1β. The IL-1β along with other cytokines in the circulation further act

on the major sites of insulin action and promote insulin resistance. C-reactive protein, which is also a marker of inflammation also helps in the development of diabetes.[60]

## INTRAUTERINE ENVIRONMENT

An interplay of the uterine environmental and genetic factors mediates the familial predisposition in the development of type 2 DM. Mutations in the gene on the mitochondrial DNA attribute to the maternal inheritance pattern where the affected mother transmits to her progeny. Several studies have shown that the intrauterine hyperglycemia is associated with increased perinatal morbidity and mortality and also expose the fetus for obesity, diabetes and metabolic and cardiovascular diseases. [61]

NON MODIFIABLE RISK FACTORS

AGE AND GENDER

Type 2 diabetes is more prevalent in older age groups. Based on Dambal *et al*[62] study diabetes is more commonly noted in elderly age groups between 40-50years.

However, age is not a defining criteria as the onset of diabetes has been reported in younger adults and children, due to imbalance between energy intake and expenditure.

## GENETIC FACTOR AND ETHNICITY

Genetic susceptibility and predisposition is associated in the pathogenesis of type 2 DM. The genetic factors are attributed by the degree of variations between ethnic groups when exposed to similar environments.[63]

The prevalence of type 2 DM is two-three times more in African and African-Caribean population and five –six times more in South Asian counterparts.[63]

Several studies done on the migrant Indians across the globe have shown that Asian Indians have an increased risk for developing type 2 DM and related metabolic syndromes when compared to other ethnic groups.

Although the definite reason is not clear, certain unique biochemical and clinical characteristics of this ethnic group collectively called the "Asian Indian phenotype" is regarded as one of the major factors contributing to the increased predilection towards diabetes.[15,64]

**FAMILY HISTORY**

The risk for the development of type 2 diabetes increases with the number of affected first degree relatives.[64]

**PREVIOUS HISTORY OF GESTATIONAL DIABETES**

Following delivery the gestational diabetes usually returns to normal glycemic state; however, these have a substantial risk for the development of type 2 diabetes in their later life.[64]

**POLYCYSTIC OVARIAN DISEASE (PCOD)**

The features of insulin resistance and compensatory hyperinsulinemia are highly prevalent in women with PCOD, and attribute to a seven-fold increased risk of developing type 2 diabetes as compared with their normal counterparts. [65]

**ADDITIONAL RISK FACTORS**

SMOKING:

Smoking has been attributed in the deterioration of glucose metabolism and has been identified as one of the risk factors for insulin resistance which may lead to the development of type 2 diabetes.[66]

HYPERHOMOCYSTEINEMIA:

Based on the European Union Concerted Action Project, "homocyteinaemia and vascular disease", elevated levels of homocysteine in poorly controlled type 2 is associated with the increased risk of development of atherosclerosis and cardiovascular disease. [67]

## NORMAL MORPHOLOGY OF THE BUCCAL EPITHELIAL CELLS

The oral cavity comprises of the palate, tongue, pharynx, floor of the mouth, and the buccal mucosa is lined by the squamous epithelium with different degrees of keratinization. The oral epithelial changes are associated with many underlying diseases. Exfoliative cytology of the oral epithelial cells is an essential technique to assess the morphological variations of these cells. It is based on the microscopic evaluation of these cells after a procedure of their fixation and staining. Cytological study of the buccal epithelial cells is a simple non aggressive technique that is compatible with patients comfort, and hence render an aid to the evaluation of the oral epithelial cells in the early diagnosis and prognosis of certain metabolic diseases like for example diabetes mellitus. However, its usage is limited in the diagnosis of oral malignancies due to its poor sensitivity and specificity.[68]

### History:

The technique of exfoliative cytology was first introduced by Johannes Muller (1801-1858), a pathologist in Berlin to demonstrate cancer cells on scrapings from the cut sections of surgically excised tumors. The 1920s was the momentous year in the field of diagnostic cytology. A clinical cytology monograph was first published in Spain. George Papanicolaou (1883-1962) published further papers for the detection of uterine cervical cancers from cytological examination of vaginal smears. Henceforth,

he introduced the staining technique named after him, what is called PAP test as a routine adjunct to diagnosis. Later Zaskin reported the use of exfoliative cytology in oral cavity for the first time. In 1951 Montgomery and Von Hmm used exfoliative cytology for the diagnosis of oral cancer.[69]

SAMPLING TECHNIQUES:

Various techniques have been employed to obtain samples from oral lesions of which worth mentioning are incisional and excisional biopsies. However, these methods are invasive ones and are associated with low patient compliance. In this regards exfoliative oral cytology technique comes into hand which is painless, less aggressive, comfortable for the patients and cost effective. The two methods most commonly used are-

i. Oral scrapings and
ii. Brush biopsy

Oral scrapings are done by using a cytobrush, a wooden spatula, cotton tip applicators, via vigorous saline rinsing, forceful aspiration of cells from the surface and aspiration of the saliva from the floor of the mouth. However, these methods carries its own pros and cons.

It consists of cleansing the surface of the oral cavity with water or saline to get rid of the debris and mucin, and select a healthy looking area in the oral mucosa and scrapping it several times by a spatula or moistened blade.

The collected material is then quickly spread evenly on a microscopic slide and fixed immediately by immersing in 95% alcohol to avoid drying up. Later the slides are stained with Papanicolaou stain and visualized under the microscope. Brush biopsy( Oral CDX) is an important method which has the advantage in obtaining the

full trans epithelial cellular samples according to some workers which they further applied the image analysis system to detect the oral epithelial cells abnormalities especially in the detection of oral cancers, candidiasis, Epstein- Barr virus in oral lesions of hairy leukoplakia. A multi centric study revealed nearly 5% of the clinically benign appearing lesions sampled by this technique and were later confirmed by the conventional scalpel biopsy to diagnose dysplastic epithelial changes or invasive cancer. [69,70]

**The cytological smears are reported in the following five classes:[70]**

**Class I (Normal):** It indicates the observation of only normal cells.

**Class II (Atypical):** The presence of minor atypia is indicated without evidence of malignant changes.

**Class III (Indeterminate):** This is an intermediary cytology that separates cancer from non-cancer diagnosis. The cells display wider atypia that may be suggestive of cancer, but they are not clear-cut and may represent precancerous lesions or carcinoma *in situ*. Biopsy is recommended.

**Class IV (Suggestive of cancer):** A few cells with malignant characteristics or many cells with borderline characteristics. Biopsy is mandatory.

**Class V (Positive for cancer):** Cells that are obviously malignant. Biopsy is mandatory.

## INDICATIONS FOR ORAL CYTOLOGICAL EXAMINATION

The principal application of exfoliative cytology technique in the oral epithelial lesions is the diagnosis and screening of occult carcinomas, not identified and not suspected on clinical inspection. Some of the important indications worth mentioning are-

- Clinically innocuous appearing lesions which otherwise would not be biopsied.

  Evaluation of extensive mucosal lesion when it is not feasible to perform enough incisional biopsies for adequate sampling.
- As a follow up of patients with either a malignant or premalignant mucosal lesion.
- If the patient's medical condition is not favorable for a biopsy to undergo.

CONTRAINDICATIONS:

- Provide limited information regarding the specific tissue changes other than cancer.
- Cannot comment on the extent of invasion of the lesion.
- Inability in identifying the degree of differentiation of malignancy.
- The limited reliability of the technique.

ADVANTAGES OF THE TECHNIQUE[69,70]:

- Painless, quick, noninvasive, feasible and cost effective.
- It can be performed easily as an outpatient procedure.
- It is feasible in patients with concomitant metabolic diseases and who are contraindicated for a biopsy.
- Post biopsy complications can be avoided.
- It can be used as a mass screening procedure.
- Early detection of oral lesion malignancy.

DISADVANTAGES:

- Limited information as compared to histological slides.
- Reliability of positive results only.
- Usage suitable only for epithelial cells.
- It provides an adjunct but cannot replace biopsy.
- Subjectivity bias of the cytological evaluation and interpretation errors.
- Tumor grading cannot be assessed.

APPLICATIONS OF ORAL EXFOLIATIVE CYTOLOGY:[69,70]

- As an aid in the study of diabetes mellitus, smoking, alcoholism, etc.
- Timely detection of oral cancer.
- In the evaluation of certain microbial diseases and dermatological lesions(pemphigus vulgaris).
- Assessment of certain nutritional deficiencies example. Iron deficiency, B12 and folic acid deficiency.
- Forensic dentistry evaluations.
- To determine sex chromatin for genetic counselling and as a source of DNA.
- Predicting the cellular response of tumor to irradiation.

**The oral epithelial cells in the absence of disease:**

The normal the squamous epithelium of the oral mucosa sheds superficial and intermediate squamous cells, similar to those of the vagina and cervix, except that nuclear pyknosis is not observed. These cells are found singly or in clusters and similar to those seen in the saliva and sputum samples. Figure 4 (A) & (B)

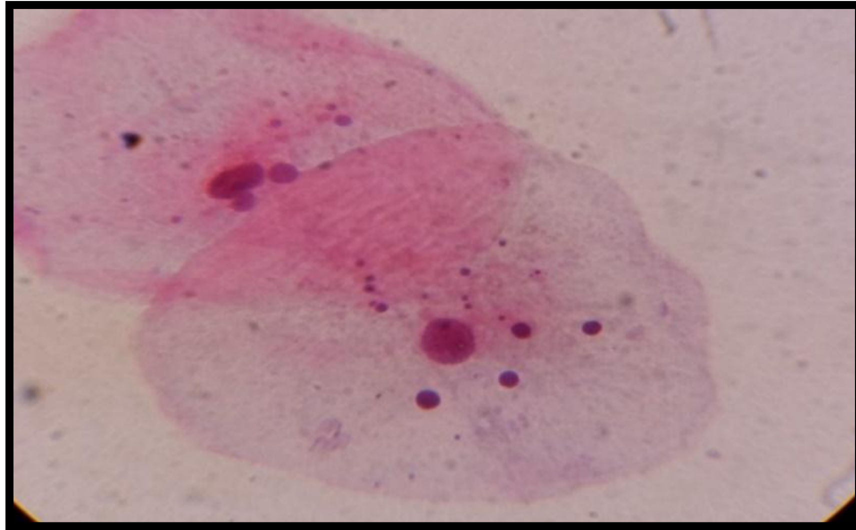


Figure: 4 (A) Normal squamous epithelial cells.

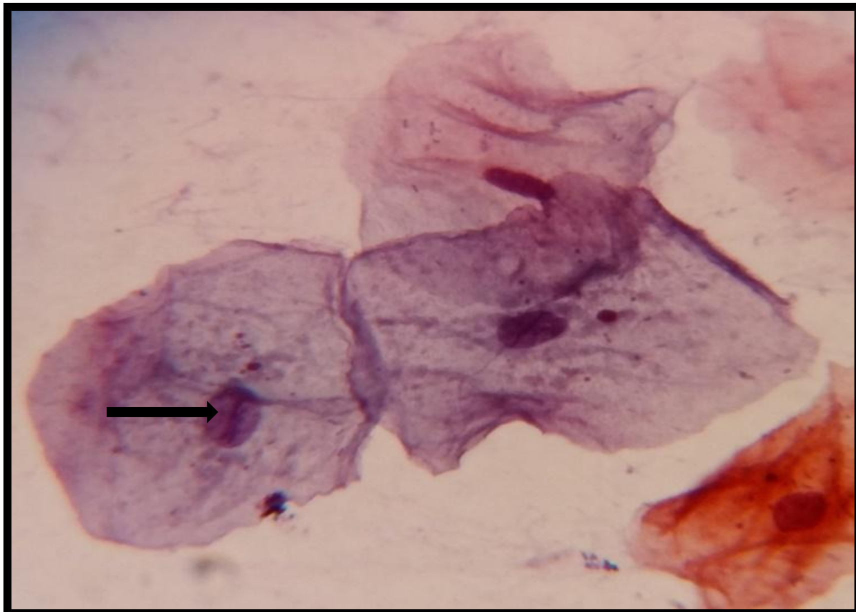


Figure: 4 (B) Normal squamous epithelial cells from oral scrapings.

The cell show a nuclear bar with lateral extensions (caterpillar nuclei) similar to Antischkow cells. (Depicted by arrow in microphotograph figure 4 B)

The study of exfoliative cytology is concern with the analysis of the cells which abrade from the body surfaces. The normal cellular physiological turnover leads to the shedding of the superficial squamous epithelial cells.

The underlying deeper cells like the intermediate cells and the parabasal cells are adherent to one another normally. Under pathological condition, these cells lose their cohesiveness and the deeper layer sheds along with superficial cells. These cells are scrapped off for cytological evaluation quantitatively and qualitatively. However, the usage of the cytologic smear depends on the proper preparation and evaluation by a cytologist.

It was observed that the cells show a nuclear bar with lateral extensions which is nothing but the longitudinal condensation of the nuclear chromatin, similar to those seen in Antischkow cells in the myocardium in rheumatic heart disease patients which are most commonly seen in cells from the floor of the mouth. This change is related to the “nuclear creases” but its significance is not known. Similar findings are noted in the mesothelial cells of the pericardium surface.

Variable degrees of keratinization of the cells are noted from fully keratinized orange colored anucleated cells to smaller parabasal cells.

**Other cells:**

Mucus-producing columnar epithelial cells originating from the nasopharynx or from the salivary gland ducts can occur occasionally. At times any vigorous scrapings from the tonsillar areas or the base of the tongue can give rise to shedding of the lymphocytes, singly or in clusters.

**Oral flora:**

The oral flora most commonly in persons with poor oral hygiene are mostly comprised of saprophytic fungi and bacteria. A protozoa, *Entamoeba gingivalis* is a common finding. An unusual organism called *Simonsiella* species which is also nonpathogenic is observed in persons with rich dietary intake of proteins and fats.

**Buccal cells in genetic counselling and as a source of DNA**

The exfoliative cytological analysis of the oral squamous cells for the detection of the Sex chromatin (Barr bodies) is one of the cost effective and feasible procedure. These can be seen as half-moon shaped chromatin concentrations at the nuclear membrane. Theoretically the nonpyknotic squamous cells all genetic females, having open vesicular nuclei should contain a Barr body.

However, in practice it can be identified in fewer than half of these cells on light microscopy of oral epithelial cells stained with Papanicolaou's stain, but one show keep in mind that the peripherally placed chromocenters and focal thickening of the nuclear membrane may mimic Barr bodies and the visible sex chromatin varies with the menstrual cycle.

The finding of about half a dozen or more cells with a clear-cut single sex chromatin body is diagnostic of the XX female chromosomal constitution. However, an excess of Barr bodies (randomly more than two in a cell) indicates an excess of X chromosomes ( "superfemales," with cells containing 47 chromosomes with XXX). Malignant cells occasional also contain two or more Barr bodies, reflecting aneuploidy.

The presence of Barr bodies in cells with a phenotypic male suggests Klinefelter's syndrome (47 XXY) and the absence of Barr bodies in a phenotypic female suggests Turner's syndrome or another form of gonadal dysgenesis.[68-72]

**CYTOMORPHOMETRY:**

Several underlying mechanisms brings about alterations in the cellular and nuclear morphology and which can otherwise be used to assess certain processes. A qualitative and a qualitative assessment of the changes in the oral epithelial cells is

still a controversial technique surrounding the usage of exfoliative cytology. The quantitative technique include the study of DNA cytomorphometry (optical quantification of the chemical substances incorporated into the DNA- the Feulgen stain) and cytomorphology (characterizes the proliferation activity of the cell populations of the oral mucosal squames ).

Studies have shown that normal cells display a diploid DNA distribution, indicating a non-replicative cell population. However, malignant cell show a variety of DNA profiles such as diploid, polyploid, aneuploidy and hyperdilpoidy.

The morphometric analysis can therefore give the changes associated with characteristic cellular changes such as senescence, apoptosis or nuclear irregularities. The cytomorphometry encompasses the study of the measurement of the Nuclear area(NA), Cytoplasmic Area(CA) and the Nuclear Cytoplasmic ratio (NCR) of a cell.[73]

**The oral epithelial cells alterations in various diseases:**

Certain conditions correlates the oral epithelial changes. Nutritional deficiencies states like megaloblastic anemia (deficiency of vitamin B12 and folic acid ) and iron deficiency anemia also attributes to the cellular alterations of the buccal mucosa. Sumanthi J *et al* [74] and Gujuraj *et al* [75] studies showed that there was a significant increase in the average nuclear area (NA) and the nuclear cytoplasmic ratio (NCR) in iron deficiency anemia patients when compared with the control group.

Similar findings were also reported in megaloblastic anemia patients. Vitamin B12 and folic acid are essential elements in the synthesis of DNA. Thus, nutritional deficiencies leads to the increase in cytoplasm and nuclear size.[76]

Interpretations of the exact causes for these changes is not exactly known, it may be attributed to the following reasons:

- Normal cells has the ability to withstand the oxidative stress by certain oxidant defense systems. The red cells in iron deficiency have a diminished activity of essential antioxidant enzymes like glutathione peroxidase and catalase which increases the lipid peroxidation which is a very lethal to the cells.
- The growth and tissue repair depend on increased rates of cellular proliferation. Folic acid and vitamin B 12 are required for the synthesis of thymidine and pyrimidine bases, while zinc participates at the catalytic site of DNA and RNA polymerases. Since iron, folate and zinc have critical roles in the regulation of the DNA synthesis these nutrients are considered as rate limiting factor for growth.[74-76]

Ogden *et al* [77] study revealed that the cytomorpho-metric changes in the buccal mucosal cells of the cigarette smokers are similar to those noted in diabetes.

In a study conducted by Patel V and Sheela G, it was found that the mean nuclear area and mean NCR ratio of keratinocytes was significantly higher in experimental groups (non-smokers and smoker diabetics) when compared with the control group. The alterations noted in the oral mucosal cells of the smokers can also be attributed by the associated chronic inflammation[78].

The inflammatory process can give rise to a similar finding to those found in type 2 diabetes patients. These detections can be understood by the presence of superficial erosions or ulcerations of the oral mucosal epithelium, with concomitant inflammatory conditions such as stomatitis and gingivitis. So the normal squamous cell populations are replaced by the cells of the deeper layers, such cells vary in size

and shapes and encompasses an increased nucleus and with poorly preserved cytoplasm. These cellular changes are characteristics of the younger germinative layer, and thus the microscopic findings in smears of the smokers associated with inflammation are attributed to the prevalence of non-keratinized cells and must not be confused with cellular atypias.[77-78]

Loss *et al*[79] study in patients with candidiasis concluded that candida-infected epithelial cells have an increase in nuclear diameter, perinuclear rings, discrete orangeophilia and cytoplasmic vacuoles and a decrease in the cytoplasmic area. An increase in the NCR ratio was also noted.

**The cytomorphometric cellular alterations in Type 2 Diabetes mellitus.**

The utilization of exfoliative cytology has expanded like for instance as an adjunct to the diagnosis oral epithelial changes in diabetes patients. Several studies have been done to evaluate the cytomorphometric changes of the oral epithelial cells and its correlation with the glycemic status of these patients. The interpretation of this technique has many shortcomings due to its subjective nature of observation, which has now been overcome by the introduction of image analysis systems to evaluate the cytomorphometric alterations at the cellular level.

In the studies conducted by Alberti *et al*[80] and Shareef *et al*[81] from the smears of buccal mucosa, floor of the mouth and dorsum of the tongue in Type2 diabetes patients it was concluded that the mean nuclear area(NA) was increased in diabetics as compared to the healthy controls and no significance change was noted in the mean cytoplasmic area(CA). They also suggested that the qualitative or morphologic changes to inflammation in diabetes patients were possibly due to decreased salivary flow. Their findings also concur with the study done by Jajarm HH *et al* study.[82]

Study by Prasad H *et al* [83] showed that the NA and Nuclear:Cytoplasmic Ratio (NCR) of cells in diabetic patients were significantly higher than those from control subjects.

Survana M *et al* [84] study on the cytomorphometric analysis of the buccal cells in 40 type 2 DM patients and 40 healthy individuals concluded the there was a statistically significant increase in the average NA and decrease in the NCR in diabetics when compared to the normal healthy subjects. However, the average CA didn't show any statistical correlation. They also attributed these changes in diabetics were due to acute inflammation.

Lamichhane RS *et al* [85] study also noted an increased in mean NA, CA and NCR in diabetic patients as compared to normal healthy groups. They also further evaluated the smears from the exfoliated cells of the buccal mucosa after staining with Acridine orange and visualized under the fluorescence microscope and noted nuclear alterations like micronuclei, nuclear budding binucleation and karyorrhexis.

In another study conducted by Karthik KR *et al* [86] they compared the buccal epithelial cell alterations and attempted to correlate the cytomorphometric parameters with Glycosylated hemoglobin ($HbA_{1c}$) and concluded that there existed correlation between $HbA_{1c}$ values with the NA and NCR.

Sahay *et al*[87] study concluded that the quantitative analysis of the overall mean CA and NCR were less and mean NA was more in type 2 diabetic patients as compared to normal subjects. They also noted microbial colonies, cytoplasmic vacuoles, karyohexis, binucleation in the diabetic oral epithelial cells.

In another In-Situ Study done by Kishore Sonawane *et al* [88] on Cytomorphometric analysis of the Oral mucosa in diabetic patients in Bhopal region, reported that the mean NA was significantly more in diabetic patients but the mean CA didn't show any statistical difference. Based on the clinical observations and results it was concluded that cytomorphometric analysis using exfoliative cytology can be used as an aid in the diagnosis of DM. The cytomorphometrical changes can be attributed to the increased cellular aging in patients with diabetes, a secondary reaction to ischemia caused by atherosclerosis in diabetic patients leading to decreased cellular turnover and limited production of young cells.

The study done by Zimmermann and Zimmermann suggested that the difference in cytomorphometry of the oral mucosa may be attributed to the variations in recovery rate of keratinizing cells of the oral tissue following systemic endocrine disorders like diabetes.[89]

## MATERIAL AND METHODS:

### Source of data:

A cross sectional hospital based study was conducted fulfilling the inclusion and exclusion criterias of diabetic patients who were from either outpatient or inpatient department attending 'Diabetic clinic' at BLDEU Shri B.M. Patil medical College, Hospital and Research Centre, Vijayapur.

Study period- From 1st November, 2015 to 30th June, 2017.

### Method of collection of data:

- A cross sectional study of 100 subjects i.e. patients with Type 2 DM (n=50) and normal control group (n=50) who are healthy individuals with no risk of diabetes and were age and sex matched were included in the study.
- The diabetic patients were selected according to the American Diabetic Association Criteria.
- Both the patients and control group were evaluated for Random blood glucose (RBS), Fasting plasma glucose (FP), 2 hour post prandial plasma glucose level (PP) and Glycosylated Hemoglobin ($HbA_{1c}$ ).
- To evaluate further the glycemic control of the two study groups $HbA_{1c}$ values were also investigated.
- Controlled diabetic patients were categorized as having $HbA_{1c} < 7\%$ and uncontrolled diabetic patients whose $HbA_{1c} > 9\%$.
- A detailed history and duration of the disease were taken with preliminary general physical examination of the patients.
- An informed consent was taken from the patients and the control groups for the procedure as a dental examination in order to check the status of the buccal mucosa.

**Material:**

- 1-2 clean non grease frosted glass slides
- Swab stick, ice cream stick
- A pair of sterile gloves
- 95% ethyl alcohol in a coupling jar for fixation
- PAP smear reagents

**Technique of smear collection as depicted by figures 5A & 5B:**

The procedure was priory informed to the patients before taking the smears. The patients name or the IP number or the OPD numbers were marked on one side of the frosted glass slides. All smears were made using a sterile wooden spatula with a gentle scraping motion, exerting little pressure from a clinically normal appearing buccal mucosa in all individuals. The collected samples were evenly smeared onto the center of the glass slides. The slides were immediately immersed in 95% ethyl alcohol to ensure proper fixation for a duration of one hour.

Air drying was avoided to avoid the cellular morphology alterations.

The technique of smear collection using a wooden spatula as depicted by Figures 5A & B

**Fig: 5 (A) Gently scrape and rotate the spatula.**

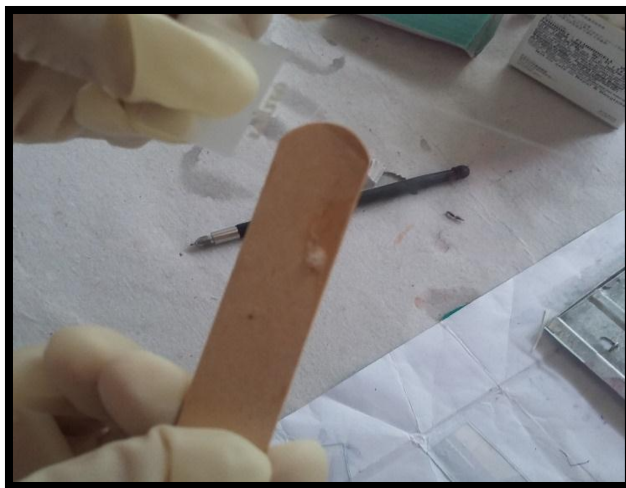


**Fig:5 (B) Spread the smear on the glass slide.**

The technique of smear collection using a wooden spatula as depicted by Figures 5A & B

**Staining**

Smears were then stained with the Papanocolaou staining technique (PAP) as follows:

**HYDRATION OF THE SLIDES**

| | | |
|---|---|---|
| 90% alcohol | ---- | 2 minutes |
| 70% alcohol | ---- | 2 minutes |
| 50% alcohol | ---- | 2 minutes |
| Water wash | ---- | 5 minutes |
| Stain with Harris' Haematoxylin | ---- | 10-15 minutes |
| Wash in water | ---- | ---- |
| Differentiate in acid alcohol | ---- | 1-2 dips |
| Water wash | ---- | ---- |
| Blue in ammonia water | ---- | 1 dip |
| Water wash | ---- | 10-15 minutes |

**DEHYDRATION OF THE SLIDES**

50% alcohol 1 dip

70% alcohol 1 dip

90% alcohol 1 dip

Stain in OG-6 2 minutes

90% alcohol 2 dips

Stain on EA-36 2 minutes

90% alcohol 2 dips

Xylene 2 minutes

Mount in DPX

**Cytomorphometric analysis (as depicted by Figures 9 &10):**

The cells with clearly defined cellular and nuclear outlines were selected prior to the procurement of the images. The cells that were clumped, overlapped or folded and covered by the inflammatory cells were avoided for the analysis.

The PAP stained smears were manually screened and the cells were selected from the field of vision beginning at one end of the smear, moving horizontally, and at the other end, moving downward to the next level and again moving horizontally in the opposite direction to avoid repeating the cell count.

- An average 20 clearly defined cells belonging to all levels of differentiation were selected in a random fashion from different fields and images were captured via AXOCAM Digital Camera under 100x magnification (oil immersion).
- The images of the selected buccal epithelial cells analyzed using the MIPS (Magnified Image Processing System) calibrated with ZEN software and subjected to morphometric analysis by the DIZIMIZER Image Analyzer 0.4.3 version.
- The measurements of the nuclear area (NA) and Cytoplasmic Area(CA) were obtained by utilizing the ‘auto trace’ function in the software. The number of pixels in the region of interest was used to derived the ‘area’ in square microns.
- The values of the NA and CA were automatically displayed and were recorded and the NCR was calculated for each cell. The above parameters were automatically calculated by the Image analysis software and thereby reducing the subjective error.

**Microphotographs:**

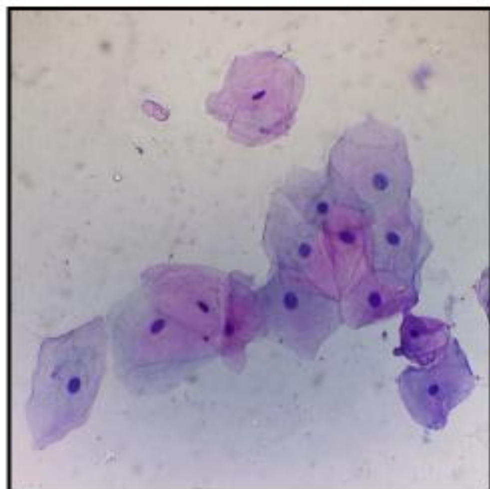


Fig: 6(A) Pap stained buccal mucosal cells representative of normal healthy group.(40x)

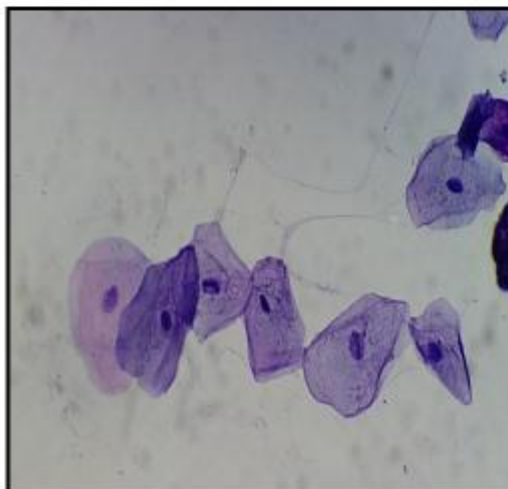


Fig: 6(B) Pap stained buccal mucosal cells representative of diabetic group.(40x)

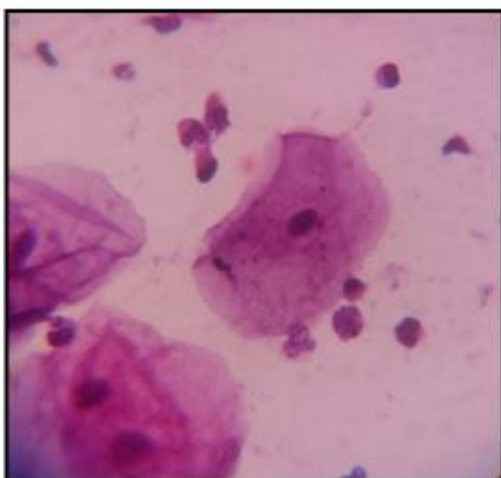


Fig:7(A) Pap stained buccal epithelium cell of diabetic group showing infiltration by inflammatory cells. (100x)

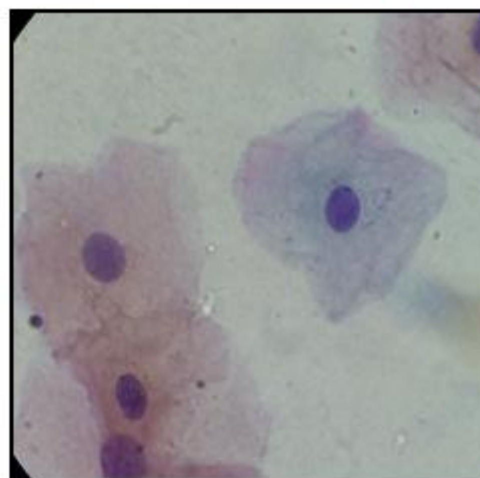


Fig:7(B) Pap stained buccal epithelium cell of healthy control group showing no inflammatory cells. (100x)

In our study we also noted the infiltration of inflammatory cells in the buccal epithelial cells in the diabetic group whereas the smears from the healthy controls were devoid of it, as depicted by (Figures 8 A & B).

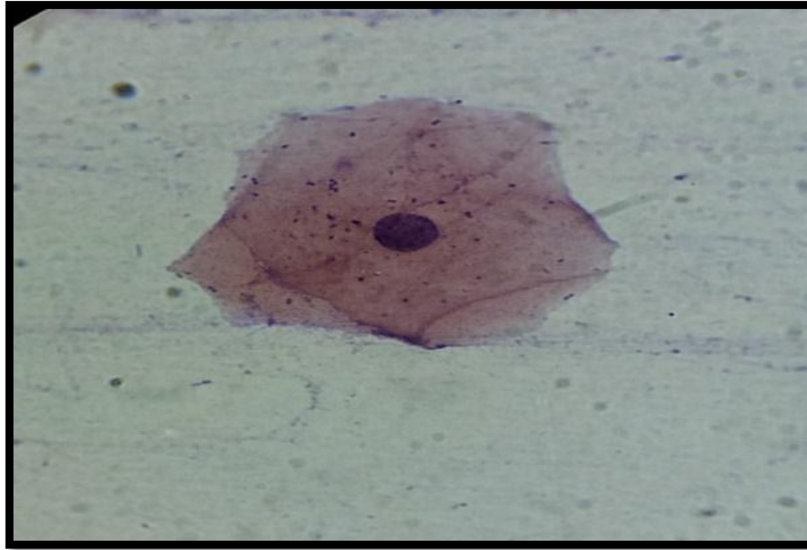


Figure 8 (A) A high power view of buccal squamous epithelial cell of control subject. (100x) Pap stained.

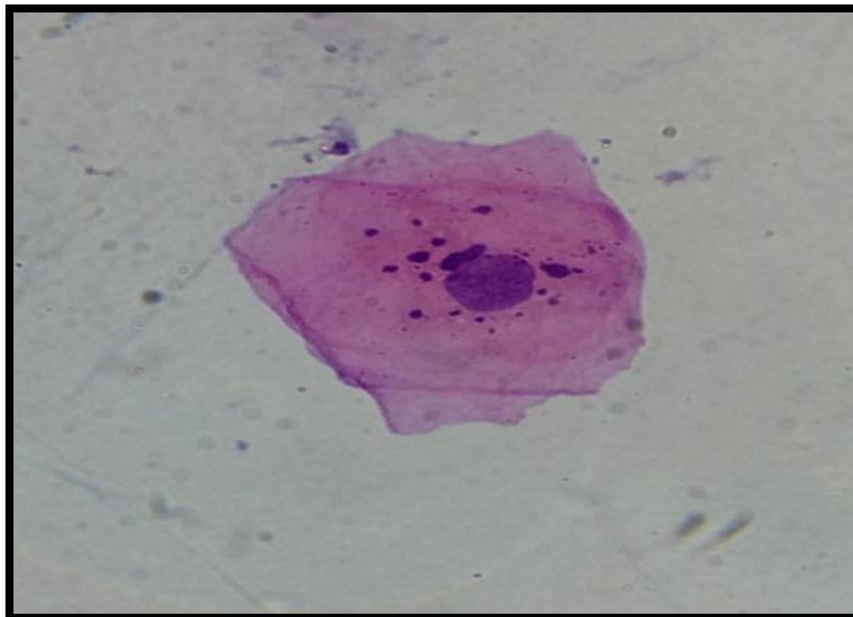


Figure 8(B) A high power view of buccal squamous epithelial cell of diabetic patient. (100x) Pap stained.

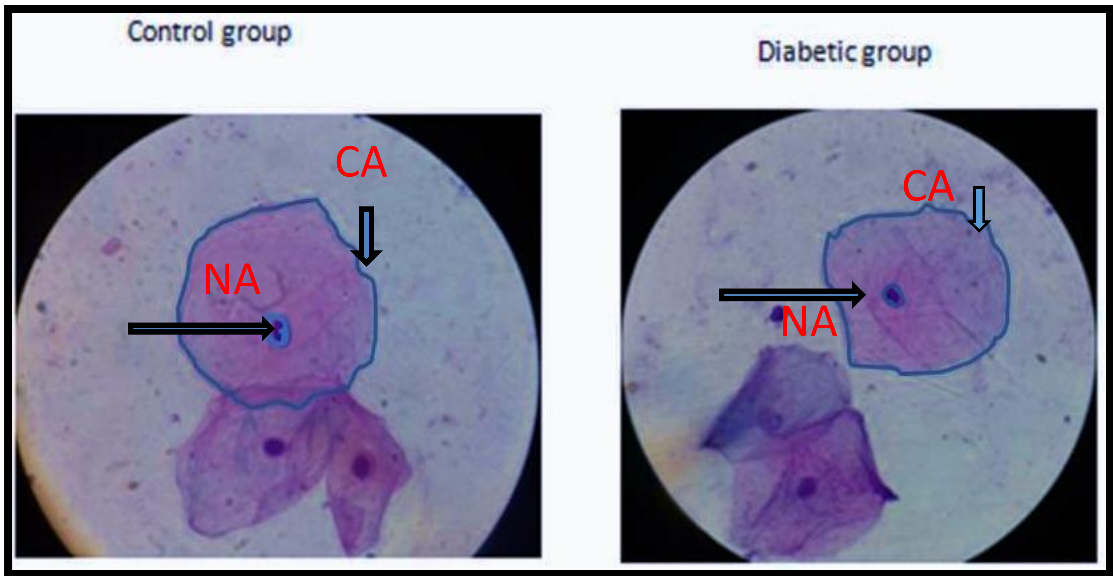



Figure 9: Cytomorphometric analysis of Pap stained buccal smears in normal control group and diabetic groups.

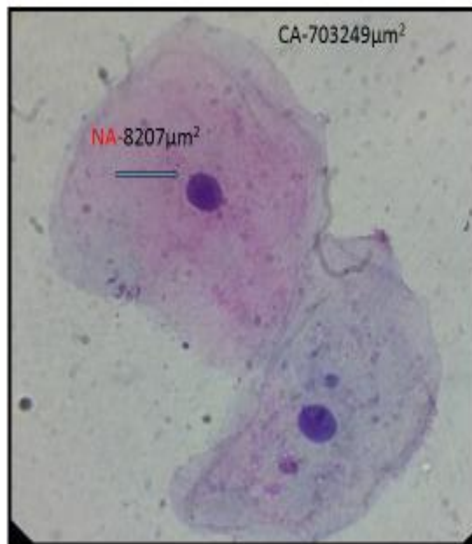



Fig: 10(A) Pap stained buccal mucosal cells representative of healthy group(100x)

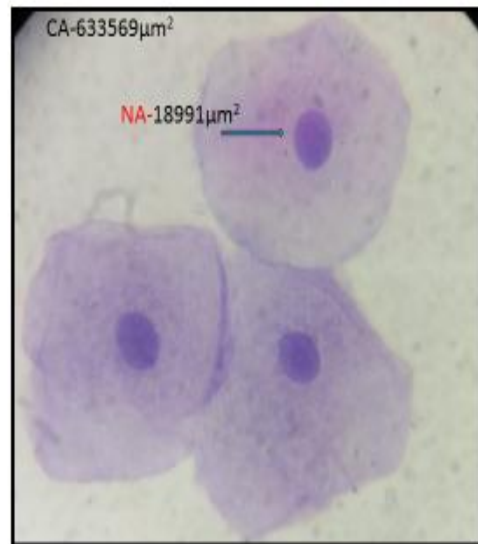



Fig: 10(B) Pap stained buccal mucosa cells representative of diabetic group.(100x)

**Statistical analysis:**

- All characteristics were summarized descriptively.
- For continuous variables, the summary statistics of mean, standard deviation (SD) were used.
- For categorical data, the number and percentage were used in the data summaries. Chi-square ($\chi^2$)/ Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data.
- The difference of the means of analysis variables between two independent groups was tested by unpaired t test.
- The difference of the means of analysis variables between more than two independent groups was tested by ANOVA and F test of testing of equality of Variance.
- Normal linear regression analysis was employed to assess the adjusted effect of determinants of continuous study variable.
- If the p-value was $< 0.05$, then the results were considered to be statistically significant otherwise it was considered as not statistically significant.
- Data were analyzed using SPSS software v.23.0. and Microsoft office.

**Inclusion criteria:**

- All the Type 2 Diabetes mellitus patients attending “Diabetic Clinic” at BLDEU Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur.

**Exclusion criteria:**

- Diabetics who are alcoholics, tobacco chewers, smokers
- Patients suffering from anemia and malignancy.

# RESULTS

50 Type 2 diabetes patients and 50 age and sex matched control subjects were included in the present study. The cytomorphological parameters i.e. NA, CA and NCR were compared between type 2 diabetic cases and non-diabetic controls. The age range of the patients in the study was from 30 to 70 years. The mean age of the diabetic patient in our study was 54.9 years and those of the control group was 49.2 years, as shown in Figure 11.

**Figure 11: MEAN AGE DISTRIBUTION OF DIABETIC AND CONTROL GROUPS**

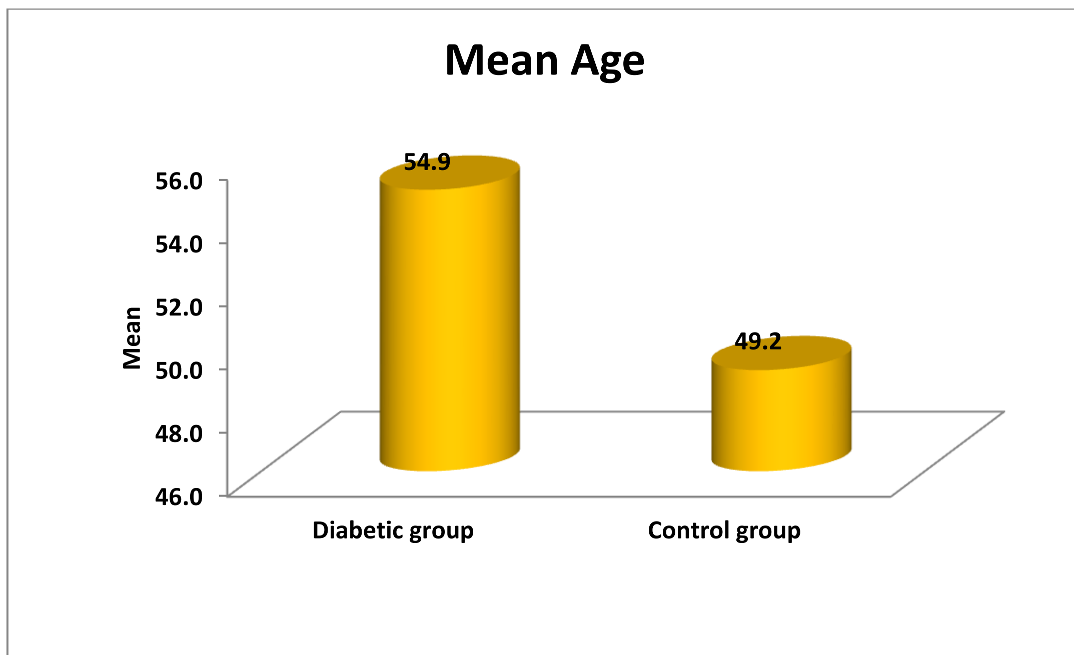
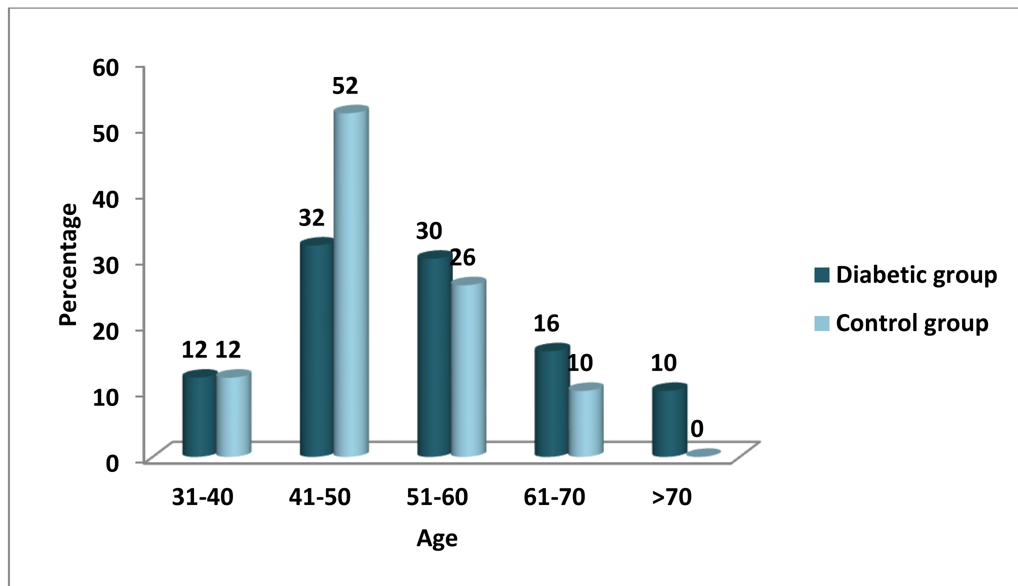


**Figure 12: AGE DISTRIBUTION OF DIABETIC AND CONTROL GROUPS**

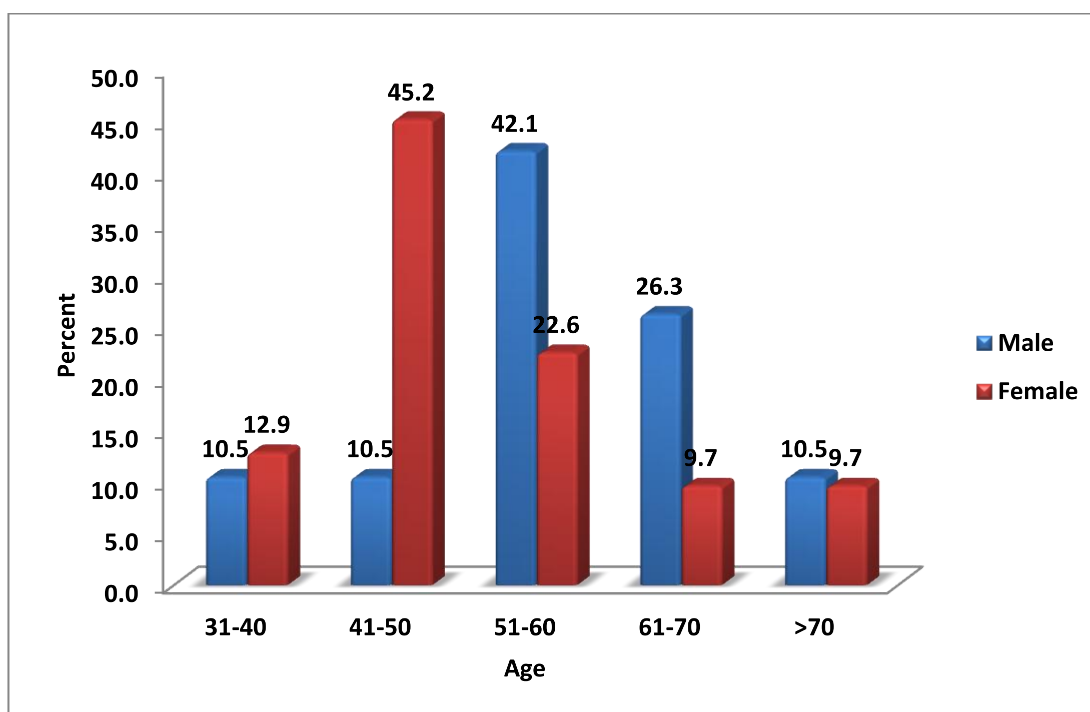



In our study the majority of the diabetic patients were in the $4^{th}$ to $5^{th}$ decades of life (depicted by figure 12).

**Table 5: AGE DISTRIBUTION OF DIABETIC GROUP**

| Age group (Yrs) | Male | | Female | | Chi sqr p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| 31-40 | 2 | 10.5 | 4 | 12.9 | 0.091 |
| 41-50 | 2 | 10.5 | 14 | 45.2 | |
| 51-60 | 8 | 42.1 | 7 | 22.6 | |
| 61-70 | 5 | 26.3 | 3 | 9.7 | |
| >70 | 2 | 10.5 | 3 | 9.7 | |
| Total | 19 | 100.0 | 31 | 100.0 | |

**Figure 13: AGE DISTRIBUTION GROUPS BY SEX.**




The majority of the diabetics in our study were females in the 4th to 5th decades of life and male diabetics were in the 5th to 6th decades of life. (Table 5 & Figure 13)

**Table: 6 AGE DISTRIBUTION OF HEALTHY CONTROLS**

| Age group (Yrs) | Male | | Female | | Chi sqr p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| 31-40 | 0 | 0.0 | 6 | 19.4 | 0.05 |
| 41-50 | 9 | 47.4 | 17 | 54.8 | |
| 51-60 | 6 | 31.6 | 7 | 22.6 | |
| 61-70 | 4 | 21.1 | 1 | 3.2 | |
| Total | 19 | 100.0 | 31 | 100.0 | |

In age and sex distribution of the healthy controls, the number of females were 54.8% and males were 47.4% both in their 4th to 5th decades of life. (Table 6 & Figure 14).

**Figure: 14 AGE DISTRIBUTION OF HEALTHY CONTROLS**

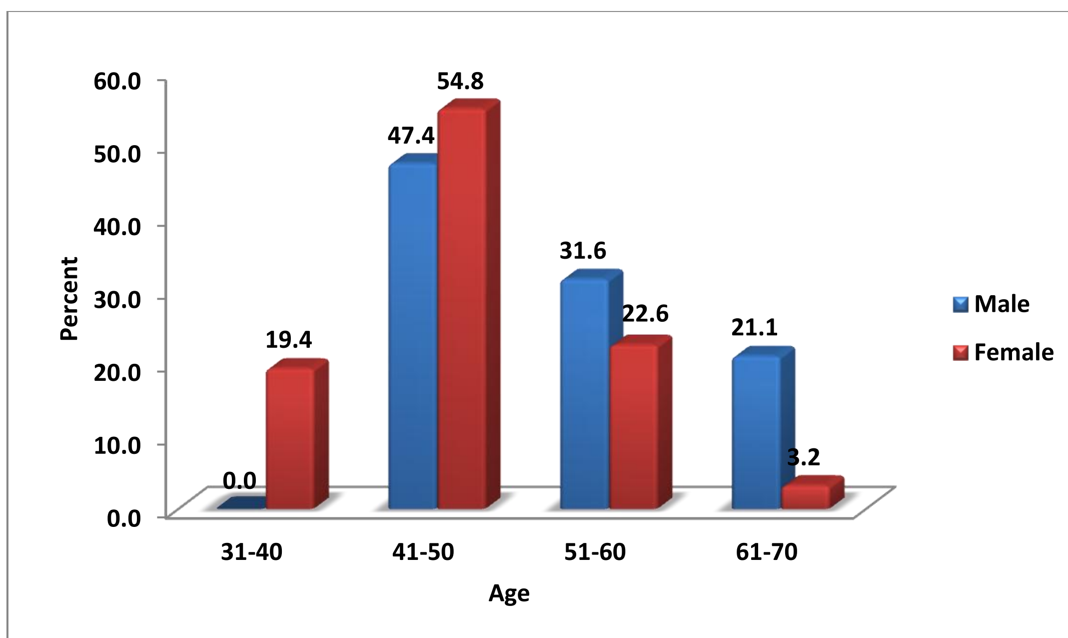
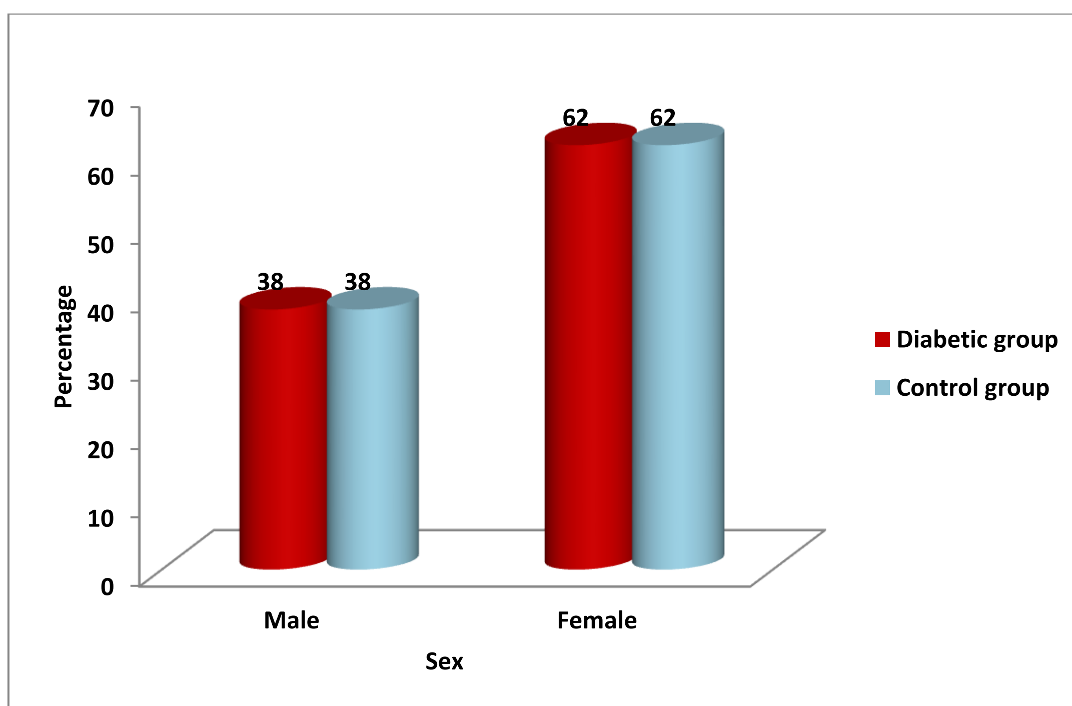


**Table: 7 SEX DISTRIBUTION BY GROUPS**

| Sex | Diabetic group | | Control group | | p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Male | 19 | 38 | 19 | 38 | 1 |
| Female | 31 | 62 | 31 | 62 | |
| Total | 50 | 100 | 50 | 100 | |

**Figure: 15 SEX DISTRIBUTION BY GROUPS.**




In the present study the total number of males both control and diabetes were 38 and number of females were 62. The total number of male patients with diabetes were 19 and females with diabetes were 31. As depicted by (Table 7& Figure15).

BLOOD SUGAR PARAMETERS.

The plasma blood sugar parameters, fasting blood sugar (FBS), post prandial 2 hours plasma glucose levels (PPBS) and $HbA_{1c}$ were evaluated among diabetics and compared to age and sex matched non diabetic control groups.

The mean FBS was 174.3 mg/dl (p value <0.001), PPBS was 283.3 mg/dl (p value <0.001). and $HbA_{1c}$ was 8.7 % (p value <0.001) in diabetic patients, depicted by Table 8 & Figure 16.

In the non-diabetic control group mean FBS was 102.6 mg/dl, PPBS was 150 mg/dl and $HbA_{1c}$ was 5.1% respectively, depicted by Table 8 & Figure 16.

**Figure: 16 MEAN BLOOD SUGAR DISTRIBUTION IN DIABETIC AND CONTROL GROUPS.**

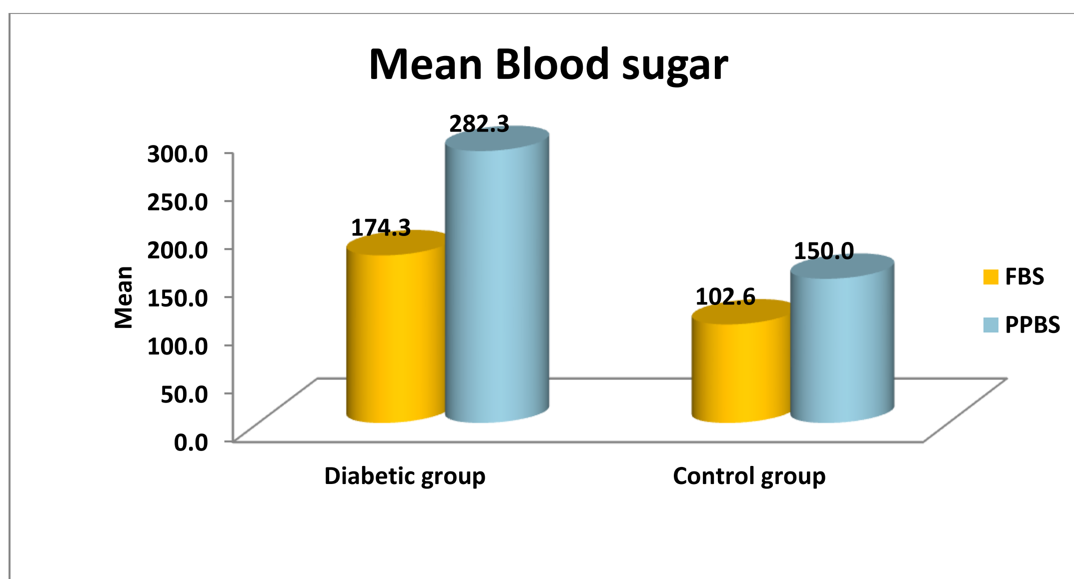


**Figure 17: MEAN WEIGHT DISTRIBUTION IN DIABETIC AND CONTROL GROUPS.**

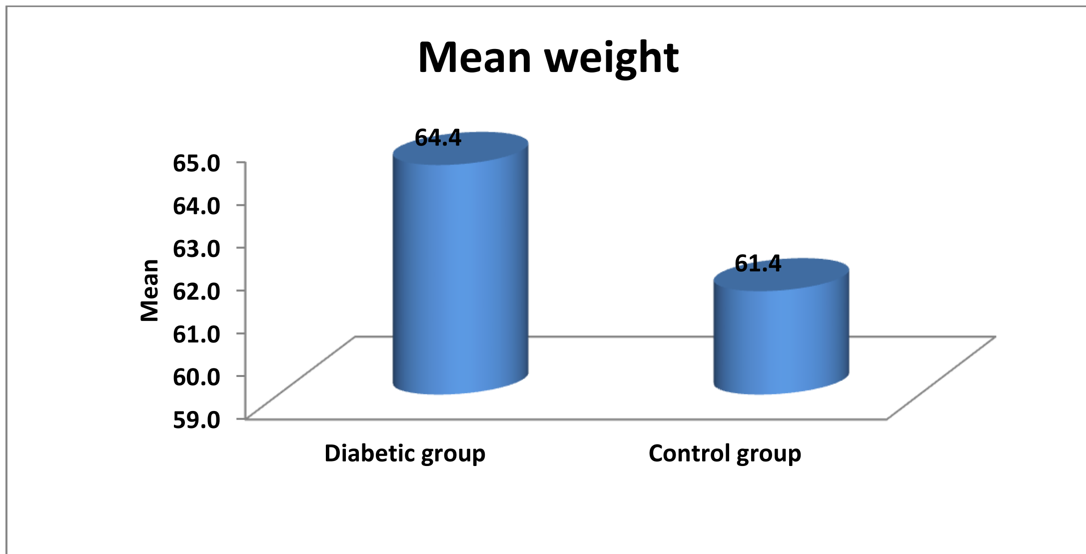
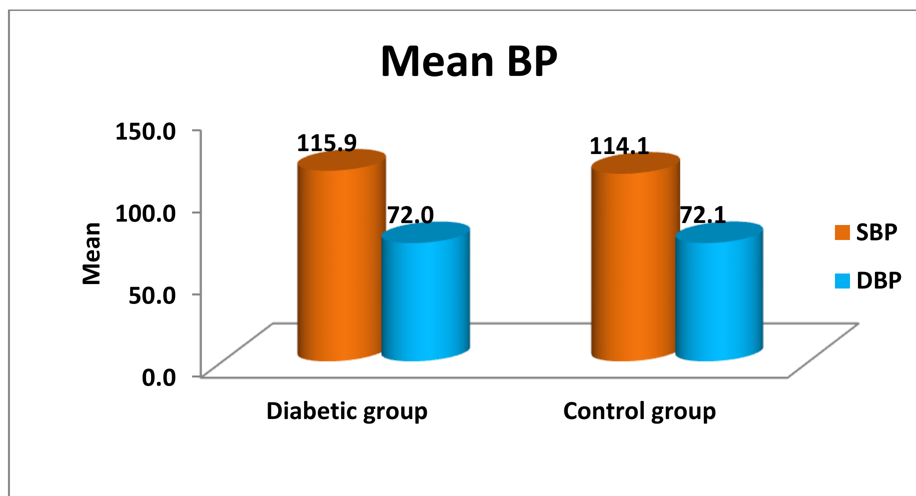



**Figure 18: MEAN BP IN DISTRIBUTION IN DIABETIC AND CONTROL GROUPS.**




The mean weight in diabetic and control groups were 64.4 kg and 61.4 kg respectively which showed no statistical significance. Figure 17.

Also the blood pressure did not show significant change between Diabetic and control groups. Figure 18.

**CYTOMORPHOMETRICAL PARAMETERS:**

The cytomorphometric parameters, **Nuclear Area** (NA), **Cytoplasmic Area** (CA) and **Nuclear cytoplasmic Ratio** (NCR) were evaluated using the DIMIZIMER IMAGE analyzer version 0.4.3 from the images of the buccal mucosal cells from both diabetic and non-diabetic control group and were compared. As depicted by (Figures 9 & 10 microphotographic images)

The average cytomorphometric parameter values along with the plasma glucose values (FBS and PPBS) the $HbA_{1c}$ values means were correlated and tabulated as depicted in Table 8.

The $HbA_{1c}$ was also determined for each individual both the control and diabetic groups and the mean $HbA_{1c}$ was calculated along with the selected parameters. The mean $HbA_{1c}$ and NCR are shown in figure 19(B).

Mean NA and CA in both the groups are depicted in figure 19(A).

**Figure: 19(A) Distribution of means of NA, CA in diabetic and control groups**

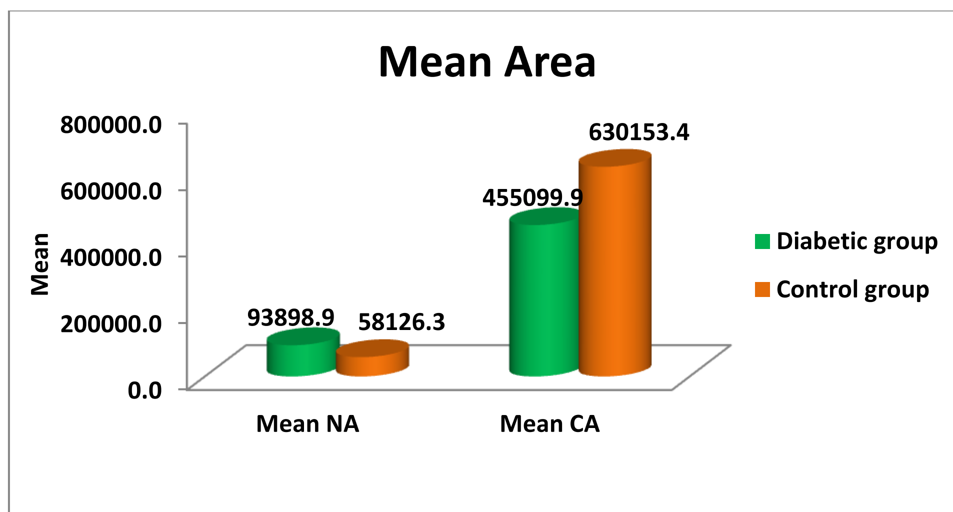


**Figure: 19(B) Means of NCR and $HbA_{1c}$ in diabetic and control groups.**

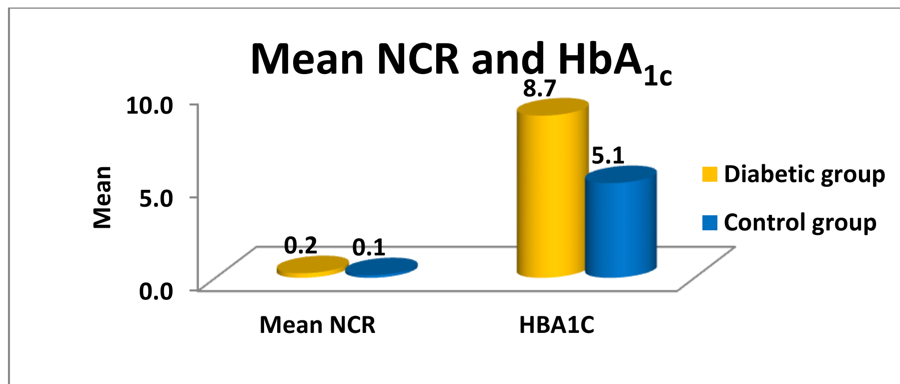



**Table: 8 COMPARISON OF MEAN PARAMETERS BETWEEN DIABETIC AND CONTROL.**

| Parameters | Diabetic group | | Control group | | p value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Age | 54.9 | 11.1 | 49.2 | 8.2 | 0.005* |
| FBS | 174.3 | 62.0 | 102.6 | 15.5 | <0.001* |
| PPBS | 282.3 | 99.5 | 150.0 | 23.9 | <0.001* |
| Weight | 64.4 | 5.9 | 61.4 | 7.9 | 0.038* |
| SBP | 115.9 | 8.6 | 114.1 | 7.5 | 0.263 |
| DBP | 72.0 | 7.0 | 72.1 | 5.9 | 0.963 |
| Mean NA | 93898.9 | 41727.6 | 58126.3 | 13410.5 | <0.001* |
| Mean CA | 455099.9 | 122960.8 | 630153.4 | 349161.9 | 0.001* |
| Mean NCR | 0.2 | 0.2 | 0.1 | 0.1 | <0.001* |
| $HbA_{1c}$ | 8.7 | 2.1 | 5.1 | 0.5 | <0.001* |

A significant increase in mean NA and NCR in Diabetic group was noted when compared to those of control group ($p<0.001$). It was also found that the Mean CA declined in Diabetic group when compared to control group. $HbA_{1c}$ was also significantly higher among diabetic patients.

We also attempted to assess the correlation between average $HbA_{1c}$ values and the average three cytomorphometric parameters (NA, CA and NC) in each group (Table 9).

We noted a significant increase in mean NA and NCR in Uncontrolled Diabetic group with compare to controlled and normal groups ($p<0.001$).

Mean CA declined in Uncontrolled Diabetic group in comparison to other groups. $HbA_{1c}$ was also significantly higher among uncontrolled diabetic patients. Blood pressure did not show significant change between study groups. As shown in table 9.

**Table: 9 COMPARISON OF MEAN PARAMETERS OF CONTROLLED, UNCONTROLLED DIABETICS AND HEALTHY NORMAL GROUP.**

| Variables | Groups | N | Mean | SD | ANOVA p value |
|---|---|---|---|---|---|
| Age | Controlled DM | 21 | 58.52 | 9.73 | 0.001* |
| | Uncontrolled DM | 29 | 52.28 | 11.48 | |
| | Normal | 50 | 49.22 | 8.23 | |
| | Total | 100 | 52.06 | 10.14 | |
| Weight | Controlled DM | 21 | 65.95 | 5.43 | 0.046* |
| | Uncontrolled DM | 29 | 63.21 | 6.05 | |
| | Normal | 50 | 61.42 | 7.94 | |
| | Total | 100 | 62.89 | 7.11 | |
| SBP | Controlled DM | 21 | 115.71 | 8.11 | 0.532 |
| | Uncontrolled DM | 29 | 116.00 | 9.13 | |
| | Normal | 50 | 114.06 | 7.50 | |
| | Total | 100 | 114.97 | 8.10 | |
| DBP | Controlled DM | 21 | 72.38 | 7.82 | 0.939 |
| | Uncontrolled DM | 29 | 71.72 | 6.52 | |
| | Normal | 50 | 72.06 | 5.93 | |
| | Total | 100 | 72.03 | 6.47 | |
| Mean NA | Controlled DM | 21 | 88226.57 | 11762.35 | <0.001* |
| | Uncontrolled DM | 29 | 98006.38 | 53913.42 | |
| | Normal | 50 | 58126.34 | 13410.52 | |
| | Total | 100 | 76012.60 | 35692.64 | |
| Mean CA | Controlled DM | 21 | 460859.61 | 123671.83 | 0.005* |
| | Uncontrolled DM | 29 | 450929.11 | 124463.50 | |
| | Normal | 50 | 630153.35 | 349161.94 | |
| | Total | 100 | 542626.63 | 274887.11 | |
| Mean NCR | Controlled DM | 21 | 0.22 | 0.14 | <0.001* |
| | Uncontrolled DM | 29 | 0.26 | 0.18 | |
| | Normal | 50 | 0.14 | 0.08 | |
| | Total | 100 | 0.19 | 0.14 | |
| $HBA_{1c}$ | Controlled DM | 21 | 6.57 | 0.32 | <0.001* |
| | Uncontrolled DM | 29 | 10.23 | 1.35 | |
| | Normal | 50 | 5.08 | 0.47 | |
| | Total | 100 | 6.89 | 2.37 | |

**Table: 10 Multiple Comparisons of mean parameters between Controlled, Uncontrolled Diabetics and Normal groups.**

| Variables | Groups | N | Mean | SD | p value |
|---|---|---|---|---|---|
| Mean NA | Controlled DM | 21 | 88226.6 | 11762.4 | 0.515 |
| | Uncontrolled DM | 21 | 98006.4 | 53913.4 | |
| | Controlled DM | 21 | 88226.6 | 11762.4 | 0.001* |
| | Normal | 50 | 58126.3 | 13410.5 | |
| | Uncontrolled DM | 21 | 98006.4 | 53913.4 | <0.001* |
| | Normal | 50 | 58126.3 | 13410.5 | |
| Mean CA | Controlled DM | 21 | 460859.6 | 123671.8 | 0.99 |
| | Uncontrolled DM | 21 | 450929.1 | 124463.5 | |
| | Controlled DM | 21 | 460859.6 | 123671.8 | 0.04* |
| | Normal | 50 | 630153.4 | 349161.9 | |
| | Uncontrolled DM | 21 | 450929.1 | 124463.5 | 0.012* |
| | Normal | 50 | 630153.4 | 349161.9 | |
| Mean NCR | Controlled DM | 21 | 0.22 | 0.14 | 0.513 |
| | Uncontrolled DM | 21 | 0.26 | 0.18 | |
| | Controlled DM | 21 | 0.22 | 0.14 | 0.042* |
| | Normal | 50 | 0.14 | 0.08 | |
| | Uncontrolled DM | 21 | 0.26 | 0.18 | <0.001* |
| | Normal | 50 | 0.14 | 0.08 | |

In this study we also attempted to analyze multiple comparison between Controlled DM, uncontrolled DM and normal cases. A significant increase in mean NA and NCR in uncontrolled and controlled diabetic group was noted as compared to normal cases ($p<0.05$).

Mean CA significantly declined in uncontrolled and controlled diabetic group with compare to normal cases. There is increase in values of Mean NA and mean NCR in uncontrolled DM group than controlled DM group but not significantly.(Table:10).

The variation of mean of NA, CA, NCR and $HbA_{1c}$ values were further evaluated in the controlled, uncontrolled diabetics and the healthy normal groups as depicted by figures 20 to 23.

**Figure 20: Variation of Mean NA between Controlled, Uncontrolled Diabetic and normal groups**

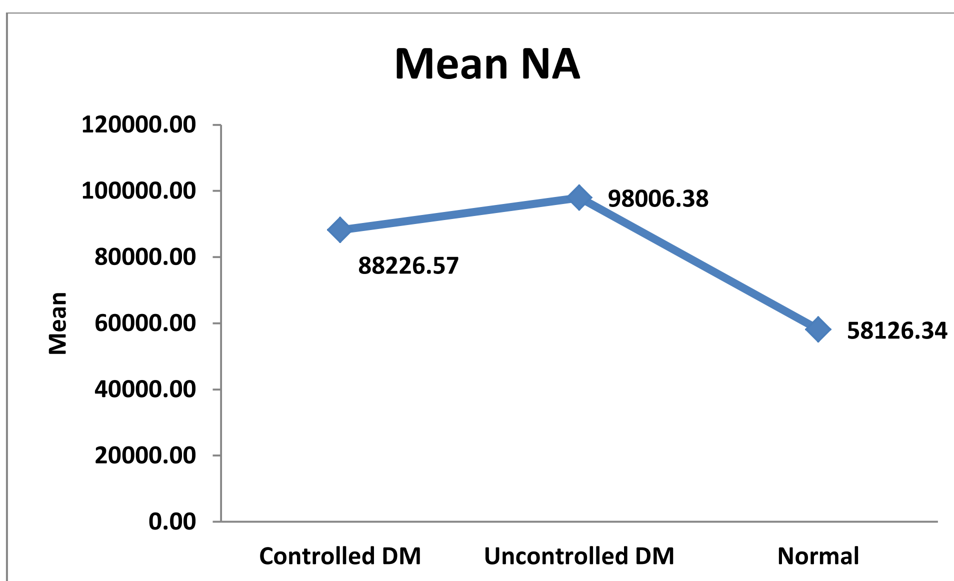



**Figure 21: Variation of Mean CA between Controlled, Uncontrolled Diabetic and normal groups**

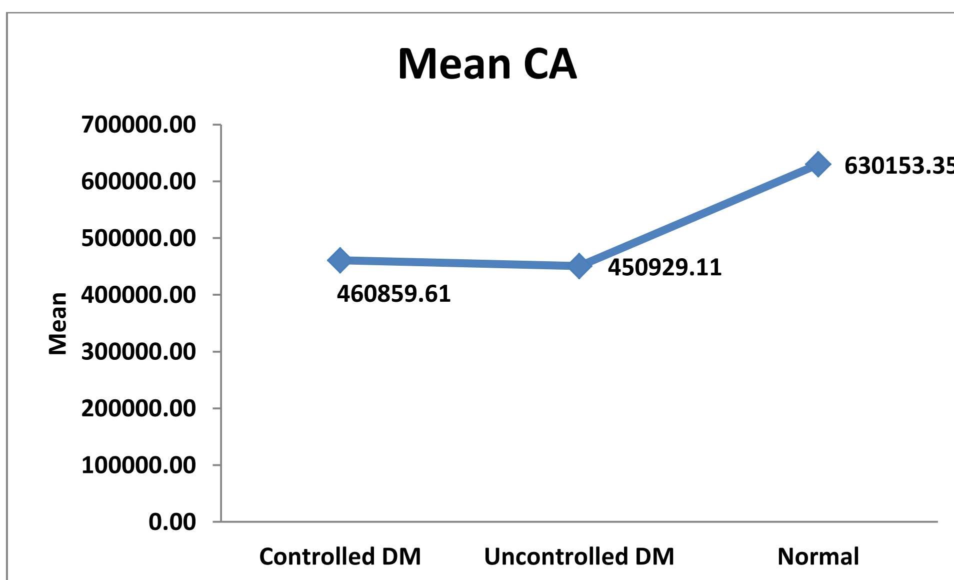


**Figure 22: Variation of Mean NCR between Controlled, Uncontrolled Diabetic and normal groups**

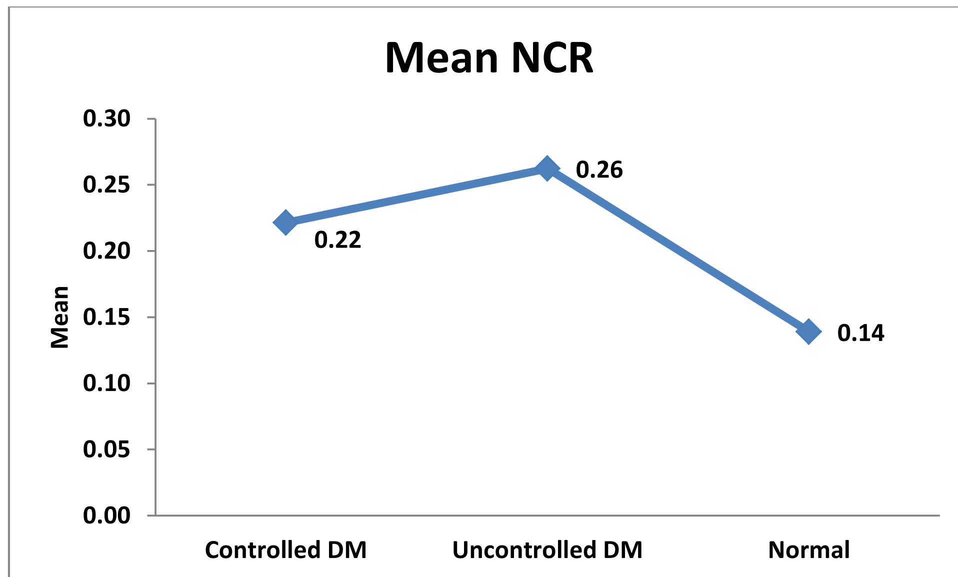



**Figure 23: Variation of Mean $HbA_{1c}$ between Controlled, Uncontrolled Diabetic and normal groups**

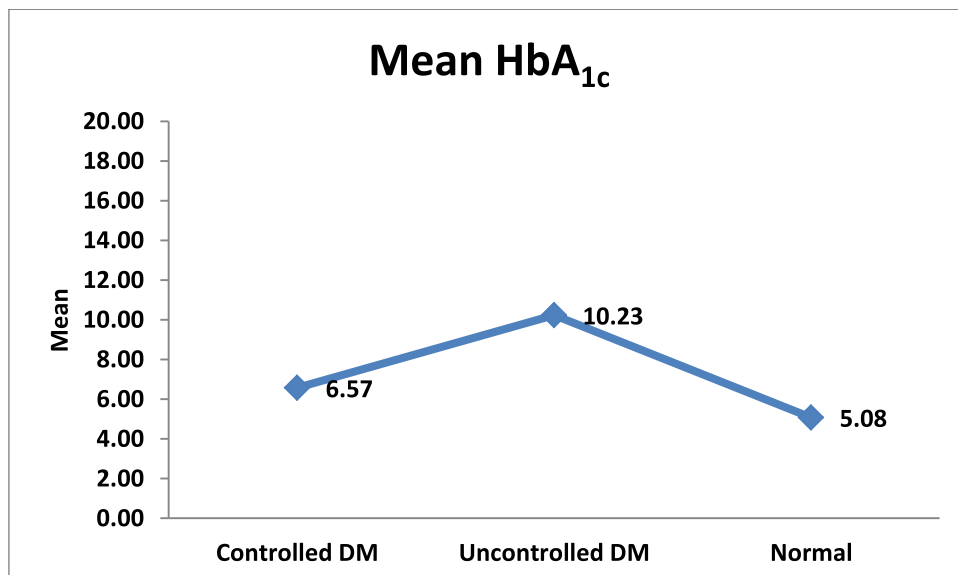


**Table: 11 Linear Regression analysis of effect of selected variables on mean NA**

| Variables | B | p value | 95.0% Confidence Interval for B | | Adjusted R Square |
|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | |
| Age | -0.012 | 0.897 | -707.3 | 620.5 | 0.243 |
| Gender | 0.099 | 0.29 | -6289.9 | 20822.9 | |
| $HBA_{1c}$ | 0.552 | 0.001* | 3282.8 | 13366.1 | |
| FBS | -0.36 | 0.126 | -509.8 | 63.9 | |
| PPBS | 0.299 | 0.182 | -51.9 | 269.6 | |

A Linear regression analysis of mean NA showed adjusted effect of $HbA_{1c}$. It is found that there is significant positive increase of 0.552 ($p<0.05$) in mean NA with unit increase in $HbA_{1c}$ values after adjusting age, sex, FBS and PPBS. (Table 11).

**Table: 12 Linear Regression analysis of effect of selected variables on mean CA**

| Variables | B | p value | 95.0% Confidence Interval for B | | Adjusted R Square |
|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | |
| Age | -0.189 | 0.075 | -10756.8 | 526.0 | 0.079 |
| Gender | -0.091 | 0.38 | -166328.1 | 64058.8 | |
| $HBA_{1c}$ | -0.049 | 0.791 | -48581.1 | 37100.3 | |
| FBS | 0.012 | 0.964 | -2381.4 | 2493.3 | |
| PPBS | -0.231 | 0.349 | -2013.1 | 718.9 | |

A Linear regression analysis of mean CA showed adjusted effect of $HbA_{1c}$. It is found that there is non-significant decrease of 0.049 ($p>0.05$) in mean CA with unit increase in $HbA_{1c}$ values after adjusting age, sex, FBS and PPBS.(Table 12).

**Table: 13 Linear Regression analysis of effect of selected variables on mean NCR**

| Variables | B | p value | 95.0% Confidence Interval for B | | Adjusted R Square |
|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | |
| Age | 0.034 | 0.748 | 0.00 | 0.00 | 0.059 |
| Gender | 0.019 | 0.859 | -0.05 | 0.06 | |
| $HBA_{1c}$ | 0.358 | 0.049* | 0.00 | 0.04 | |
| FBS | -0.093 | 0.722 | 0.00 | 0.00 | |
| PPBS | 0.046 | 0.854 | 0.00 | 0.00 | |

A Linear regression analysis of mean NCR showed adjusted effect of $HbA_{1c}$. It is found that there is significant increase of 0.358 ($p<0.05$) in mean NCR with unit increase in $HbA_{1c}$ values after adjusting age, sex, FBS and PPBS.(Table 13).

# DISCUSSION

Diabetes mellitus is fast emerging as a silent epidemic and a global health problem affecting millions of people irrespective of age and sex. In present scenario, India leads the world with the largest number of people with diabetes being referred the "diabetes capital of the world". It is one of the major causes of morbidity affecting both youth and middle aged people in India.[14,15]

The aetiology of diabetes mellitus is multifactorial and it brings about many secondary pathophysiological changes in multiple organ system including the oral cavity. Over the years several methods have been implemented to evaluate the oral epithelial mucosa in DM which includes incisional and excisional biopsy but it rarely used. [7-10]

However, the usage of exfoliative cytology is fast emerging as a more simplified and less aggressive procedure to evaluate the epithelial cell changes of the buccal mucosa, with the implementation of MIPS calibrated with ZEN software whereby we did the morphometric analysis of the cells using the parameters, NA, CA and N/C Ratio with the help of DIZIMIZER image analyzer and a qualitative and quantitative evaluation of the buccal epithelial cells were studied.

The present study included 100 subjects, 50 patients with Type2 DM and 50 age and sex matched healthy control individuals.

AGE:

Type 2 DM is more typically prevalent with increasing age. However, it is also being diagnosed more often in children and younger adults, mostly in obese adolescents. The onset of diabetes in the Indian population precedes a decade earlier s compared to the western counterparts.[15,18]

In the present study, the age ranged from 40 – 60 years. The mean age of patients in our study was 54.9±11.1 years. Majority of the patients diagnosed with Type 2 DM were in the 5th decade of life. This is in accordance with other studies conducted by Jajarm *et al*,[82] Survana *et al* [84] and Lamichane *et al*. [85]

**Type 2 diabetes mellitus and cytomorphometry:**

Exfoliative cytology imparts its role in the diagnosis, based on the principle that any change in the superficial cells can be the reflection of the change in the immediate underlying tissue. Several studies have analyzed the detrimental effects of diabetes on the oral mucosal cells with respect to the changes in the morphology of these cells which in turn may undermine the tissue function supporting the proneness to oral infections.[39-41]

Exfoliative cytology with the incorporation of the quantitative image analysis systems enables the assessment of the cytomorphometric cellular changes, and this study was conducted to evaluate the associated changes in the buccal mucosal cells in diabetes mellitus.

Chronic hyperglycemia in diabetes leads to the deterioration of the oxidative equilibrium which further leads to the depression of the oxidative scavengers and enzymes functions, favoring the unrestrained formation of free radicals and protein glycation products.[43-46]

These deleterious changes can lead to the injury of the biological structures at the cellular and molecular levels which can be acknowledged by oral exfoliated cytology with the incorporation of the image analyzer system increasing its reliability and reproducibility.

In the present study we evaluated the cytomorphometric alterations in the exfoliated cells of apparently normal buccal mucosa in the Type 2 DM patients and a comparison was made with those of the non-diabetic group in accordance to the parameters mentioned earlier.

There was a significant increase in the mean NA and NCR in diabetic group when compared to control group ($p < 0.001$). A decline in the CA was noted in the diabetic group. It was also noted that the in uncontrolled diabetics, the NA was significantly higher when compared with the controlled diabetics.

These findings were in concordance with the findings of studies done by Alberti *et al*[80], Shareef *et al* [81] and Prasad *et al*. [83]

Alberti *et al* [80] studied the oral smears of buccal mucosa, floor of the mouth, and dorsum of the tongue in Type 2 diabetes patients and found that the mean NA was higher in diabetics than the healthy control and no significant difference was noted with respect to the CA. It was attributed that these morphological alterations were due to inflammation of the oral mucosa in diabetics, possibly due to decreased salivary flow.

The increase in the NA in the diabetic group can be attributed by the delay in keratinization of the oral epithelium, effects of cellular ageing, dehydration/atrophy and associated inflammatory processes. The delay in keratinization is due to the glycation changes. Long term hyperglycemia leads to the accumulation of the advanced glycation end products of abnormal glycation of proteins, lipids and nucleic acids in the walls of the larger blood vessels and basement membrane of the microvasculature.[85,86]

The luminal narrowing of the vessels leads to the decreased tissue perfusion and deteriorating the cellular turnover attributing the delay in the keratization process of the epithelium, which further affects the epithelial differentiation process leading to the increase in the number of mature cells which have a larger nucleus.[84] The present study also showed similar observations.

Diabetic patients also suffer from dehydration due to the decreased salivary flow rates which further enhances the mucosal cell atrophy. So it was noted that the non-keratinized cells of the parabasal layers which are smaller in cell size with relatively larger nuclei were seen, giving the impression of nuclear enlargement without pleomorphism.[80]

The above changes were also observed in our study.

Various age- related studies showed insignificant statistical alteration in the CA in the oral epithelial cells of normal healthy subjects. Gender based studies noted that gender factor has no influence on either NA or CA of buccal epithelial cells.[89,90]

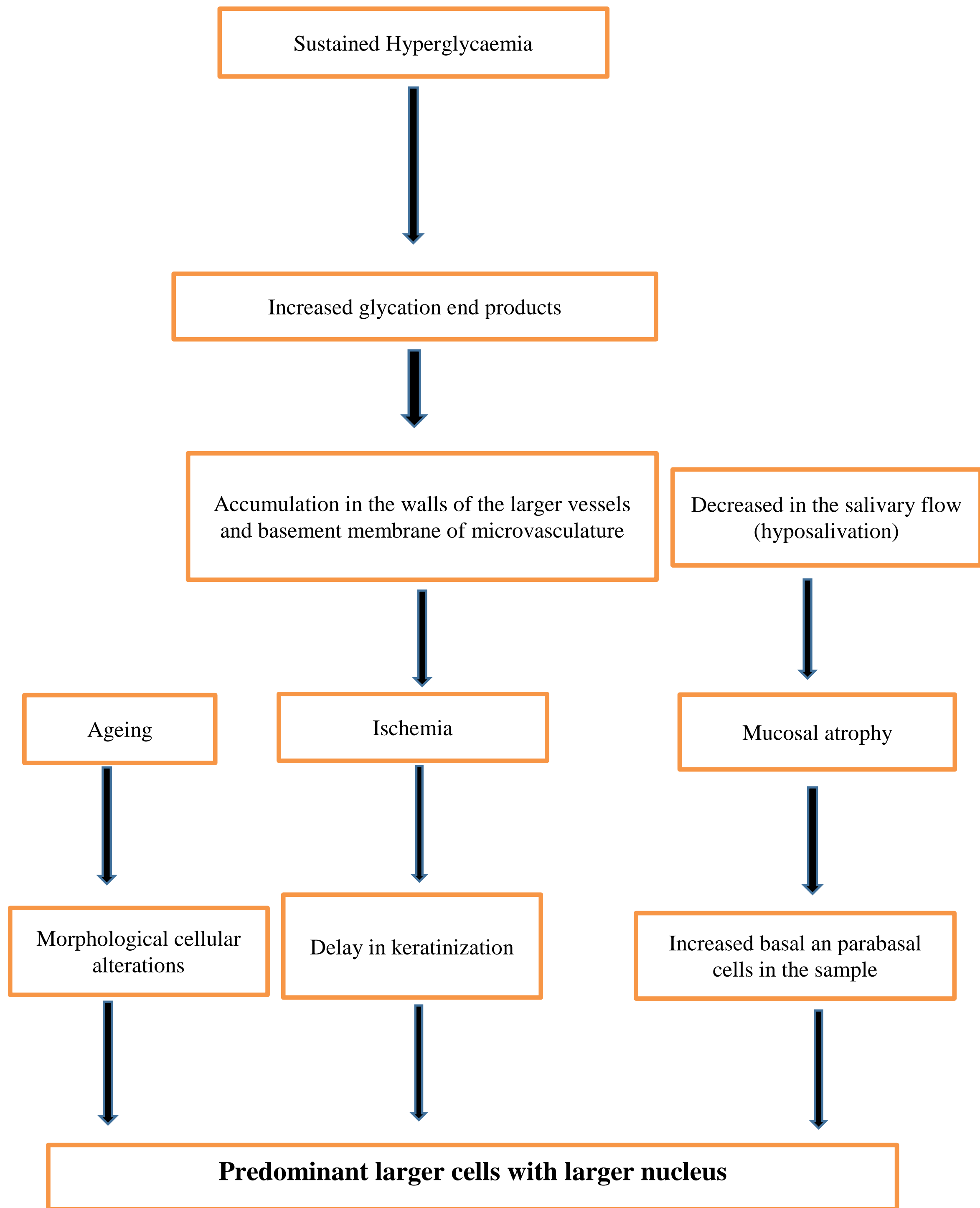



**Figure: 24 Flow chart depicting the mechanism of nuclear size alteration in DM.**

It was suggested that the increase in the NA in these individuals were due to the sustained chronic irritation of the oral mucosa caused by the tobacco consumption. Further it was suggested that the smoking leads to the delay epithelial cell differentiation leading to the predominance of the cells of the basal layer. [78]

Ogden *et al* [77]study also noted similar findings in the oral mucosal cells of the smokers similar those in diabetics.

Other conditions such as nutritional deficiencies like megaloblastic anaemia (vitamin B12 and folic acid) and iron deficiency, the NA changes in the buccal epithelial cells were found to be similar to those seen in diabetic individuals. This is suggested by the possible derangement in the DNA synthesis.[74] In our study the exclusion criteria were strictly kept into consideration to avoid all other conditions that give rise to increase in NA.

The possible hypothesis in correlating the increase in the NA in diabetic patients are as follows:

- Increases susceptibility of trauma of the oral mucosa in diabetic attributed to decreased salivary flow leading to xerostomia as well as increase in proneness to oral infections.
- The decrease in cellular turnover, the enhancement of the activity of the basal cells to compensate the loss of cells(atrophy) giving rise to the increase population of actively dividing cells with prominent nuclei.[75,76]

However, the concomitant increase in the CA along with the increased NA seen in actively proliferating cells was not noted in the current study this can be possibly attributed by the relative insulin deficiency state that hampers the glucose uptake by the cells required for their growth. Hence, the amount of cytoplasm a cells makes decreases relatively to the amount of nucleoplasm.

**Table:14 Comparison of the cytomorphometric parameters with other studies:**

| Author | N | Nuclear Area | Cytoplasmic area | N/C Ratio |
|---|---|---|---|---|
| Alberti *et al* [80] 2003 | Cases 10<br>Control 10 | Increased<br>(p=<0.05) | Decreased<br>P=NS | Increased<br>(p=<0.05) |
| Jajarm HH *et al* [82] 2008 | Cases 30<br>Control 30 | Increased<br>(p=<0.0001) | Increased<br>(p=<0.0001) | Increased<br>(p=<0.0001) |
| Shareef *et al* [81] 2008 | Cases 10<br>Control 10 | Increased<br>(p=<0.014) | Decreased<br>(p=<0.705) | Increased<br>(p=<0.002) |
| Prasad *et al* [83] 2010 | Cases 50<br>Control 5 | Increased<br>(p=<0.0367) | Decreased<br>(p=<0.0934) | Increased<br>(p=<0.1164) |
| Survana M *et al* [84] 2012 | Cases 40<br>Control 40 | Increased<br>(p=<0.001 | P=NS (p=0.826) | Decreased |
| Lamichane RS *et al* [85] 2015 | Cases 30<br>Control 30 | Increased<br>(p=<0.00079) | Increased<br>(p=<0.0027) | Increased<br>P=NS |
| Karthik KR *et al* [86] 2015 | Cases 20<br>Control 20 | Increased<br>(p=<0.00001) | NS(p=<0.99) | Increased<br>(p=<0.00002) |
| Sahay K *et al* [87] 2017 | Cases 30<br>Control 30 | Increased<br>(p=<0.001) | Decreased | Increased<br>(p=<0.001) |
| Our study | Cases 50<br>Control 50 | Increased<br>(p=<0.001) | Decreased | Increased<br>(p=<0.001) |

The significant increase in the NA and the decrease in the CA as in our study concurs with the findings of studies conducted by Alberti *et al*[80], Shareef *et al* [81], Prasad H *et al* [83] and Sahay K *et al.*[87]. In addition Prasad H *et al* [83] study further observed that the degree of glycaemia significantly affected the NA and N/C ratio i.e the severity of diabetes gradually increases the NA and NCR. However, Jajarm HH *et al*[82] study reported an increase in CA in diabetic group when compared with the control group.

The Survana M *et al* [84]study also noted an increase in the NA and significant decrease in NCR in diabetics but the CA showed no statistical difference between the diabetics and healthy individuals.

Lamichane RS *et al*[85] study evaluated the parameters from two sites, the buccal mucosa and tongue and they enumerated an increase in the NA, CA and NCR in Type 2 DM patients when compared to the control group. They further concluded that there was only mild correlation with these parameters with the glucose levels, NA (R=0.27) showed slight correlation, CA (R=0.34) showed mild correlation while N/C ratio (R=0.083) showed no correlation with glucose levels.

Karthik *et al* [86]study evaluated the cytomorphometric parameters of the buccal epithelial cell changes in diabetic (n=40) and control group (n=20), they further attempted to correlate the $HBA_{1c}$ values and concluded that there existed significant correlation between $HbA_{1c}$ and average NA and NCR. However, they noted that no significant correlation with CA.

In our study among the diabetic group (n=50) there were more number of individuals with uncontrolled glycemic control where $HbA_{1c}$ was more than 9%

(n=29).This may be attributed as a result of poor dietary practices and lack of adequate awareness and knowledge regarding the diet and exercise regimens that are ought to be followed in diabetics.

We also made an attempt to correlate the $HbA_{1c}$ value and the average of the three cytomorphometric parameters( NA,CA and NCR) with the intra groups of heathy individuals, controlled and uncontrolled diabetics and the mean Hb $A_{1c}$ values in these groups were 5.08, 6.57 and 10.23 respectively.

A linear regression analysis of the mean of NA, CA and N/C ratio with adjusted effects of Hb $A_{1c}$ along with the selected variables like age, gender, FBS and PPBS was conducted. It was found that there exist a significant positive increase of 0.552($p<0.05$) in mean NA, increase of 0.358($p<0.05$) in mean NCR with unit increase in $HbA_{1c}$ values after adjusting with age, sex, FBS and PPBS.

However, we found a non-significant decrease of 0.049($P>0.05$) in mean CA with unit increase in Hb $A_{1c}$ values after adjusting age, sex, FBS and PPBS. In general, it was concluded that with the increasing severity of diabetes a gradual increase in the NA and NCR was noted.

This finding concur with the studies conducted by Shareef *et al*[81] , Prasad *et al*[83] and Karthik KR *et al.*[86]

Our data suggest that cytomorphometric evaluation of the parameters can emerge as an efficient aid to comprehend the magnitude of the cellular changes that occur in the buccal epithelial cells as a result of diabetes and its concomitant secondary pathophysiological processes like microvascular changes and oxidative stress at the molecular level.

We further enumerated that diabetes can attribute to these cytomorphometric changes in the buccal cells and may occur independently of factors such as age, sex, smoking and nutritional deficiencies states.

Hence, cytomorphometric analysis of the buccal mucosal cells with the incorporation of the computer based image processing system can complement as a non-invasive procedure for monitoring the disease status in diabetic patients.

# CONCLUSION

The study was undertaken to evaluate the cytomorphometric parameters nuclear area (NA), cytoplasmic area (CA) and nuclear cytoplasmic of the exfoliated buccal epithelial cells in Type 2 diabetes mellitus.

We observed that there was a significant increase in the NA and NCR in Type 2 diabetes patients when compared to the healthy control group. In the context of the $HbA_{1c}$ values correlation with the individual parameters, it was noted that with the degree of severity of diabetes there existed a gradual increase in the NA and the NCR. The changes in the parameters in can further enhance our understanding and knowledge of the disease process at a cellular level and correlating these changes with the progressive stages of Type 2 DM. The objective demonstration of the nuclear and cytomorphometric alterations indicates the existence of the cytological changes despite the normal clinical presentations.

Oral exfoliative cytology can be implemented as a reliable, cost effective, non-invasive, simple procedure to screen the diabetes for microscopic evaluation of the mucosa and in monitoring the glycemic control keeping in view of the correlation between the $HbA_{1c}$ and the cellular parameters.

# SUMMARY

- This study was undertaken in Shri B.M.Patil Medical College, Vijyapur, Karnataka to evaluate the nuclear and cytomorphometric analysis of exfoliative buccal mucosal cells in Type 2 Diabetes Mellitus.
- A total of 100 cases were studied and further divided into two groups of 50 each, who were diagnosed with Type 2 Diabetes Mellitus and age and sex matched healthy controls.
- Majority of the patients with diabetes were in the $4^{th}$ to $5^{th}$ decades of life.
- The total number of males in Type 2 DM group were 19 and females were 31.
- In the present study, the diabetic cases were further divided into based on the $HbA_{1c}$ values, controlled diabetics HbA1c level <7% (n=21) and uncontrolled diabetics with Hb $A_{1c}$ level >9 (n=29). The mean $HbA_{1c}$ in controlled and uncontrolled DM were 6.75% and 10.23% respectively.
- The mean FBS was 174± 62 mg/dl (p value < 0.001), PPBS was 282.3± 99.5 mg/dl (p value < 0.001) in diabetic patients which was significantly higher than the non- diabetics.
- The mean NA in diabetic cases was 93898.9±41727.6 μm$^2$ compared to the non- diabetics 58126.3± 13410.5 μ m$^2$ where it was statistically increased significantly (p value < 0.001).
- The mean CA in diabetic cases was 455099.9±122960.8 μm$^2$ when compared to the heathy group which was 630153.4±349161.9 μm$^2$ where it showed no statistical significance.

- The mean N/C ratio in the diabetic group was 0.2±0.2 and those of the control group was 0.1±0.1 where it showed statistical significance increase (p value <0.001)
- Multiple comparison between controlled DM, uncontrolled DM and normal healthy subjects showed a significant increase in mean NA and N/C ratio in controlled and uncontrolled diabetics when compared to healthy group($p<0.05$). However, mean CA declined in uncontrolled and controlled diabetic groups when compared to healthy group.
- Furthermore a linear regression analysis of effect of selected variables (age, gender, $HbA_{1c}$, FBS and PPBS) with each of the cytomorphometric parameters was evaluated, which showed significant positive increase of 0.552($p<0.05$) in mean NA and a significant increase of 0.358($p<0.05$) in mean NCR with unit increase in $HbA_{1c}$ values after adjusting with age, sex, FBS and PPBS.
- On the other hand, the linear regression analysis of mean CA with adjusted effect of $HbA_{1c}$ found that there was a non-significant decrease of 0.049($p>0.05$) in mean CA with unit increase in $HbA_{1c}$ values after adjusting with age, sex, FBS and PPBS.

# LIMITATIONS OF THE STUDY

- Follow up of the cases was not possible to determine the prognostic significance of our findings.
- However, these alterations in the cytomorphometric parameters are not unique to diabetes and so they cannot be considered as predictive and diagnostic but can be taken care of by eliciting a detailed history and proper clinical examination.

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**ANNEXURE-I**

**ETHICAL CLEARANCE CERTIFICATE**

B.L.D.E.UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103

INSTITUTIONAL ETHICAL COMMITTEE No/SEf/2015 20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

*The Ethical Committee of this college met on* 17-11-2015 *at* 03 pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

*Title* "Nuclear Morphometric and Morphological study of exfoliated buccal mucosal cells in type-2 Diabetes mellitus patients"

*Name of P.G. Student :* Dr Wahengbam Jyotsna Devi, Dept of Pathology

*Name of Guide/Co-investigator :* Dr. B.R. Yelikar. Prof & HoD.

DR.TEJASWINI VALLABHA
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization

1)Copy of Synopsis/Research Project

2)Copy of informed consent form.

3)Any other relevant documents.

## ANNEXURE-II

## SAMPLE INFORMED CONSENT FORM

| | | |
|---|---|---|
| **TITLE OF THE PROJECT** | **:** | NUCLEAR MORPHOMETRIC AND MORPHOLOICAL STUDY OF EXFOLIATIVE BUCCAL MUCOSAL CELLS IN TYPE-2 DIABETES MELLITUS PATIENTS. |
| **PRINCIPAL INVESTIGATOR** | **:** | Dr. WAHENGBAM JYOTSNA DEVI<br>P.G.<br>DEPARTMENT OF PATHOLOGY |
| **P.G. GUIDE** | **:** | Dr. B.R.YELIKAR M.D.<br>PROFESSOR & HEAD<br>DEPARTMENT OF PATHOLOGY |

**PURPOSE OF THE REASERCH:** I have been informed that this study is being conducted to evaluate the cytomorphometric parameters in the exfoliative buccal mucosal cells in Type-2 Diabetes mellitus which can substantiate in the diagnosis and prognosis of DM.

**PROCEDURE:** I am aware that smears will be taken from the buccal mucosa after properly explaining the procedure under aseptic precautions.

**RISK AND DISCOMFORTS:** I am aware that, there is no risk pertaining from the procedures performed.

**BENEFITS:** I understand that my participation in the study will help to know the implications of the associated changes brought about by DM and can be useful as an aid in the diagnosis.

**CONFIDENTIALITY:**

I understand that the medical information obtained from the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is utilized for publication purposes the identity of patient will not be disclosed.

**REQUEST FOR MORE INFORMATION:**

I am aware of the fact that I may be ask relevant questions concerning the study at any point of time.

**REFUSAL FOR WITHDRAWAL OF PARTICIPATION:**

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any point of time. I am also aware that Dr. WAHENGBAM JYOTSNA DEVI may withdraw my participation in the study after she has explained the reasons for doing so.

**INJURY STATEMENT:**

I understand that in the unlikely event of injury inflicted to me during the study I will get medical treatment but no further compensations.

I have explained the patient regarding the implications of the study, the required procedure and the possible risk ad benefits to the best of my ability in the conversational language.

_______________________________ ___________________

**Dr. WAHENGBAM JYOTSNA DEVI** Date

(Investigator)

**STUDY SUBJECT CONSENT STATEMENT:**

I hereby confirm that Dr. WAHENGBAM JYOTSNA DEVI has explained to me the purpose of the research, the study procedure, that I will undergo and the possible discomforts as well as the benefits that I may experience. I have been explained all the above in detail in my own dialect and I understand the same. Therefore I agree to render the consent to participate as a subject in this research project.

_______________________ __________________

(Participant) Date

_________________

(Witness to signature) Date

# ANNEXURE-III

## PERFORMA FOR THE STUDY OF THE CYTOMORPHOMETRIC EVALUATION

**Demographic Details:**

1. Name: 2. Age : 3. Sex: M/F 4. OPD / IPD no.:
5. Present history:
6. Past history :
7. Family history :
8. **History of intake of drugs:**

9. General physical examination**:**

Pallor : Icterus :

Built : Nourishment :

BMI :

10. Vitals:-

Pulse: BP: RR:

Temperature: Weight:

11. Systemic Investigation:
12. Biochemical Investigations:

- Random blood glucose (RBS)
- Fasting plasma glucose (FPG)
- Two -hour plasma glucose (PP)
- Hb $A_{1c}$

13.Clinical diagnosis:

14.Smear collection and staining with Pap stain reagents.

15.Cytomorphometric analysis of exfoliative cytology.

| Parameters | Measurements. |
|---|---|
| Nuclear area ($\mu m^2$) | |
| Cytoplasmic area($\mu m^2$) | |
| Nuclear : Cytoplasm ratio | |

**KEY TO MASTER CHART**

| | |
|---|---|
| $HbA_{1c}$ | Glycosylated hemoglobin |
| BP | Blood pressure |
| FBS | Fasting blood sugar |
| PPBS | Post prandial blood sugar |
| DBP | Diastolic blood pressure |
| SBP | Systolic blood pressure |
| Mean NA | Mean nuclear area |
| Mean CA | Mean cytoplasmic area |
| Mean NCR | Mean nuclear cytoplasmic ratio |

# MASTER CHART

| Sn | Name | Group | OP/IP No. | Age | Gender | FBS | PPBS | Weight | SBP | DBP | Mean NA of 20 cells | Mean CA of 20 cells | Mean NCR Of 20 cells | $HbA_{1c}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Jayshree | DM | 54920 | 45 | Female | 147 | 200 | 50 | 120 | 80 | 73836 | 266724.7 | 0.19 | 9.3 |
| 2 | Parvati | DM | 55484 | 43 | Female | 250 | 407 | 55 | 110 | 70 | 77299 | 837962.8 | 0.22 | 10.4 |
| 3 | Sidawwa | DM | 45174 | 45 | Female | 245 | 352 | 60 | 120 | 70 | 66741 | 541521.8 | 0.16 | 9.2 |
| 4 | Mahadevi | DM | 45156 | 58 | Female | 158 | 320 | 67 | 120 | 75 | 51635 | 273701.8 | 0.17 | 9 |
| 5 | Kasturibai | DM | 55053 | 55 | Female | 130 | 190 | 70 | 110 | 67 | 82234 | 451680.3 | 0.21 | 6.7 |
| 6 | Neelabai | DM | 55243 | 45 | Female | 126 | 189 | 60 | 110 | 60 | 93109 | 356116.8 | 0.25 | 6.2 |
| 7 | Shantabai | DM | 45165 | 60 | Female | 126 | 202 | 55 | 120 | 80 | 82217 | 497333.8 | 0.16 | 6.4 |
| 8 | Malamma | DM | 55272 | 47 | Female | 128 | 210 | 60 | 100 | 60 | 101940 | 485837 | 0.17 | 7 |
| 9 | Sobha | DM | 55247 | 40 | Female | 130 | 204 | 67 | 110 | 66 | 80658 | 606324 | 0.18 | 9 |
| 10 | Tarabai | DM | 54970 | 70 | Female | 127 | 200 | 65 | 120 | 70 | 93136 | 573387.8 | 0.24 | 6.3 |
| 11 | Kashibai | DM | 41784 | 80 | Female | 138 | 210 | 60 | 110 | 75 | 93373 | 461931.5 | 0.23 | 9.4 |
| 12 | Gurubai | DM | 54941 | 50 | Female | 128 | 200 | 65 | 120 | 80 | 91140 | 340089.3 | 0.19 | 7.2 |
| 13 | Parvati | DM | 54955 | 75 | Female | 129 | 200 | 56 | 110 | 70 | 66494 | 347031.8 | 0.17 | 6.5 |
| 14 | Ramesh | DM | 45155 | 54 | Male | 196 | 240 | 70 | 120 | 80 | 75849 | 390976 | 0.29 | 10 |
| 15 | Aravind | DM | 45153 | 56 | Male | 151 | 264 | 73 | 110 | 70 | 61815 | 319343.3 | 0.24 | 9.6 |
| 16 | Kalidas | DM | 54965 | 40 | Male | 200 | 359 | 60 | 120 | 76 | 79725 | 290723.8 | 0.21 | 10.3 |
| 17 | Birader G | DM | 54963 | 76 | Male | 125 | 200 | 70 | 120 | 80 | 85010 | 390298.8 | 0.21 | 6.4 |
| 18 | Malappa | DM | 8352 | 65 | Male | 128 | 368 | 67 | 110 | 70 | 72347 | 399401.8 | 0.15 | 6.5 |
| 19 | Basavaraj | DM | 8555 | 70 | Male | 285 | 400 | 67 | 120 | 80 | 74550 | 384275.5 | 0.22 | 9.8 |
| 20 | B.Masali | DM | 65595 | 58 | Male | 127 | 244 | 70 | 126 | 70 | 67718 | 481561.5 | 0.4 | 9.6 |
| 21 | Guruppa | DM | 65594 | 52 | Male | 116 | 203 | 68 | 120 | 76 | 92453 | 539784.5 | 0.15 | 6.5 |
| 22 | Laxmibai | DM | 65606 | 71 | Female | 132 | 191 | 68 | 130 | 80 | 124394 | 446084.5 | 0.49 | 9.4 |
| 23 | Kashimsab | DM | 65502 | 72 | Male | 82 | 210 | 66 | 130 | 85 | 78303 | 377355.5 | 0.19 | 6.1 |

| 24 | Anil Hudar | DM | 73835 | 59 | Male | 124 | 208 | 70 | 120 | 76 | 107114 | 779173.8 | 0.17 | 6.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | Solochana | DM | 73854 | 46 | Female | 246 | 309 | 58 | 110 | 70 | 96082 | 478534.5 | 0.21 | 9.2 |
| 26 | Pramila K | DM | 73868 | 70 | Female | 156 | 270 | 66 | 120 | 70 | 93314 | 532328.5 | 0.17 | 9 |
| 27 | Akubai | DM | 73899 | 47 | Female | 175 | 236 | 55 | 110 | 60 | 105265 | 466731.5 | 0.35 | 9.3 |
| 28 | Timanna | DM | 73880 | 65 | Male | 251 | 342 | 68 | 120 | 70 | 71801 | 428032.3 | 0.16 | 9.3 |
| 29 | Nagappa | DM | 73954 | 68 | Male | 200 | 298 | 70 | 120 | 75 | 95650 | 596393.5 | 0.19 | 10 |
| 30 | Manjula M | DM | 73997 | 48 | Female | 305 | 513 | 58 | 100 | 60 | 89818 | 439083.8 | 0.2 | 13.3 |
| 31 | Irappa K | DM | 73960 | 70 | Male | 105 | 220 | 69 | 120 | 80 | 74056 | 400456.3 | 0.25 | 6.4 |
| 32 | K.L.Kumnar | DM | 74150 | 55 | Male | 115 | 210 | 73 | 120 | 70 | 90404 | 463640.5 | 0.21 | 7 |
| 33 | Mangala B | DM | 74160 | 40 | Female | 262 | 550 | 57 | 110 | 65 | 96281 | 351361.5 | 0.26 | 11.5 |
| 34 | Subhadra | DM | 125512 | 60 | Female | 225 | 380 | 66 | 100 | 65 | 69844 | 310762 | 0.32 | 9.5 |
| 35 | S.V.Dulage | DM | 125151 | 57 | Male | 128 | 186 | 71 | 130 | 80 | 88197 | 341170.8 | 0.81 | 7.2 |
| 36 | Nigamma | DM | 125307 | 48 | Male | 130 | 208 | 66 | 120 | 75 | 240746 | 339271 | 0.25 | 10 |
| 37 | Laxmibai | DM | 125119 | 50 | Female | 227 | 438 | 67 | 130 | 80 | 107589 | 647169.3 | 0.17 | 11 |
| 38 | Malawwa | DM | 125616 | 48 | Female | 261 | 380 | 59 | 110 | 70 | 112610 | 606485.5 | 0.17 | 6.5 |
| 39 | Kamala H | DM | 125358 | 54 | Female | 133 | 170 | 64 | 100 | 60 | 90701 | 438088.8 | 0.27 | 6.5 |
| 40 | Kasturi B | DM | 125367 | 56 | Female | 133 | 160 | 70 | 110 | 60 | 77327 | 417016.8 | 0.16 | 6.4 |
| 41 | Kasturibai | DM | 125190 | 40 | Female | 224 | 400 | 55 | 100 | 63 | 85352 | 562380.8 | 1.1 | 9.5 |
| 42 | Parvati | DM | 125397 | 64 | Female | 104 | 248 | 74 | 120 | 76 | 103971 | 716251.5 | 0.16 | 7 |
| 43 | Ramijan S | DM | 125131 | 50 | Male | 252 | 460 | 65 | 120 | 80 | 320382 | 387858.8 | 0.17 | 12.1 |
| 44 | Tipanna | DM | 125196 | 40 | Male | 304 | 430 | 70 | 120 | 80 | 85604 | 441839.5 | 0.2 | 14 |
| 45 | Renuka S | DM | 116541 | 57 | Female | 212 | 298 | 69 | 128 | 70 | 86355 | 465295.3 | 0.19 | 13 |
| 46 | Rajesjwari | DM | 116388 | 45 | Female | 213 | 295 | 56 | 130 | 75 | 88571 | 445538.8 | 0.2 | 11.2 |
| 47 | Shoba N | DM | 116658 | 45 | Female | 120 | 210 | 63 | 110 | 70 | 80053 | 446000.3 | 0.18 | 6.3 |
| 48 | Sushila M | DM | 116386 | 40 | Female | 203 | 265 | 58 | 110 | 70 | 76216 | 501908.8 | 0.22 | 10.3 |
| 49 | Shruthi H | DM | 116864 | 42 | Female | 295 | 450 | 62 | 100 | 60 | 105722 | 381323.8 | 0.25 | 9.6 |

| 50 | Shamu S | DM | 116732 | 54 | Male | 109 | 220 | 70 | 120 | 80 | 89942 | 311450.8 | 0.17 | 6.4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | Bhimawwa | Healthy control | 4508 | 62 | Female | 120 | 170 | 50 | 120 | 75 | 48924.7 | 624615 | 0.07 | 5.1 |
| 52 | Chanlawwa | Healthy control | 4501 | 60 | Female | 123 | 150 | 45 | 110 | 65 | 42218.7 | 494337.8 | 0.08 | 5.6 |
| 53 | Kasturibai | Healthy control | 4514 | 60 | Female | 120 | 145 | 52 | 110 | 70 | 47610.5 | 605267.5 | 0.12 | 4.8 |
| 54 | Mahantama | Healthy control | 4518 | 45 | Female | 125 | 147 | 57 | 125 | 68 | 53688.5 | 451466 | 0.1 | 4.9 |
| 55 | Katumbe | Healthy control | 4233 | 43 | Female | 120 | 156 | 47 | 110 | 73 | 63608.5 | 429162 | 0.15 | 5 |
| 56 | Shantabai | Healthy control | 45159 | 52 | Female | 124 | 170 | 54 | 120 | 76 | 34003.71 | 449330.5 | 0.07 | 4.7 |
| 57 | Bouramma | Healthy control | 45133 | 35 | Female | 110 | 150 | 56 | 110 | 67 | 36924 | 407594.8 | 0.1 | 5.2 |
| 58 | Kamabai | Healthy control | 45390 | 42 | Female | 109 | 167 | 64 | 120 | 80 | 41514.31 | 501267 | 0.12 | 4.8 |
| 59 | Rama | Healthy control | 55220 | 37 | Female | 115 | 170 | 55 | 110 | 68 | 59967.2 | 408117 | 0.12 | 4.7 |
| 60 | Sangamma | Healthy control | 45597 | 40 | Female | 107 | 160 | 50 | 120 | 76 | 49636.47 | 542938.8 | 0.1 | 4.8 |
| 61 | Bharati | Healthy control | 55477 | 40 | Female | 90 | 127 | 56 | 110 | 70 | 49636.47 | 542938.8 | 0.33 | 5.1 |
| 62 | Laxmibai | Healthy control | 54545 | 38 | Female | 100 | 120 | 50 | 110 | 70 | 76678 | 2161978 | 0.25 | 5.4 |
| 63 | Basalingama | Healthy control | 42105 | 45 | Female | 70 | 156 | 60 | 120 | 76 | 76438.9 | 667045.3 | 0.12 | 5 |
| 64 | Apasab | Healthy control | 45154 | 65 | Male | 68 | 125 | 60 | 110 | 70 | 68932.2 | 306930.3 | 0.34 | 5.1 |
| 65 | Hantamtray | Healthy control | 45163 | 48 | Male | 120 | 143 | 66 | 120 | 80 | 60260.2 | 633752 | 0.11 | 5.3 |
| 66 | Anandappa | Healthy control | 45383 | 70 | Male | 83 | 159 | 66 | 110 | 75 | 80058.2 | 626139.3 | 0.09 | 4.2 |
| 67 | Chandappa | Healthy control | 45822 | 65 | Male | 80 | 145 | 70 | 110 | 80 | 51534.7 | 522021.8 | 0.09 | 5.2 |
| 68 | Subas Bapad | Healthy control | 55071 | 60 | Male | 97 | 147 | 83 | 108 | 73 | 53155.5 | 655772 | 0.11 | 4.7 |
| 69 | Irappa | Healthy control | 55103 | 65 | Male | 85 | 162 | 70 | 120 | 80 | 45164.19 | 621677 | 0.09 | 4.9 |
| 70 | Basamma | Healthy control | 47508 | 56 | Female | 118 | 188 | 67 | 110 | 70 | 53031.3 | 447984.3 | 0.09 | 6 |
| 71 | Sharanama | Healthy control | 125129 | 42 | Female | 119 | 166 | 50 | 110 | 68 | 45554 | 713235.3 | 0.05 | 4.7 |
| 72 | Boramma | Healthy control | 125126 | 45 | Female | 112 | 187 | 65 | 130 | 80 | 50113 | 533082.5 | 0.07 | 5.3 |
| 73 | B.S.Masali | Healthy control | 125175 | 46 | Male | 92 | 126 | 68 | 120 | 70 | 80602 | 692092 | 0.08 | 6 |
| 74 | Lakawwa | Healthy control | 125111 | 40 | Female | 99 | 126 | 60 | 110 | 65 | 36387 | 690477.8 | 0.15 | 5.1 |
| 75 | Mallappa | Healthy control | 125149 | 46 | Male | 112 | 187 | 64 | 105 | 60 | 37036 | 545242.3 | 0.05 | 6.3 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 76 | Bhimarao | Healthy control | 125113 | 47 | Male | 93 | 100 | 60 | 135 | 70 | 40481 | 510477.3 | 0.09 | 4.7 |
| 77 | Tyawwa | Healthy control | 125125 | 45 | Female | 109 | 187 | 58 | 120 | 80 | 54494 | 662879.3 | 0.07 | 5.3 |
| 78 | Savitri | Healthy control | 125145 | 52 | Female | 75 | 156 | 60 | 110 | 72 | 54968 | 682906.8 | 0.07 | 4.8 |
| 79 | Laxmiba | Healthy control | 125142 | 50 | Female | 119 | 185 | 65 | 110 | 70 | 61683 | 795642 | 0.09 | 4.4 |
| 80 | Chandu | Healthy control | 125664 | 45 | Male | 119 | 159 | 67 | 110 | 70 | 69156 | 662434.5 | 0.23 | 4.7 |
| 81 | Ratnabai | Healthy control | 12554 | 52 | Female | 95 | 165 | 55 | 120 | 80 | 78231 | 456460.5 | 0.2 | 5.2 |
| 82 | Shanagouda | Healthy control | 107966 | 48 | Male | 106 | 118 | 60 | 110 | 76 | 46307 | 732136.5 | 0.07 | 5.5 |
| 83 | Malabai | Healthy control | 107945 | 42 | Female | 102 | 182 | 65 | 120 | 80 | 72137 | 776303 | 0.07 | 6 |
| 84 | Kamalabai | Healthy control | 107938 | 45 | Female | 102 | 150 | 58 | 120 | 80 | 68291 | 488876.5 | 0.06 | 4.8 |
| 85 | Neelamma | Healthy control | 107958 | 43 | Female | 100 | 126 | 64 | 110 | 70 | 68672 | 333986 | 0.22 | 4.6 |
| 86 | Rukhmini | Healthy control | 116356 | 51 | Female | 83 | 139 | 60 | 110 | 60 | 53164 | 455191 | 0.31 | 4.7 |
| 87 | B.S.Biradar | Healthy control | 116853 | 55 | Male | 125 | 158 | 68 | 100 | 60 | 71415 | 256989.3 | 0.42 | 5.1 |
| 88 | Shivagappa | Healthy control | 116366 | 50 | Male | 104 | 186 | 70 | 110 | 65 | 38675 | 319076.8 | 0.18 | 5.3 |
| 89 | Pushpa | Healthy control | 116362 | 41 | Female | 105 | 131 | 67 | 110 | 70 | 65929 | 880137 | 0.07 | 4.7 |
| 90 | Vijaykumar | Healthy control | 116396 | 48 | Male | 99 | 159 | 70 | 100 | 70 | 82902 | 718318 | 0.08 | 4.6 |
| 91 | Huvamma | Healthy control | 116413 | 50 | Female | 110 | 130 | 57 | 110 | 70 | 67291 | 448970.5 | 0.22 | 4.9 |
| 92 | Bhimasanur | Healthy control | 116387 | 55 | Male | 76 | 160 | 73 | 110 | 75 | 82902 | 718318 | 0.19 | 5 |
| 93 | Devendrapa | Healthy control | 116378 | 58 | Male | 117 | 172 | 70 | 130 | 80 | 67291 | 448970.5 | 0.21 | 5.2 |
| 94 | Vijayalaxmi | Healthy control | 47503 | 42 | Female | 80 | 102 | 50 | 120 | 80 | 61125 | 830549 | 0.09 | 6 |
| 95 | Pushpa B | Healthy control | 47519 | 41 | Female | 106 | 123 | 57 | 110 | 70 | 61961 | 623401.5 | 0.09 | 4.7 |
| 96 | Neelamma | Healthy control | 47770 | 44 | Female | 86 | 95 | 55 | 100 | 60 | 62672 | 2207853 | 0.2 | 5.3 |
| 97 | Yalawwa | Healthy control | 47752 | 50 | Male | 93 | 118 | 68 | 120 | 70 | 54412 | 532867.5 | 0.09 | 4.7 |
| 98 | Noora | Healthy control | 47517 | 49 | Female | 92 | 137 | 65 | 110 | 70 | 66364 | 618135.8 | 0.21 | 4.6 |
| 99 | S.S.Nimbal | Healthy control | 47483 | 53 | Male | 107 | 151 | 70 | 120 | 80 | 50239 | 594890.5 | 0.22 | 5.3 |
| 100 | Devappa | Healthy control | 47506 | 58 | Male | 108 | 164 | 74 | 120 | 70 | 62877 | 477433.5 | 0.18 | 6.1 |