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# CYTOMORPHOMETRY AS A DIAGNOSTIC TOOL FOR ASSESSING THE ALTERED NATURE OF EXFOLIATED BUCCAL MUCOSAL CELLS IN DIABETIC AND HYPERTENSIVE PATIENTS

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**ABSTRACT:Aim:** To study the cytomorphometric analysis of cells in the cytologic smears from buccal mucosal cells of diabetic and hypertensive patients. **Materials and Methods:** Smears were collected from clinically normal mucosa of 30 diabetes and 30 hypertensive patients and 30 healthy controls. Smears were stained using papanicolauo method. Cell diameter, nuclear diameter, cell area, nuclear area and nuclear: cytoplasmic ratio was obtained for each patient.**Results:** Compared to normal it was found that there were significant changes in diameter of cells (P<0.01), nucleus diameter (P<0.01), cell area(P<0.01) and nuclear area (P<0.001) in diabetes patients but not in hypertensive patients (P>0.01). **Conclusion:** Diabetes produces definite cytomorphometric changes in buccal mucosal cells. This research area is worthy for further exploration for public health implications.

**KEYWORDS:** O**ral exfoliative Cytology; Cytomorphomerty; Diabetes; Hypertension.**

## INTRODUCTION

Diabetes and Hypertension are the most common worldwide diseases afflicting humans. Diabetes is a chronic metabolic disorder characterized by hyperglycaemia associated with disturbances in metabolism of carbohydrates, proteins and lipids, as a result of insulin deficiency[1].Glycosylated haemoglobin (GHb) is not affected by factors like diet or medication intake, and it gives an accurate and objective measure of glycaemic control over the past 3 months. Hence GHb estimation is preferred by many to monitor diabetic patients[2]. There is higher prevalence and severity of gingivitis, periodontitis, candidiasis, other opportunistic infections and may also cause various changes in the cells of the oral mucosa[3]. Hypertension has a multifactorial and highly complex pathology. Multiple factors modulate blood pressure which include vascular calibration, blood viscosity, cardiac output, blood vessel elasticity, humoral mediators (T lymphocytes and T cell derived cytokines 17 and TNF alpha)[4]. One hypothesis suggests that hypertension results in oxidation and altered mechanical forces that lead to formation of neoantigens which activates T cells and infiltrate around vessels promoting vascular dysfunction leading to hypertension. This oxidation and vasoconstricition of capillaries alters the microcirculation[5] and may lead to atrophy which may cause cellular alteration in oral mucosa [6].

A study of cytomorphometry in oral exfoliative cytology was taken up to assess the usefulness of this procedure for assessing the altered nature of buccal cell in diabetic and hypertensive patients. Alterations in the superficial cells can serve as a reliable indicator of hyperplastic and dysplastic changes[7]. Exfoliative cytology is a painless, non-invasive, non- aggressive procedure and is very well accepted by patients and allows quick and accurate assessment[8].

## MATERIALS AND METHOD:

Approval for this study was obtained from MNR Dental College and Hospital Ethical Committee, Andhra Pradesh. All individuals signed a post-informed consent before inclusion in the study. A total of 60 diabetic patient, 60 hypertensive patient and 10 control subjects were included in the study.

Inclusion criteria for the study:

- Patients with a known history of diabetes at least for the past 1 year.
- Hypertensive patient with recently monitored blood pressure are included in the study.

- Control group includes normal healthy adult individuals with no history of diabetes, hypertension or any other illness.
- Diabetic , hypertensive and control individuals should have with clinically normal mucosa.

Exclusion criteria for the study include:

- Smoking, alcohol
- Medications other than used for diabetes and hypertension
- Anemia

Diabetic patients were also grouped into the following three categories for further analysis based on their GHb [2] levels:

- Well-controlled diabetics (WCD) – GHb ≤ 8%
- Moderately controlled diabetics (MCD) – GHb > 8% and ≤ 12%
- Uncontrolled diabetics (UCD) – GHb > 12%

Based on physicians consent and AHA [9] recommendations hypertensive patients were grouped into following three categories:

| | |
|---|---|
| Mild (pre) hypertensive: | 120 - 139mmhg (systolic) ,80-89mmhg (diastolic) |
| Moderately hypertensive: | 140-150mmhg(systolic) ,90-99mmhg(diastolic) |
| Severely hypertensive: | >160mmhg (systolic) , >100(diastolic) |

Smears were taken from clinically normal buccal mucosa of the patients using wooden spatula moistened in distilled waters. The scrapings were then transferred to clean glass previously marked with patient's reference number, and spread thinly and uniformly over the slide, after air drying they are fixed and stained with Papanicolaou method.

*Cytomorphometric analysis:*

Fifty clearly defined cells were selected; images of cells under high power objective of 40 x magnification were captured with the help of digital camera. The images were uploaded for computer analysis. The cytomorphometric analysis (**Fig.1**) was done using the windows based image analyser software (ImageJ 1.34s) [10] developed by National Institute of Health, USA [11].

*Nuclear and cell area:*

Cell diameter ( CD) and nucleus diameter ( ND) are measured (microns) by taking mean of two perpendicular planes **(Fig.2).**

*Nuclear and cell area:*

Cell and nucleus diameter was traced on screen and the software calculated the cell area ( CA) and nuclear area ( CA) in square microns. (Fig.3, Fig.4).

**STATISTICAL ANALYSIS:**

For this study statistical analysis was done using student t test. Comparison of mean nuclear and cell diameter; and nuclear and cellular area between different groups was done. The *P*- value < 0.05 was considered to be significant.

**RESULTS: (Fig.5,Fig.6)**

Cytomorphometric analysis **(Table.1)** showed that compared to control group, well controlled diabetics and moderately controlled diabetics showed no significant changes in ND, NA, CD, CA and N:C ratio [ ND (P>0.01), CD (P>0.01), NA (P>0.01), CA (P>0.01) and N: C (P>0.01)], whereas when compared to control uncontrolled diabetics showed significant in increase in ND, NA and decrease in CD, CA and increased N:C ratio. [NA (P<0.01), CA (P<0.001), ND (P<0.01), CD (P<0.01) and N:C (P<0.001)]. Cytomorphometric analysis **(Table.2)** in mild, moderate and severe hypertensive patients compared to control group showed no significant changes in ND, NA, CA, CD and N:C ratio [ ND (P>0.01), NA (P>0.01), CD (P>0.01), CA (P>0.01) and N: C ratio (P>0.01)].

**DISCUSSION:**

Cytomorphometric analysis of buccal mucosal cells obtained from patients with diabetes and hypertension were compared with healthy individuals. The purpose of this study is to determine if any significant differences in parametric like ND, CD and N: C ratio are present in diabetic and hypertensive patients and if it can play any important role in diagnosing, prevention and control of disease.

In this study uncontrolled diabetics showed significantly high ND, decreased CD and increased N: C ratio compared to control group. The findings of this study are analogous with those of Prasad H et al (2010) who performed cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients which showed statistically significant increase in ND (*P*=0.0367) as the findings of the present study suggested significant increase in nuclear diameter [12]. A study on type II diabetics by Alberti S, Spadella et al (2003) showed that ND was markedly higher in diabetic group but no significant change in cytoplasmic area and these results are parallel with the present study [7].Similarly, study conducted by Ummuhan Tozogul et al (2010) on type I diabetics demonstrated significantly high NA compared to controls and the result of this study supports our study [13].

Ban Tawfeek Shareef et al (2008) study on type II diabetics revealed cytomorphometric changes in NA in diabetics compared to those of normal and no changes in CA however in the present study NA showed similar result but there was decrease in CA as well [14]. Where as in a study conducted on type II diabetics by Hassan Hosseinpour Jajaram et al (2008) showed a significant increase in both NA & CA in diabetics compared to controls in buccal mucosa and dorsum of tongue[15] which showed similar results as the present study. Gaurav Sapra (2009) study on type II diabetics results showed that mean NA was significantly higher ($p < 0.001$) in study group where as mean CA did not exhibit a statistically significant difference ($p > 0.001$) [16] and the statistically significant of NA are similar to the NA results of the present study. In another study conducted on type II diabetics by Suvarna M, et al (2012), there was a statistically significant increase in average NA and significant decrease in the C/N ratio in diabetics when compared to non-diabetic healthy individuals and the average CA did not show any statistical difference between the two groups in comparision [17] with our study the decreased NA result was similar. Results by Rivera et al (2013) on type II diabetics study showed that the cell diameter and the nuclear-cytoplasmic ratio was significantly higher compared to those patients without the disease [18] where as in the present study the results where decreased cell diameter and increased N : C ratio which is not analogous with the study results of Rivera et al. Study on diabetics by Sankhla Bharat et al (2010) showed that mean NA was significantly higher ($p < 0.001$) in study group where as mean CA did not exhibit a statistically significant difference ($p > 0.001$) [19] which partially supported the presented study with similar result of decreased NA.

These variations can be as a result of numerous underlying pathogenesis in diabetes like ischemia that is caused by atherosclerosis in diabetic patients may result in decreased cellular turnover rate and limit the production of young cells which indicates that majority of cells are old or aged, which is indicated by increased nuclear size. Progressive decline in proliferative capacity and life span results in cellular ageing [20]. They stated that nutritional deficiencies like vitamin $B_{12}$ and folic acid may alter the size of nucleus as these nutrients are essential for DNA synthesis [13]. Inflammation is one of the factors that can increase NA and can lead to a poorly preserved cytoplasm[[21].

Xerostomia in diabetic patients due to dehydration may result from hypofunction of salivary glands secondary to adverse hormonal microvascular and neuronal change which may result in inflammation of oral mucous membrane resulting in increase ND [22]. Decrease in salivary flow rates may not maintain the oral cavity clean which may cause superadded infections like bacterial and candidiasis which may evoke a chronic inflammatory response resulting in increase ND and decrease CD [20,23]. Dehydration due to xerostomia, polyuria or medications like diuretics may lead to cytomorphometric changes of oral mucosal cells[13]. This may also lead to mucosal atrophy increasing the changes that basal and parabasals cells may be included in cytologic smears[24].

We aimed at assessing the changes in buccal mucosal cells of hypertensive patients as this disease also responsible for many oral mucosal changes [25] due to aforementioned factors; however the cytomorphometric analysis did not show any significant changes in ND or CD either of the groups of hypertensive patients compared to control groups.

**CONCLUSION:**

Cytomorphometry in exfoliated buccal mucosal cells can be used for routine application in population screening programmes. The results observed might contribute to understanding of alterations in cellular morphology of oral epithelium in diabetic patients. However these alterations cannot be predictive or diagnostic for diabetes because they are not unique to this disease. This research area is worthy for further exploration for public health implications.

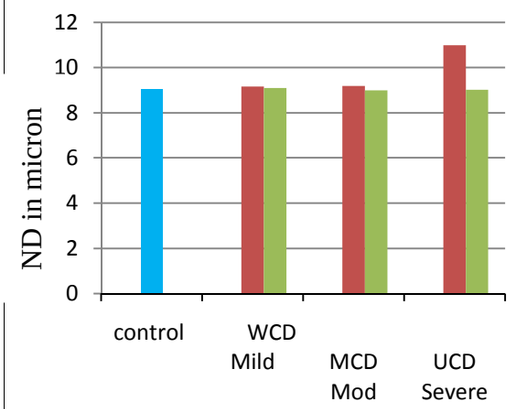


Figure.5. There is an increase in ND in uncontrolled group of diabetes (Red) compared to control group (Blue)($P<0.01$).However no significant change was seen in mild and moderately controlled groups ($P>0.01$). In all groups of hypertensive patients (Green) there is no significant change in ND compared to control group ($P>0.01$

**Table 1. Comparison of parameters between control and various group of Diabetes**

| Parameters | Group | Mean | P value |
|---|---|---|---|
| ND | Control | 9.05 | |
| ( In microns) | Well controlled | 9.17 | 0.621 |
| | Moderately controlled | 9.19 | 0.481 |
| | Uncontrolled | 10.99 | 0.01 |
| CD | Control | 54.14 | |
| ( In microns) | Well controlled | 54.04 | 0.29 |
| | Moderately controlled | 53.9 | 0.65 |
| | Uncontrolled | 50.2 | 0.01 |
| NA | Control | 43.89 | |
| ( sq.microns) | Well controlled | 39.27 | 0.3 |
| | Moderately controlled | 42.89 | 0.4 |
| | Uncontrolled | 52.1 | 0.01 |
| CA | Control | 1396.86 | |
| ( sq.microns) | Well controlled | 1456.4 | 0.312 |
| | Moderately controlled | 1410.1 | 0.305 |
| N:C | Control | 0.013 | |
| | Well controlled | 0.01 | 0.33 |
| | Moderately controlled | 0.015 | 0.41 |
| | Uncontrolled | 0.05 | 0.001 |

ND- Nuclear Diameter: CD- Cell Diameter: CA- Cell area; NA- nuclear area;

**Table 2. Comparison of parameters between control and various group of hypertensive patient**:

| Parameters | Group | Mean | P value |
|---|---|---|---|
| ND | Control | 9.05 | |
| In microns | Mild | 9.1 | 0.4 |
| | Moderate | 9.0 | 0.5 |
| | Severe | 9.02 | 0.421 |
| CD | Control | 54.14 | |
| In microns | Mild | 54 | 0.27 |
| | Moderate | 54.2 | 0.64 |
| | Severe | 53.9 | 0.65 |
| NA | Control | 43.89 | |
| ( sq.microns) | Mild | 44.1 | 0.4 |
| | Moderate | 43.7 | 0.3 |
| | Severe | 43.9 | 0.3 |
| CA | Control | 1396.86 | |
| ( sq.microns) | Mild | 1390.2 | 0.305 |
| | Moderate | 1420.7 | 0.305 |
| | Severe | 1401.2 | 0.300 |
| N:C | Control | 0.013 | |
| | Mild | 0.01 | 0.33 |
| | Moderate | 0.015 | 0.25 |
| | Severe | 0.015 | 0.33 |

ND- Nuclear Diameter: CD- Cell Diameter: CA- Cell area; NA- nuclear area

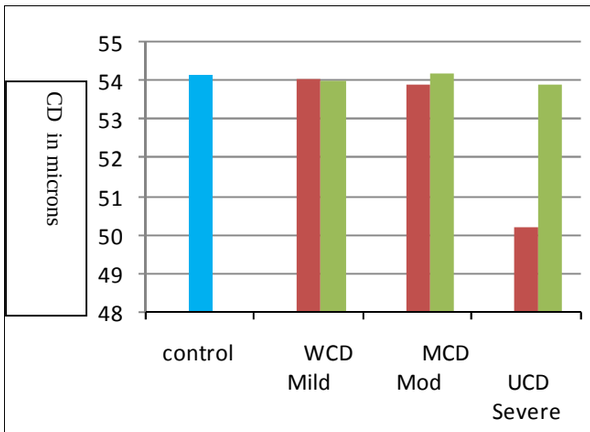


Figure .6. There is a decrease in CD in uncontrolled group of diabetes (Red) compared to control group (Blue)(P<0.01).However no significant change was seen in mild and moderately controlled group (P>0.01). In all groups of hypertensive patients (Green) no significant change in CD compared to control group (P>0.01).

**Figure.1. Cytomorphometric analysis using image J analysis**

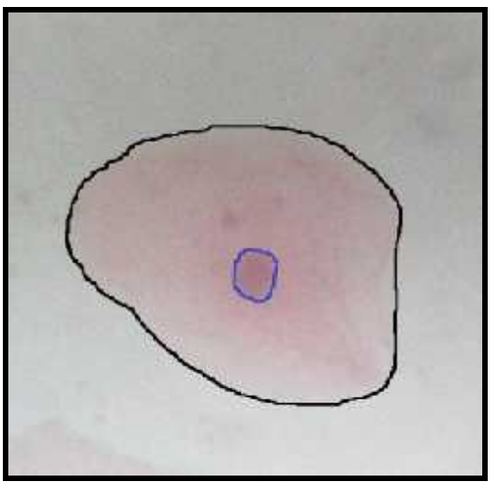

**Figure.4. Cell diameter**

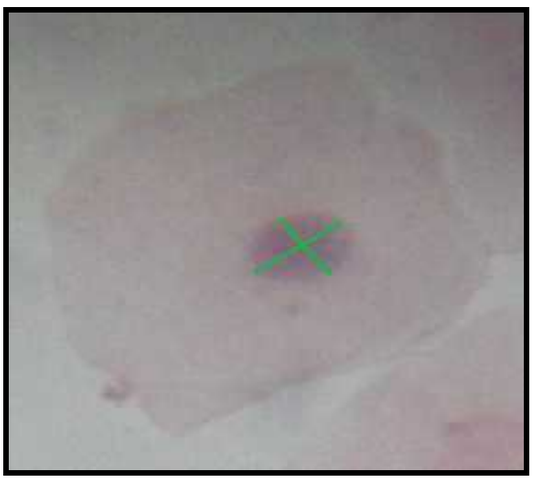

**Figure.3 Nuclear diameter**

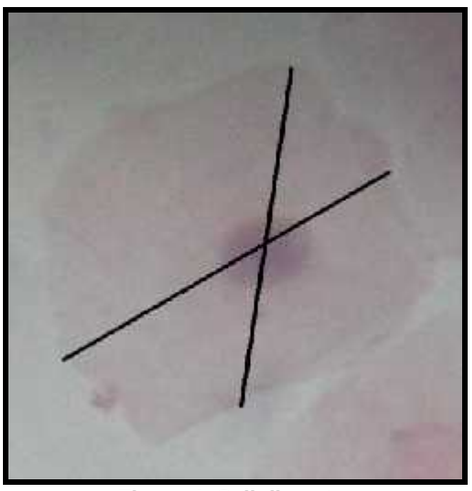

**Figure.4. Cell diameter**

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