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Research Article

A COMPARATIVE STUDY OF LIQUID BASED CYTOLOGY AND EXFOLIATIVE CYTOLOGY OF ORAL EPITHELIAL CELLS AMONG TYPE II DIABETES MELLITUS INDIVIDUALS

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A R T I C L E I N F O

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A B S T R A C T

Aim: To find agreement between Liquid based Cytology and Exfoliative Cytology of oral epithelial cells among Type II Diabetes Mellitus individuals. **Materials and method:** The study was conducted on 68 known diabetic patients visiting to the Department of Oral Pathology and Microbiology at Bapuji Dental College and Hospital, Davanagere. Both qualitative and quantitative examination of the oral epithelial cells obtained were done following PAP stain. **Results:** The quality of smears obtained using Liquid based Cytology was superior both qualitatively and quantitatively compared to Exfoliative cytology smear. **Conclusion:** Number of satisfactory results obtained was significantly higher in Liquid Based Cytology smear than Exfoliative cytology smear.

INTRODUCTION

"Oral cavity is considered to be the mirror of rest of the body"- James Osler. The changes occurring in and around the oral cavity do not seem to have equal appreciation by the physician. The oral health serves to be a sensitive indicator of the general health status of an individual[1]. Diabetes mellitus (DM), a rapidly emerging epidemic ailment that has required the potential health care services in the world exerts its deleterious effects on multiple organs such as kidneys, heart, blood vessels, eyes, nerves and also the oral cavity[2]. Numerous studies have demonstrated the harmful role of DM on the oral mucosa, that have shown the morphological alterations of oral mucosa leading to retarded tissue function, oral infection and oral neoplasia. In diabetes, alterations in the living structures can be identified with cytological studies. The changes are attributed to the loss of oxidation equilibrium causing depression of antioxidant scavenger and enzyme activity because of the increased concentration of glucose, increased synthesis of free radicals, and protein glycation. Early diagnosis helps control of blood sugar level and prevent various complications. Data from recent studies depicts that there are around 500 million prevailing cases of T2DM across the globe and the figure is expected to rise to 693 million by 2045[3].

The present study was conducted with the aim to find agreement between Liquid Based Cytology (LBC) and Exfoliative Cytology (EC) of oral epithelial cells among T2DM individuals and compare cytomorphometric changes with controlled and uncontrolled diabetes mellitus using HbA1c.

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MATERIALS AND METHOD

The present study was conducted in the Department of Oral Pathology and Microbiology at Bapuji Dental College and Hospital, Davanagere. Study group comprised of 68 individuals having T2DM of more than 2 years. Ethical clearance was obtained from Institutional Review Board. 30-70 year age group individuals having T2DM of more than 2 years with clinically healthy oral mucosa were included. The patients were positioned with the arm extending to form a straight line from shoulder to the wrist, then the tourniquet was applied 3-4 inches above the puncture site. The puncture site was prepared by gently rubbing a cotton dipped in spirit in a circular motion. 5 ml of blood was drawn from the median cubital vein and sent for glycated haemoglobin examination. The method used for HbA1c estimation was High Performance Liquid Chromatography (HPLC). This was followed by scraping of buccal mucosa using cytobrush in a rotatory motion with gentle pressure. Scraping from one cytobrush was used to prepare conventional smear and fixation using cytospray followed by PAP stain and the second scraping was dipped in fixative liquid comprising of 20 ml of 95% ethanol, 6 ml of glacial acetic acid and 74 ml normal saline followed by centrifugation at 2500rpm for 15mins. The supernatant solution was rejected leaving behind a few drops of suspension in the bottom. A small amount of this specimen was then taken and applied over the glass slide evenly with the help of another glass slide, fixed using cytospray and subjected to PAP stain.

Both the cytosmears were then observed by an expert Oral Pathologist.

RESULTS

The smears were observed and assessed using 4x, 10x, 20x and 45x under LEICA DMRB microscope equipped with JENOPTIK GERMANY PROGRESS CAPTURE PRO VERSION 8.0.8 image analysis software and image J 1.46 r Java 1.6.0_20 (64bit) software. For each slide, 50 epithelial cells were selected randomly in different fields. The slide was evaluated for following *Staining quality:* Staining (adequate/inadequate), Number of cells (adequate/inadequate), Distribution of cells (uniform/non uniform), Background (clear/non clear), Mucous/Debris (present/absent) and *Microscopic observations:* Nuclear area, Cytoplasmic area and Cytoplasmic area/Nuclear area ratio.

The data obtained were statistically analysed to measure the agreement between LBC and EC using McNemar's test and cytomorphometric changes between controlled and uncontrolled diabetes mellitus using HbA1c with correlation analysis.

Table 1

Liquid based cytology stain	Exfoliative cytology stain				Total	
	Adequate		Inadequate			
	Count	%	Count	%	Count	%
Adequate	48	96.0	17	94.4	65	95.6
Inadequate	02	4.0	1	5.6	3	4.4
Total	50	100.0	18	100.0	68	100.0

Table 2

Liquid based cytology number of Cells	Exfoliative cytology number of Cells				Total	
	Adequate		Inadequate			
	Count	%	Count	%	Count	%
Adequate	27	71.1	23	76.7	50	73.5
Inadequate	11	28.9	7	23.3	18	26.5
Total	38	100.0	30	100.0	68	100.0

Table 3

Liquid based cytology distribution of cells	Exfoliative cytology Distribution of Cells				Total	
	Non Uniform		Uniform			
	Count	%	Count	%	Count	%
Non Uniform	6	10.2	1	11.1	7	10.3
Uniform	53	89.8	8	88.9	61	89.7
Total	59	100.0	9	100.0	68	100.0

Table 4

Liquid based cytology Background	Exfoliative cytology Background				Total	
	Clear		Non-Clear			
	Count	%	Count	%	Count	%
Clear	18	100.0	45	90.0	63	92.6
Non-Clear	0	0.0	5	10.0	5	7.4
Total	18	100.0	50	100.0	68	100.0

Table 5

Liquid based cytology Mucous/Debris	Exfoliative cytology Mucous/Debris				Total	
	Absent		Present			
	Count	%	Count	%	Count	%
Absent	17	100.0	43	84.3	60	88.2
Present	0	0.0	8	15.7	8	11.8
Total	17	100.0	51	100.0	68	100.0

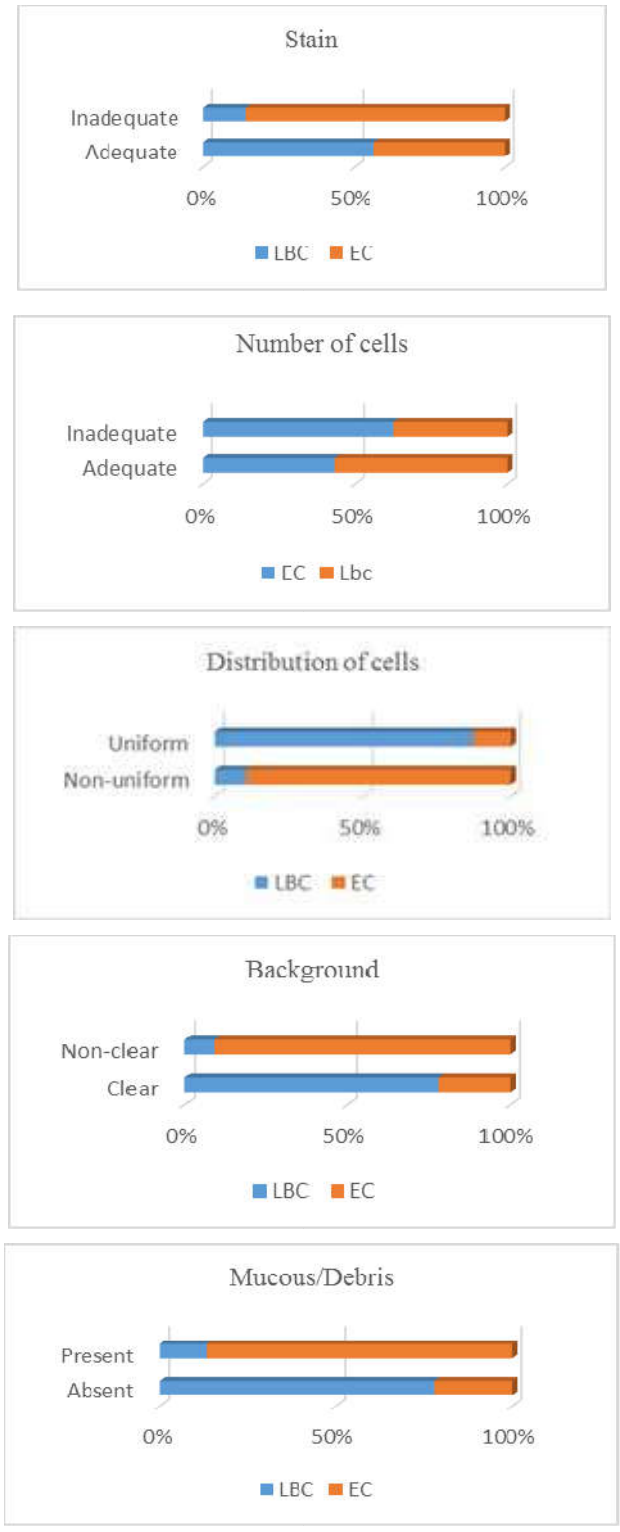
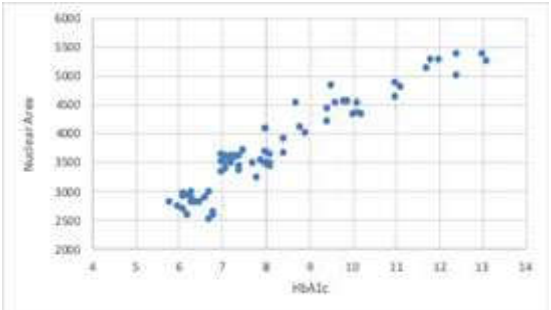


Figure 1 Qualitative analysis of staining quality between EC and LBC



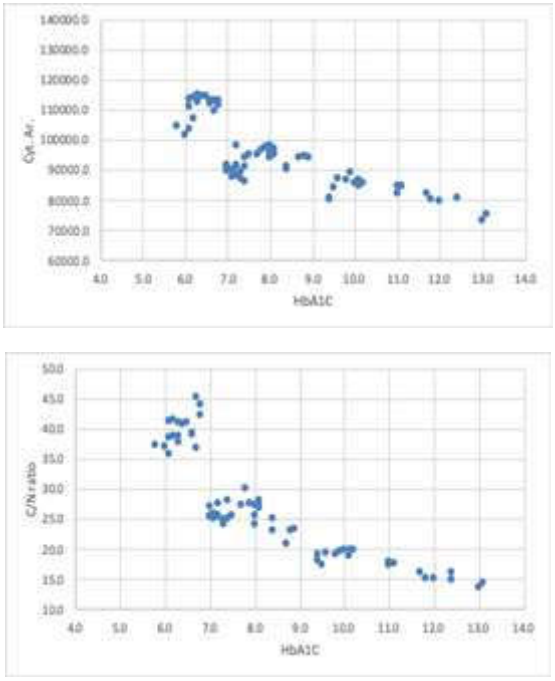


Figure 2 Cytomorphometric observations obtained between controlled and uncontrolled diabetes using HbA1c.

DISCUSSION

Results from the study conducted showed that the quality of smears obtained using LBC was superior both qualitatively and quantitatively compared to EC smear. It was found that for quality of stain p =0.001, number of cells obtained p =0.058, distribution of cells p < 0.001, background p < 0.001 and mucous/debris p < 0.001 hence, allowing for greater precision for diagnosis compared to conventional methods. Also, there was significant variation in cytomorphometry of oral epithelial cells between controlled and uncontrolled DM (HbA1c and Nuclear area - Pearson correlation coefficient r = 0.950; HbA1c and cytoplasmic area - Pearson correlation coefficient r = -0.795; HbA1c and cytoplasmic area/nuclear area - Pearson correlation coefficient r = -0.845). Hence, proving the vitality of LBC in diagnostic therapeutics. Results of the present study was similar to previous studies conducted by Ganesh *et al* [6], Pawar *et al* [8], and Hegde *et al* [9].

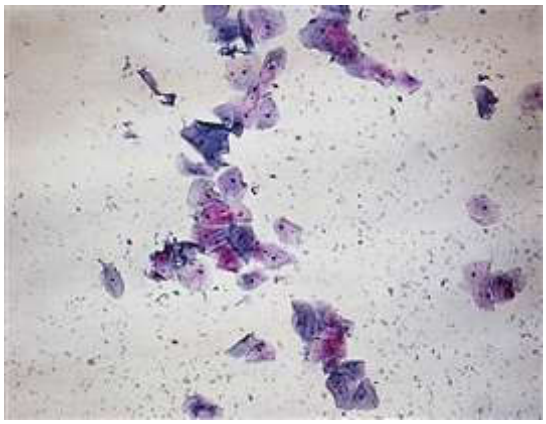


Figure 3 Photomicrograph showing PAP stained epithelial cells using Exfoliative cytology (10x)

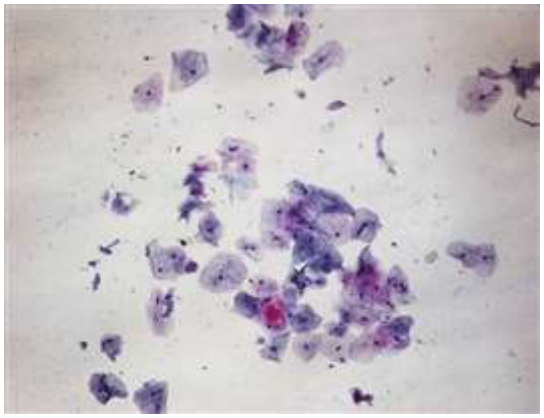


Figure 4 Photomicrograph showing PAP stained epithelial cells using Liquid Based cytology (10x)

Various screening and diagnostic tests for DM are readily available. Usually, FPG, 2-h PG during 75-g OGTT, and A1C are equally suitable for diagnosis.

Criteria for the diagnosis of diabetes (According to American Diabetes Association 2018)

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.* *OR*

2-h PG ≥200mg/dL (11.1mmol/L) during OGTT. The test should be done as designated by the WHO, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.* *OR*

A1C ≥6.5% (48 mmol/mol). The test should be conducted in a laboratory using a NGSP certified method and DCCT standardized assay.* *OR*

In a patient with classical symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

*In the absence of unequivocal hyperglycemia, results should be established by repeating the test[4].

A1C is a widely used marker for hyperglycaemia, depicting average blood glucose levels over 2 to 3 month duration. This test has an important function in monitoring patients with diabetes, since it has a good association both with micro vascular and macro vascular complications and is broadly used as the standard biomarker for an adequate glycaemic control[5].

However, since these invasive procedures are relatively contraindicated in diabetic patients it soughts pathway for non-invasive diagnostic procedure such as EC. However, with sporadic failure of cervical smear prepared with conventional EC method evolution of LBC was seen in the early 1970s in Germany, and favoured the progress of computer guided cervical screening[6, 7]. Two technologies-namely ThinPrep (Hologic, Marlborough, MA, U.S.A.) and BD SurePath (BD Diagnostics-TriPath, Burlington, NC, U.S.A.) had extensively been used[8]. However, these required highly sophisticated and expensive equipment's. Hence, an economical replacement for automated LBC along with a definitive qualitative replacement of conventional smear cytology, Centrifuged liquid-based cytology (CLBC) technique was introduced[6]. CLBC is an adaptation to LBC. Being cost-effective, yet efficient technique it uses readily available equipment's, removes debris, blood and provides microbe free background. Also, it facilitates in attaining a single layer of cell with increase in

contrast between nucleus and cytoplasm with a clearer background[9].

In our study we found better staining quality of smears with greater number of cells uniformly distributed in a clear background with minimal evidence of debris using CLBC. Better quality results in CLBC is attributable to the fixative liquid reagents used and centrifugation of sample that allows settling down of the RBCs, mucous, debris, and microbes.

CONCLUSION

CLBC is a modification of LBC, which is a cost-effective yet efficient technique with better results in comparison to conventional cytology. Being a non-invasive procedure, it acts as an adjunct to the early diagnostic aid for presently available methods. Number of satisfactory results obtained was significantly higher in LBC smear than EC smear.

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