Original Article

Markers of cytotoxicity and oxidative DNA damage in Diabesity: A new age illness

Ankita Tandon[1], Nikita Gulati[2], Narendra Nath Singh[1]

[1]Department of Oral Pathology, Microbiology and Forensic Odontology, Dental Institute, RIMS, Ranchi, Jharkhand, [2]Department of Oral Pathology and Microbiology, ITS-CDSR, Ghaziabad, Uttar Pradesh, India

Abstract

Background: The oral mucous membrane is particularly sensitive to certain types of systemic disorders such as anemia, vitamin deficiencies, infectious diseases, hormonal disturbances and can be objectively reproduced through definite measurements using cytomorphometry.

Objectives: The objective of the study is to evaluate the quantitative and qualitative changes in cytological buccal smears of obese individuals with type II diabetes (Group 1 = 20), obese individuals without type II diabetes (Group 2 = 20), individuals with type II diabetes without obesity (Group 3 = 20) by comparing with controls (individuals without obesity and without type II diabetes) (Group 4 = 20).

Materials and Methods: Buccal mucosal cells were scraped from study participants and were subjected to morphometric analysis (Magnus Pro software). Clinical history, hemoglobin A1c, heights and weights of participants were measured and consequently, their body mass index was calculated. Quantitative parameters (nuclear area, cytoplasmic area, nucleo-cytoplasmic ratio) and qualitative parameters (micronuclei [MN], nuclear budding, nuclear disintegration, apoptosis, necrosis) were assessed among the groups. The data were statistically interpreted using SPSS software version 20.0.

Results: There is an increase in nuclear diameter and nuclear: cytoplasmic ratio of Groups 1 and 3 relative to Group 2. The qualitative assessment revealed MN and nuclear disintegration in Group 1 and 3 individuals. In addition, other qualitative changes such as nuclear budding and apoptotic bodies were evident in patients with type II diabetes.

Conclusion: The aforementioned qualitative and quantitative parameters facilitate early diagnosis and identification of individuals at risk of developing new age systemic illnesses such as diabetes and obesity.

Keywords: Cytomorphometry, diabetes mellitus, obesity

Address for correspondence: Dr. Ankita Tandon, Department of Oral Pathology, Microbiology and Forensic Odontology, Dental Institute, RIMS, Ranchi, Jharkhand, India.
E-mail: drankitatandon7@gmail.com

Submitted: 03-May-2021, **Revised:** 04-Sep-2021, **Accepted:** 05-Sep-2021, **Published:** 22-Dec-2022

INTRODUCTION

Obesity accounts for annually 2.8 million deaths worldwide. In addition, 44% of the diabetes burden is attributable to being obese. The pathophysiology of twin epidemics connecting obesity and diabetes is chiefly attributed to two factors: insulin resistance and insulin deficiency. Diabetes is a global health-care problem that threatens to reach pandemic levels by in the forthcoming decade.

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_132_21

How to cite this article: Tandon A, Gulati N, Singh NN. Markers of cytotoxicity and oxidative DNA damage in diabesity: A new age illness. J Oral Maxillofac Pathol 2022;26:589-90.

Type 2 diabetes mellitus (T2DM) represents approximately 90% of all cases of diabetes, and its frequency is similar to that of obesity.[1] The increase in oxidative stress in buccal mucosal cells of obese and diabetic subjects may result in genotoxic damage. It is estimated that about 90% of T2DM is attributable to excess weight.[2]

A vicious circle of progressive microvascular dysfunction contributes to and is exacerbated by worsening insulin resistance. Capillary recruitment is an important mechanism by which insulin promotes uptake of glucose from the blood.[3] Microvascular function is found to be negatively correlated with adiposity. There are various theories put forward to support the common pathogenetic mechanism linking the two diseases such as obesity-associated oxidative stress, surplus adiposity is associated with a chronic state of vascular inflammation, with increased levels of proinflammatory cytokines, particularly, production of tumor necrosis factor, which is negatively correlated with skin capillary recruitment and insulin sensitivity leading to impaired perfusion and insulin resistance and increased fat mass leading to prolonged elevation of free fatty acid levels in the blood, which can impair capillary recruitment.[4]

Oral mucous membrane is particularly sensitive to such systemic disturbances which can be objectively reproduced through definite measurements using cytomorphometry. The knowledge of quantitative and qualitative changes using cytomorphometry in obese and/or Type II diabetic patients could contribute to the understanding of disease process at a cellular level. The presence of micronuclei has long been studied as a biomarker of chromosome breakage or loss; of nucleoplasmic bridges as a biomarker of misrepair of DNA strand-breaks or telomere-end fusions; and of nuclear buds as a biomarker of elimination of amplified DNA or DNA-repair complexes. In addition, this method assesses the mitotic (mononucleated, metaphase, anaphase, binucleated, multinucleated) and the viability status (necrosis, apoptosis) of the cell.[5,6]

T2DM adversely affects the morphology of oral mucosa, which compromises tissue function to favor the occurrence of oral infections and epithelial cell degeneration; hence, exfoliative cytology being simple, noninvasive and with the advancement in the field of computer-aided morphometry, can be used as a diagnostic tool. Thus, the present study determines that the mean nuclear area (NA), cytoplasmic area (CA) and N/C (ratio) and various other aforementioned morphological changes which can be used as an effective tool in the diagnosis and identification of individuals at risk of developing new age systemic illnesses such as diabetes and obesity and their consequential cell disruptions. Recent advances in technology facilitate the use of reliable quantitative techniques such as cytomorphometry, and they may increase the sensitivity of exfoliative cytology for the early diagnosis as they are precise, objective and reproducible. The quantitative measurement of cellular features, inputting the data points into a computer via a graphic interface, with the intent of standardizing image analysis removes the subjectivity.

MATERIALS AND METHODS

Study cases

The study was conducted in the Department of Oral Pathology and Microbiology, ITS-CDSR, Muradnagar, Ghaziabad after gaining consent from the institutional review board. Informed consent was obtained from all subjects. The study excluded participants who reported alcohol drinking, smoking, tea and/or coffee consumption (more than 3 cups/day), had any tumor, inflammatory disease, viral infection, had currently or previously been taking drugs known to cause mutations, had received vaccinations or been exposed to radiation in the past 6 months, had a history of occupational or environmental exposure to known genotoxic chemicals, different dietary habits, intake of vitamin preparations or participating in intensive sport activities a week before blood sampling. Those who had chronic diseases such as hypertension, hypercholesterolemia and cardiovascular disease were also excluded from the study.

In each PAP and H&E-stained smears, binucleation, inflammation, cytoplasmic vacuolation, karyorrhexis, karyolysis, pyknosis, microbial colonies were assessed. Scrapings were smeared onto the slide. A minimum of four smears samples were taken from each subject that would be sufficient to give 200 cells per subject (50 cells per smears). To gather cells from all the layers of the epithelium, a moderate pressure was applied while taking the smear. Collected smears were immediately fixed using spray fixative (RAPID PAP® Spray fixative, Biolab Diagnostics (I) Pvt. Ltd., India) to avoid air drying. Papanicolaou technique (RAPID PAP® stain, Biolab Diagnostics (I) Pvt. Ltd., India) was used to stain the smear. Stained smears were examined under a microscope equipped with a ×40 objective. In each PAP-stained smear, 50 clearly defined unfolded cells with adequate staining were selected by systematic sampling, moving the microscope stage from left to right followed by down and across to avoid repetition of cells from different fields at ×40.

The mean difference of qualitative data between for groups was tested by one-way ANOVA and association

of qualitative data with for groups was tested by Pearson's Chi-square test was used. The interrater agreement between two observers, for qualitative, we were calculated Kappa statistics (κ = 0.959) and for qualitative variables, the interrater reliability was tested by Cronbach's alpha 0.711 and interclass correlation coefficient with 95% confidence interval (CI) are 0.750 (0.627–0.775). Based on the literature review and consultation with expert statistician, the sample size has been estimated by the G-Power analysis at 95% confidence level, i.e., *t*-tests - Means: difference between two independent means (two groups) and power of 80% in consultation with expert statistician and was found to be significant for outcome of the study.

Recording of body mass index

Heights and weights of participants were measured. When the subjects were weighed, they were asked to remove their shoes and other items of attire that could possibly add extra weight. Body mass index (BMI) was calculated by dividing the weight (kg) by the height in meters squared. A BMI below 18.50 kg/m^2 was classified as corresponding with underweight, a BMI between 18.50 and 24.99 kg/m^2 was classified as normal. BMI values between 25.00 and 29.99 kg/m^2 were associated with overweight and BMIs of 30.00 kg/m^2 or higher with obesity. Obesity is further classified as Class I if the BMI is between 30.00 and 34.99 kg/m^2, as Class II, if it is between 35.00 and 39.99 kg/m^2 and as Class III, if the BMI is ≥40.00 kg/m^2. All subjects were classified into three groups according to BMI values as follows: normal-weight Score 1 (18.50–24.99 kg/m^2), Score 2 over-weight (25.00–29.99 kg/m^2) and obese Score 3 (≥30.00 kg/m^2).[7]

Recording of waist-to-hip ratio

Waist circumference (cm) was measured using a tape measure from the point midway between the costal margin and iliac crest in the mid-axillary line, with the subject standing and breathing normally. Hip circumference (cm) was measured at the widest point around the greater trochanter. The waist-to-hip ratio (WHR) was calculated as the waist measurement divided by the hip measurement. Those with a WHR above 0.90 for men and above 0.85 for women were considered to be abdominally obese.[8] In our study, we categorized WHR as low risk (Score 1), moderate risk (Score 2) and high risk (Score 3). For males, range was <0.90, 0.90–1 and >1 whereas for females, it was <0.80, 0.80–0.85 and >0.85.

Recording of glycated hemoglobin

Participants need to have normal fasting plasma glucose before being considered as control. Participants were comparable for age, gender, smoking habit, diet and physical activity. Measurements of blood glucose level and glycated hemoglobin (HbA1c) were noted. For people without diabetes, the normal range for the HbA1c level between 4% and 5.6% (Score 1), HbA1c levels between 5.7% and 6.4% which means having higher chance of getting diabetes (Score 2) and levels of 6.5% or higher meaning having diabetes (Score 3).

Preparation of smear

Buccal mucosal cells were scraped from obese individuals with type II diabetes (Group 1 = 20), obese individuals without type II diabetes (Group 2 = 20), individuals with type II diabetes without obesity (Group 3 = 20) and controls (individuals without obesity and without type II diabetes) (Group 4 = 20). PAP stain was used for quantitative analysis whereas both H&E and PAP were used for qualitative analysis. Patients were asked to rinse the oral mucosa with saline; the mucosa was cleaned and dried with a gauze swab to remove surface debris and excess saliva. Eight smears (two from each site) were prepared by a wet wooden spatula by gentle scraping and then transferred to clean dry glass slides. The slides were then fixed in 95% ethyl alcohol for 15 min. Out of the eight smears, four each were stained in PAP and H&E stains. Quantitative parameters (NA, CA, N/C) and qualitative parameters (nuclear budding, nuclear disintegration and apoptosis) were assessed among the groups. In each PAP and H&E-stained smears, binucleation, inflammation, cytoplasmic vacuolation, karyorrhexis, karyolysis, pyknosis, microbial colonies were assessed [Figure 1]. The smears were subjected to morphometric analysis (Magnus Pro software) [Figure 2].

Statistical analysis

The data were statistically interpreted using Statistical Package for the Social Sciences (SPSS, IBM Inc. California,

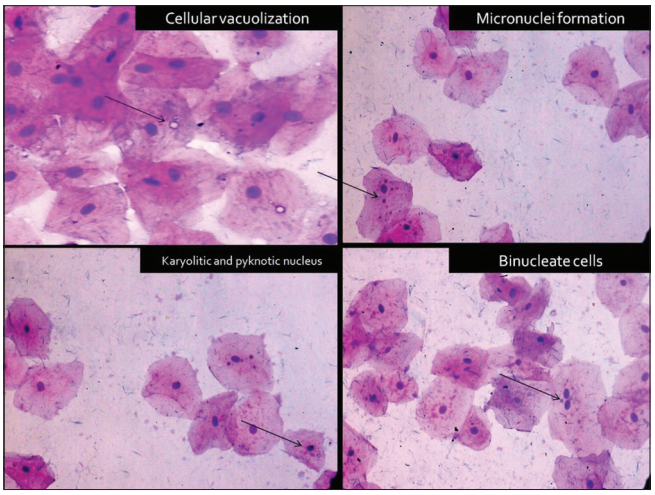

Figure 1: Cytosmears depicting various cytoplasmic and nuclear disintegrating features such as vacuolization, budding and micronuclei formation

USA) version 20.0. Then, the data were subjected to descriptive and interferential statistics to generate mean and standard deviation. For intergroup and intragroup gender comparison of means, Student's *t*-test was used at 95% CI. The mean difference of qualitative data between for groups was tested by one-way ANOVA and association of qualitative data with for groups was tested by Pearson's Chi-square test was used. The interrater agreement between two observers, for qualitative, we were calculated Kappa statistics ($\kappa = 0.959$) and for qualitative variables, the interrater reliability was tested by Cronbach's Alpha 0.711, and interclass correlation coefficient with 95% CI is 0.750 (0.627–0.775). Based on the literature review and consultation with expert statistician, the sample size has been estimated by the G-Power analysis at 95% confidence level, i.e., *t*-tests - Means: difference between two independent means (two groups) and power of 80% in consultation with expert statistician and was found to be significant for outcome of the study.

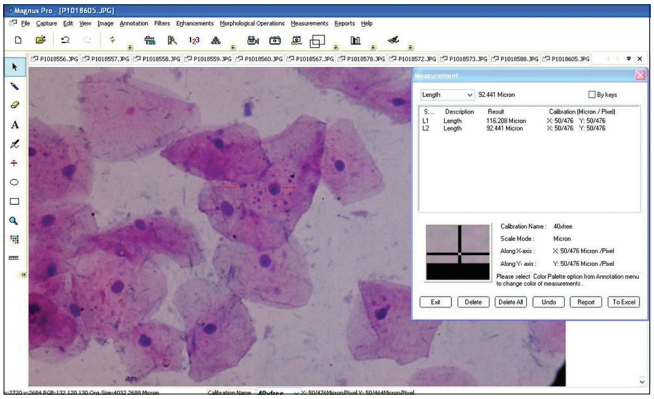

Figure 2: Morphometric measurements of nuclear and cellular area in buccal cytosmears

RESULTS

Demographic data

The overall mean age of the study cases was 51.44 years. The overall mean age of the study cases was 51.44 years. Please specify the age and sex of each group. In addition, whether age and sex have an impact on the experimental results? The cases included in the study had a higher female representation, that is, 51.2%. Group 1 had 35.0% females, Group 2 had 55% females, Group 3 had 65% and Group 4 had 50% female preponderance. HbA1c was highest in Group 1 and 3, WHR was highest in Group 2 and BMI was highest in Group 1 [Figure 3a].

Quantitative assessment of cytological buccal smears of study groups

NA was highest in Group 1 (86.203) and lowest

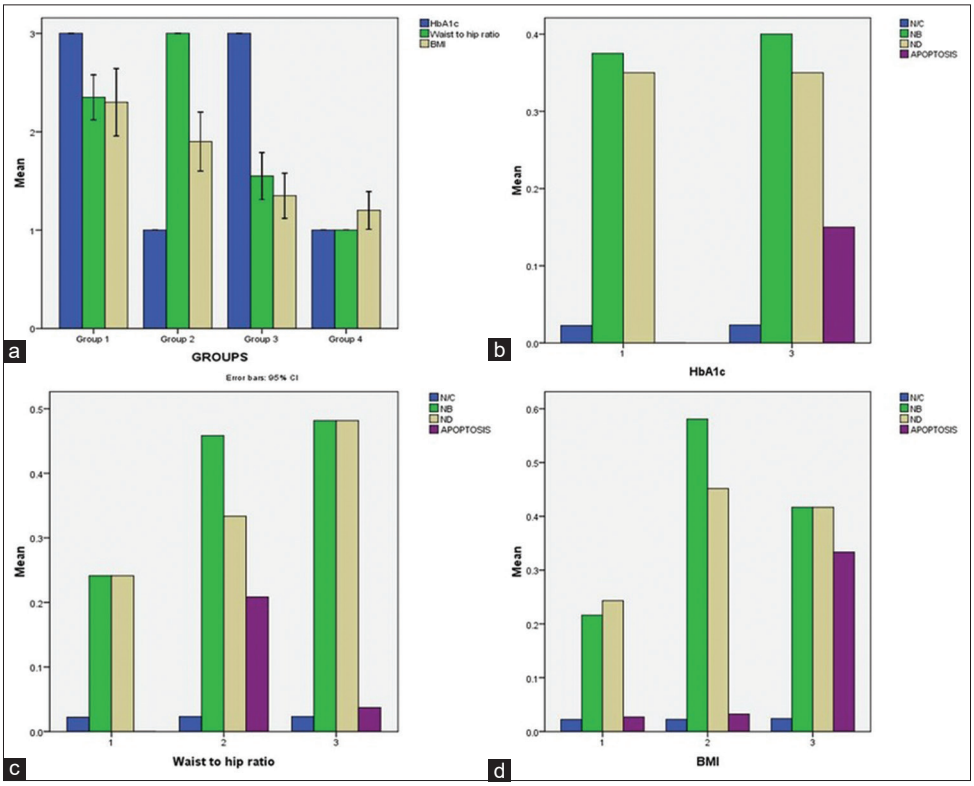

Figure 3: (a) Distribution of HbA1c, WHR and BMI in study cases, (b) Correlation of HbA1c with nuclear budding, nuclear disintegration and apoptotic bodies, (c) Correlation of WHR with nuclear budding, nuclear disintegration and apoptotic bodies, (d) Correlation of BMI with nuclear budding, nuclear disintegration and apoptotic bodies. WHR: Waist-to-hip ratio, BMI: Body mass index, HbA1c: Glycated hemoglobin

in Group 3 (79.330) whereas CA was highest in Group 3 (3601.40) and lowest in Group 2 (3373.25). N/C ratio was comparatively high for Groups 1 (0.02420) and 2 (0.02270) and slightly low in Group 3 (0.02216). The results were statistically significant [Table 1].

Qualitative assessment of cytological buccal smears of study groups

Nuclear budding was highest in study cases with HbA1c >6.5%, WHR >1 for males and >0.85 for females and BMI in the range of 25.00–29.99 kg/m^2 [Figure 3b]. On the other hand, nuclear disintegration was similar irrespective of HBA1c range but highest for WHR >1 for males and >0.85 for females and BMI in the range of 25.00–29.99 kg/m^2 [Figure 3c]. Apoptotic bodies were higher in study cases with HbA1c >6.5%, highest for WHR 0.90–1 for males and 0.80–0.85 for females and BMI ≥30.00 kg/m^2 [Figure 3d].

Table 1: Comparison of nuclear area, cytoplasmic area and N/C ratio in various study groups

Parameters	Study groups	*n*	Mean	SD	*P*
NA	Group 1	20	86.203	3.2383	0.00
	Group 2	20	76.006	3.9046	
	Group 3	20	79.330	2.5386	
	Group 4	20	67.011	2.3027	
	Total	80	77.137	7.5714	
CA	Group 1	20	3564.84	99.981	0.00
	Group 2	20	3373.25	318.328	
	Group 3	20	3601.40	264.887	
	Group 4	20	3064.79	311.206	
	Total	80	3401.07	335.736	
N/C	Group 1	20	0.02420	0.001236	0.09
	Group 2	20	0.02270	0.002237	
	Group 3	20	0.02216	0.002018	
	Group 4	20	0.02212	0.002770	
	Total	80	0.02279	0.002263	

NA: Nucleus area, CA: Cytoplasmic area, SD: Standard deviation

Correlation of N/C ratio with hemoglobin A1c, waist-to-hip ratio and body mass index

There is a positive correlation and steady increase in N/C ratio and all the three parameters considered, i.e., HbA1c, WHR and BMI. There is a positive correlation and steady increase in N/C ratio and all the three parameters considered, i.e., HbA1c, WHR and BMI [Table 2]. There is almost equal density of case distribution with high N/C ratio correlating with score 3 HbA1c and WHR whereas there was less distribution of cases with score 3 BMI [Figures 4 and 5].

DISCUSSION

Diabesity is of major health concern since persistent stress within the cell induces cytotoxicity and genomic instability.[4] The aforementioned qualitative and quantitative parameters and their correlation with morphologic features may facilitate early diagnosis and identification of individuals at risk of developing new age systemic illnesses thereby comforting a prompt

Table 2: Comparison of correlation coefficients and *P* values of nuclear area, cytoplasmic area and N/C ratio in various study groups

	Correlations					
	N/C	HbA1c	WHR	BMI	NA	CA
N/C						
Pearson correlation	1	0.172	0.134	0.227*	0.422**	−0.518**
P (two-tailed)		0.126	0.234	0.043	0.000	0.000
n	80	80	80	80	80	80
HbA1c						
Pearson correlation	0.172	1	−0.030	0.192	0.748**	0.546**
P (two-tailed)	0.126		0.792	0.089	0.000	0.000
n	80	80	80	80	80	80
WHR						
Pearson correlation	0.134	−0.030	1	0.445**	0.429**	0.278*
P (two-tailed)	0.234	0.792		0.000	0.000	0.013
n	80	80	80	80	80	80
BMI						
Pearson correlation	0.227*	0.192	0.445**	1	0.435**	0.180
P (two-tailed)	0.043	0.089	0.000		0.000	0.111
n	80	80	80	80	80	80
NA						
Pearson correlation	0.422**	0.748**	0.429**	0.435**	1	0.544**
P (two-tailed)	0.000	0.000	0.000	0.000		0.000
n	80	80	80	80	80	80
CA						
Pearson correlation	−0.518**	0.546**	0.278*	0.180	0.544**	1
P (two-tailed)	0.000	0.000	0.013	0.111	0.000	
n	80	80	80	80	80	80

*Correlation is significant at the 0.05 level (two-tailed), **Correlation is significant at the 0.01 level (two-tailed). HbA1c: Glycated hemoglobin, WHR: Waist-to-hip ratio, BMI: Body mass index, NA: Nucleus area, CA: Cytoplasmic area

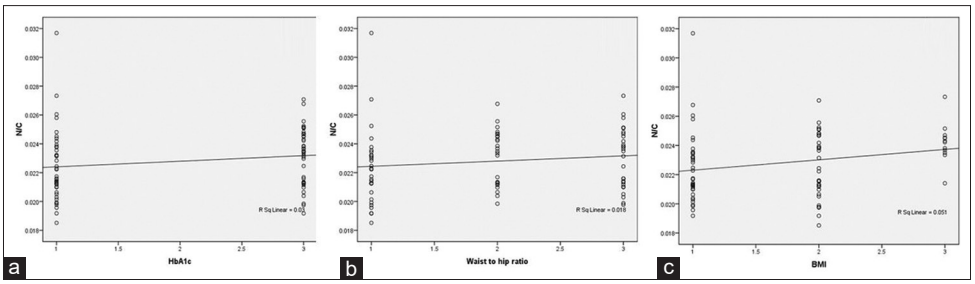

Figure 4: (a) Positive correlation coefficient between N/C ratio and HbA1c, (b) Positive correlation coefficient between N/C ratio and WHR, (c) Positive correlation coefficient between N/C ratio and BMI. WHR: Waist-to-hip ratio, BMI: Body mass index, HbA1c: Glycated hemoglobin

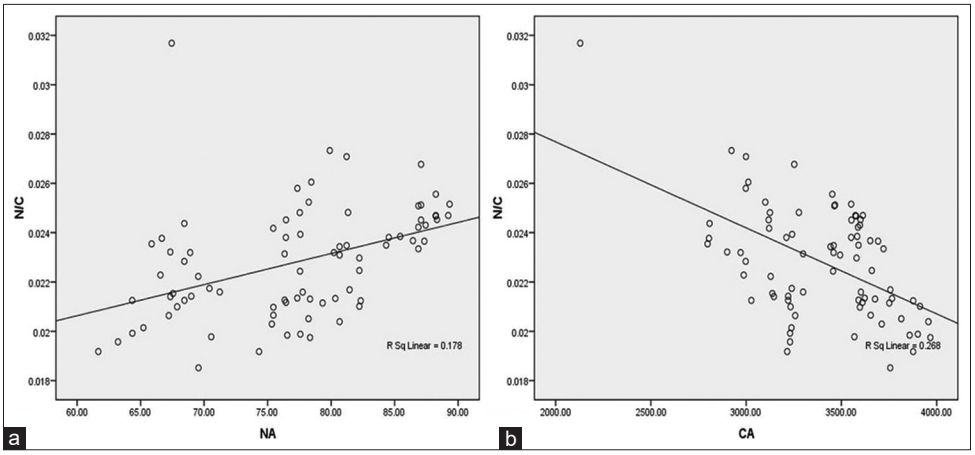

Figure 5: Comparison of correlation coefficients and P values of NA, CA and N/C ratio in various study groups. (a) NA: Nuclear area, (b) CA: Cytoplasmic area

intervention. Normally, a cell maintains proper proportion and quantities of different cellular constituents by genetic and enzymatic regulation.[9]

Hence, in a cell, the enzymes which are inactive can be activated as per requirement. Morphological alteration due to xerostomia results in a dry, atrophic mucosa accompanying mucositis as well as opportunistic infections with an increase in inflammatory response to microbial colonization. It is a well-established fact that there is a neutrophil chemotactic defect in DM. In an attempt to overcome this deficiency, a positive feedback mechanism results in an increased inflammation. Adverse hormonal, microvascular and neuronal changes may also serve as the cause behind increased inflammation.[10]

Cytokines produced as a result of inflammation are apparently redundant and pleiotropic; as a result, they can share functions and act on many cell types since a cell can have receptors for more than one cytokine. Alternatively, they may act as paired agonist/antagonist ligands in several cell functions.[11,12]

This leads to ischemia and atherosclerosis which in turn causes progressive narrowing of the vessel lumen, decreased tissue perfusion and decrease in cell turnover. This causes delay in the keratinization process of the epithelium. This delay in the cell differentiation process leads to an increase in the number of cells which usually have a large nucleus.[13]

An aging cell also shows nuclear degeneration such as karyolysis which presumably occurs secondary to DNAse activity; pyknosis which is characterized by nuclear shrinkage, condensed chromatin and increased basophilia; and karyorrhexis where there is fragmentation of pyknotic nucleus, along with nuclear vacuolization and perinuclear halo.[14]

Quantitative assessment showed less mean CA, meaner NA and highest N/C ratio in Group 1 followed by Group 3 and Group 2. As far as diabetes is considered, it is an established fact that nuclear size increases and is supported by multiple studies witnessed in literature.[10,15-19] Furthermore, it is a well-established fact that there is an inflammatory role superadded to decreased tissue perfusion because of advanced glycation end products formation in diabetes mellitus. Along with diabetes, superadded obesity can have adverse impact on the cellular and nuclear stress which is primary cause of any disease progression. Adverse hormonal, microvascular

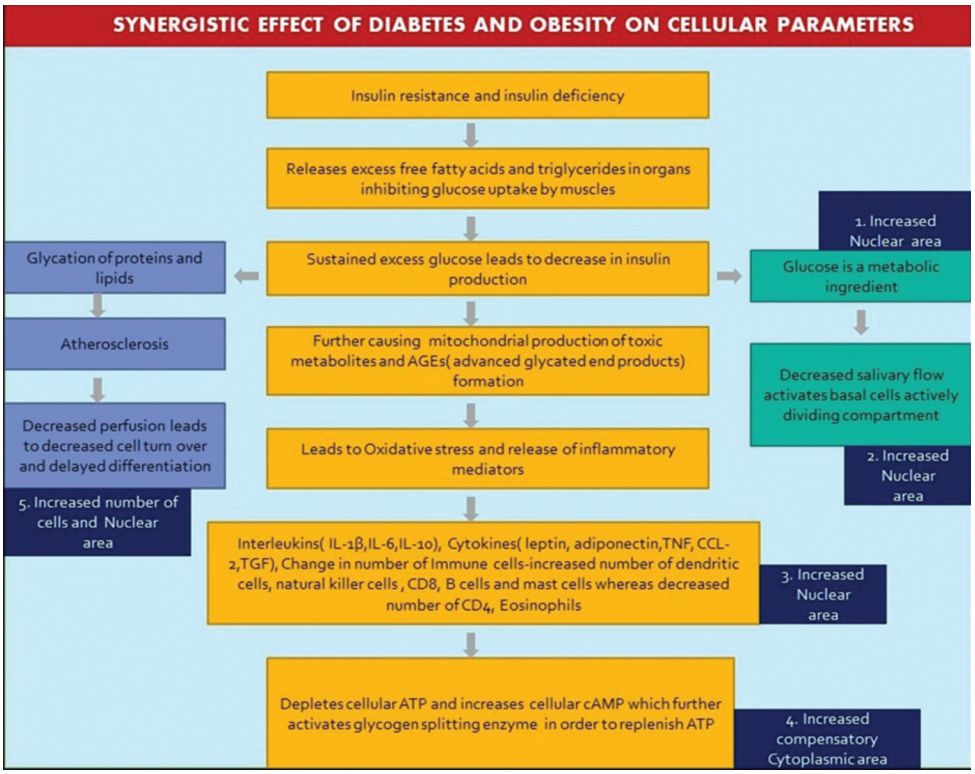

Figure 6: Synchronous pathophysiology of diabetes and obesity in bringing cytomorphometric changes

and neuronal changes may also serve as the cause behind increased inflammation. Hyperglycemia being the major sequel of T2DM exacerbates the inflammatory processes and promotes the accumulation of RAGE and TLR4 ligands within the oral mucosa through induction of advanced glycated end product formation and colonization of microbes[20] [Figure 6].

CONCLUSION

The need of the hour is combating the twin epidemic of diabetes and obesity which requires a comprehensive approach including dietary modifications and regular physical activity, augmented by newer pharmaceutical options which completely rely on early diagnostic changes at cellular and nuclear levels which may be easily established using cytomorphometry.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Pereira SS, Alvarez-Leite JI. Low-grade inflammation, obesity, and diabetes. Curr Obes Rep 2014;3:422-31.
2. Felber JP, Golay A. Pathways from obesity to diabetes. Int J Obes Relat Metab Disord 2002;26 Suppl 2:S39-45.
3. Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, *et al.* Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake *in vivo*. Diabetes 2004;53:1418-23.
4. Verma S, Hussain ME. Obesity and diabetes: An update. Diabetes Metab Syndr 2017;11:73-9.
5. Fenech M. Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. Mutat Res 2006;600:58-66.
6. Fenech M. Cytokinesis-block micronucleus Cytome assay. Nat Protoc 2007;2:1084-104.
7. World Health Organization, WHO Technical Report Series 894. Obesity: Pre-venting and Managing the Global Epidemic. Report of a WHO Consultation. Geneva: World Health Organization; 2000.
8. World Health Organization, Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation Geneva; 8–11 December, 2008, Available from: http://whqlibdoc.who.int/publications/2011/9789241501491eng.pdf. [Last updated on 2021 Sep 05; Last accessed on 2013 Jul 09].
9. Guyton, A.C. and Hall, J.E. (2006) Textbook of medical physiology. 11th Edition., Elsevier Saunders, Philadelphia. WB Saunders Co; 2006.
10. Suvarna M, Anuradha C, Kumar KK, Sekhar PC, Chandra KL, Reddy BV. Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. J NTR Univ Health Sci 2012;1:33-7.
11. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, *et al.* Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. Cell 2008;132:259-72.
12. Court N, Vasseur V, Vacher R, Frémond C, Shebzukhov Y, Yeremeev VV, *et al.* Partial redundancy of the pattern recognition receptors, scavenger receptors, and C-type lectins for the long-term control of *Mycobacterium tuberculosis* infection. J Immunol 2010;184:7057-70.
13. Hallikerimath S, Sapra G, Kale A, Malur PR. Cytomorphometric analysis and assessment of periodic acid Schiff positivity of exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients. Acta Cytol 2011;55:197-202.
14. Kumar V, Cotran RS, Robbins SL. Robbins Basic Pathology. 7th ed.

Philadelphia: Saunders; 2003.
15. Jajarm HH, Mohtasham N, Moshaverinia M, Rangiani A. Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method. J Oral Sci 2008;50:335-40.
16. Sonawane K, Jain S, Gupta I, Karthik BV, Singaraju S, Singaraju M. Cytomorphometric analysis of oral mucosa in diabetic patients in Bhopal region an *in situ* study. IJCDS 2011;2:12-5.
17. Alberti S, Spadella CT, Francischone TR, Assis GF, Cestari TM, Taveira LA. Exfoliative cytology of the oral mucosa in type II diabetic patients: Morphology and cytomorphometry. J Oral Pathol Med 2003;32:538-43.
18. Shareef BT, Ang KT, Naik VR. Qualitative and quantitative exfoliative cytology of normal oral mucosa in type II diabetic patients. Med Oral Patol Oral Cir Bucal. 2008;13: E693-6.
19. Sahay K, Rehani S, Kardam P, Kumra M, Sharma R, Singh N. Cytomorphometric analysis and morphological assessment of oral exfoliated cells in type 2 diabetes mellitus and healthy individuals: A comparative study. J Cytol 2017;34:27-33.
20. Amir J, Waite M, Tobler J, Catalfamo DL, Koutouzis T, Katz J, *et al*. The role of hyperglycemia in mechanisms of exacerbated inflammatory responses within the oral cavity. Cell Immunol 2011;272:45-52.