Promising Role of Exfoliative Cytology in the Evaluation of Glycaemic Status of Type II Diabetics: A Pilot Study

Yasmin Satpathy · Praveen S. Kumar ·
Navneet Singh

Received: 8 June 2013 / Accepted: 14 October 2013 / Published online: 29 October 2013
© Association of Oral and Maxillofacial Surgeons of India 2013

Abstract

Background The degree of metabolic control in diabetes mellitus influences the susceptibility of patients to oral diseases. It is mandatory to regularly monitor glycaemic status, however invasive methods may be contraindicated or intolerable to diabetic individuals. Thus, cytology, being a simple, non-invasive and rapid procedure, is a promising protocol for assessing diabetic status and assisting in management.

Aim To assess the number of PAS positive glycogen containing cells and associated cellular changes in buccal smears of type II diabetics and correlate the findings with their serum glucose levels.

Settings and design The study was conducted at the outpatient Department of Oral and Maxillofacial Pathology, KLES Institute of Dental Sciences, Belgaum.

Materials and methods Fifty known cases of type II diabetes mellitus and 50 healthy individuals were included in the study. Fasting blood glucose levels were estimated and buccal smears stained with Periodic Acid Schiff stain. The observed cellular changes were correlated with the glycaemic status of each patient.

Statistical analysis Statistical evaluations such as Student's *t* test ($P < 0.01$ —very significant; $P < 0.001$ —highly significant), correlation-coefficient and probability values were computed.

Results Smears of diabetic patients depicted an increase in the number of PAS positive cells in significant correlation to their glycaemic status. Cellular and nuclear morphological alterations were also found in squames of diabetic individuals.

Conclusion Cytological findings and clinical observations, suggest a correlation between the extent of these changes and clinical parameters like glycaemic control. Further studies in this aspect can help in improving the reliability of oral cytology as a diagnostic tool in diabetes.

Keywords Diabetes mellitus · Glycaemic status · Exfoliative cytology

Introduction

India has earned the dubious distinction of 'Diabetes capital of the world'. The 'Asian Indian Phenotype' is an important contributing factor which makes Indians more susceptible to diabetes. Thus early screening protocols for high risk groups and periodic monitoring of glycaemic status of known diabetics will greatly reduce the burden of this multi system disorder [1, 2].

In diagnostic technology, the race for the next generation of bloodless, painless and accurate techniques in evaluation of glycaemic status has begun. Exfoliative Cytology, a simple, rapid and inexpensive procedure used to study physiologically desquamated cells has proved an efficient diagnostic adjunct in some oral lesions [3, 4].

Glycogen a polysaccharide of glucose which serves as an energy reserve in cells is regulated by two hormones—epinephrine and glucagon. In diabetes these hormones fail to break down glycogen, resulting in its erratic accumulation [5, 6].

Y. Satpathy · N. Singh
Department of Oral Pathology, Surendra Dental College and
Research Institute, Sri Ganganagar, India

P. S. Kumar (✉)
Department of Oral and Maxillofacial Surgery, Goa Dental
College and Hospital, Bambolim 403202, Goa, India
e-mail: praveen1931@gmail.com

Various cyto-chemical studies have been done to evaluate the expression of glycogen in normal embryonic tissue, wound healing, keratinization and inflammation, but with varying results [7].

This study was undertaken to assess the presence (quantity and quality) of glycogen and other cellular changes in the exfoliated buccal cells of Type II diabetics and correlate them with the serum glucose levels, to establish exfoliative cytology as a diagnostic adjunct in Diabetes Mellitus.

Materials and Method

Fifty diagnosed cases of type II Diabetes Mellitus (study group) and fifty healthy, age and sex matched volunteers (control group) were included in the study. The patients were selected randomly from those visiting the out patient department of Oral and Maxillofacial Pathology, KLES Institute of Dental Sciences, Belgaum, for a routine blood examination. Patients with frank oral lesions and other systemic disorders were excluded from the study. The patients were briefed on the study and a detailed case history with specific details on duration of diabetes and mode of glycaemic control were recorded. A signed consent was obtained and the patients were recalled for a fasting blood sample collection.

Two ml of the patient's venous blood was drawn under aseptic measures and glucose levels estimated via enzymatic photo-colorimetry (Biozyme Liquid Glucose Kit, Biomedix, Bangalore). The values were undisclosed to the observers to eliminate bias.

A thorough examination of the hard and soft tissues of the oral cavity was done and the buccal mucosa was wiped using moistened gauze to remove excess debris and saliva. Scrapings were taken using a disposable wooden spatula and transferred on to a clean glass slide. Smears were prepared in a circular pattern and stained with Periodic Acid Schiff technique.

Smears were analyzed for—

1. *PAS positive cells* Uniformity in area assessed was maintained in each slide. 20 separate fields and an approximate 100 cells were viewed at a magnification of 100 $\times$. Magenta colored glycogen positive cells (Fig. 1) were counted with the common consensus of two observers and the results tabulated.
2. *Cellular alterations* Cells were screened under the oil immersion objective and features such as cytoplasmic changes, nuclear changes, inflammatory cells and microbial colonies were analyzed and the observations tabulated.

The obtained results were then compared with serum glucose levels for each patient.

Fig. 1 Periodic Acid Schiff stain of a buccal smear of a diabetic patient ($\times 40$)

Results

The obtained results of the study group were tabulated under seven sub headings as per the observations made. The entries were recorded in increasing order of the glycaemic status of the patients (Tables 1, 2).

The fasting serum glucose levels of the diabetic patients ranged from 135 to 165 mg/dl with an exceptional case of uncontrolled glycaemic status (295 mg/dl). The study group was divided into three class intervals based on the estimated glucose levels. As per the observations recorded in the buccal smears, each feature was then compared to and correlated with the serum glucose levels (Table 3).

The PAS positive cells stain a dark magenta pink color. Only those cells that stained intensely were taken into account. In some cells, the entire cytoplasm took up the stain while others depicted a speckled distribution. The glycaemic status was also evaluated based on these two staining patterns (Table 4).

Few inflammatory cells, predominantly lymphocytes were found in close proximity to and infiltrating the buccal squames (Table 5).

Cells with nuclear size double or more comparatively, than a standard nucleus were taken into account (Table 6).

Micro-organisms mainly cocci and bacilli were found in most buccal smears. They were accounted for, depending on the number of squames colonized by microbes (Table 7).

Discussion

In 1997, the expert committee on 'Diagnosis and Classification of Diabetes Mellitus' had enlisted certain diagnostic criteria. Positive findings include symptoms such as polyuria, polydipsia and unexplained weight loss and a RBS ≥ 200 mg/dl or a FBS ≥ 126 mg/dl or a PBS ≥ 200 mg/dl. The normal values fall within the range 80–110 mg/dl for FBS and less than 140 mg/dl for PBS [8].

Table 1 Master chart of diabetic patients, case numbers one to twenty-five

Case no.	PAS +ve cells/ staining pattern	Increased nuclear size	Karyolysis/ karyorrhexis	Binucleation	Inflammatory cells	No. of microbes	Serum glucose levels (mg/dl)
1	28/S	0	0	0	0	10	138
2	30/S	3	0	1	7	11	139
3	30/S	3	1	0	6	15	140
4	32/S	4	0	1	7	20	140
5	30/S	3	0	1	6	28	140
6	35/S	5	1	1	6	25	140
7	35/S	4	0	0	8	20	140
8	32/S	5	0	0	7	25	143
9	32/S	4	0	0	7	35	143
10	35/S	4	0	1	7	30	145
11	34/S	6	1	1	8	25	145
12	32/S	5	1	0	7	22	145
13	32/S	3	0	0	7	25	145
14	45/S	5	1	0	7	32	145
15	30/S	5	1	0	8	30	145
16	35/S	4	1	0	7	35	145
17	45/S	5	0	1	8	28	145
18	32/S	5	0	0	9	30	145
19	30/S	6	0	0	8	32	146
20	34/S	4	1	1	8	30	146
21	30/S	6	1	0	8	30	147
22	45/D	6	0	0	9	35	149
23	47/S	5	0	1	9	32	149
24	45/S	5	0	0	9	35	149
25	44/S	6	1	1	10	35	150

The ADA recommendations for diagnosis of DM focus on the FBS, while WHO focuses on the Oral Glucose Tolerance Test. The glycated hemoglobin (HbA1c) and fructosamine is also still useful for determining blood sugar control over time. However, practicing physicians frequently employ other measures in addition to those recommended. In July 2009, the International Expert Committee (IEC) recommended the additional diagnostic criteria of an HbA1c result for DM. This committee identified the range of HbA1c levels 6.0 % and <6.5 % to categorize those at high risk of developing DM [9].

In this study, 50 known cases of type II diabetes (diagnosed using ADA criteria), 25 males and 25 females in the age group 35–50 years were included. The duration of diabetes ranged from 2 to 10 years and the FBS levels ranged from 135 to 165 mg/dl. All 50 patients were on oral hypoglycemic drugs. Fifty age and sex matched, healthy individuals were included in the control group. Serum glucose levels were evaluated and buccal smears prepared and stained for each patient.

In the study group, the smears revealed a minimum of 28 and a maximum of 62 PAS positive cells in patients

with corresponding glucose levels ranging between 135 and 165 mg/dl. The varying numbers of PAS positive cells were consistent with the serum glucose levels ($r = 0.4420$ and $P = 0.0013$). Although the significance is unclear, the implication is that these cellular changes lower the mucosal barrier, promoting infections and affecting regeneration and repair. Advanced glycosylation end products (AGEs) observed in diabetic patients accelerate cell ageing and aged cells are known to exhibit such changes [10].

Two staining patterns of PAS positivity were observed; speckled and diffuse (Figs. 2, 3). In patients with blood glucose levels ranging between 135 and 150 mg/dl, the cells predominantly took up a speckled appearance, while in patients with blood glucose levels higher than 150 mg/dl, the cells took up a more intense and diffuse stain. Literature search revealed no relevant data.

A significant increase in inflammatory cells (Fig. 4) were found in buccal smears of patients with higher glycaemic status ($r = 0.4457$ and $P = 0.0012$). This may be due to diminished salivary flow in diabetes related to hypo function of salivary glands secondary to hormonal, vascular and neuronal changes [10, 11].

Table 2 Master chart of diabetic patients, case numbers twenty-six to fifty

Case no.	PAS +ve cells/ staining pattern	Increased nuclear size	Karyolysis/ karyorrhexis	Binucleation	Inflammatory cells	No. of microbes	Serum glucose levels (mg/dl)
26	47/S	6	1	0	9	37	150
27	47/S	6	1	1	8	38	150
28	44/S	5	1	0	8	30	150
29	46/S	6	1	0	8	30	150
30	48/D	5	1	1	8	37	150
31	44/D	6	4	0	8	39	150
32	45/D	6	1	0	9	35	150
33	50/D	5	1	1	8	30	150
34	48/D	6	1	0	8	30	150
35	50/D	5	1	1	8	38	151
36	45/D	6	2	1	9	38	155
37	50/D	6	1	0	10	36	155
38	56/D	8	1	1	9	35	157
39	51/D	5	3	1	9	35	158
40	52/D	6	1	0	10	36	159
41	56/D	8	0	1	12	39	160
42	55/D	8	1	1	11	38	160
43	51/D	5	0	1	11	35	160
44	55/D	8	1	1	12	38	160
45	57/D	8	2	0	12	40	160
46	55/D	6	1	0	10	35	160
47	59/D	7	1	1	13	38	161
48	58/D	8	0	0	15	39	162
49	62/D	9	0	1	17	43	165
50	54/D	7	0	0	12	33	295

S speckled, D diffuse

Table 3 Comparison of number of PAS positive cells with serum glucose levels

PAS positive cells	Serum glucose levels (mg/dl)
28–45	135–145
30–50	146–155
51–62	156–165

Table 4 Comparison of staining pattern of PAS positive cells with serum glucose

PAS positive cells	Staining pattern	Serum glucose levels
28–47	Speckled	135–150
45–62	Diffuse	>150

It is known that in the epithelium, stratum intermedium has the highest glycogen reserve. An increase in cellular glycogen is a result of reactionary acanthosis, often associated with chronic inflammation. In diabetic patients there

Table 5 Comparison of number of inflammatory cells with serum glucose levels

Inflammatory cells	Serum glucose levels (mg/dl)
0–9	135–145
8–10	146–155
9–17	156–165

Table 6 Comparison of cells with increased nuclear size and serum glucose levels

Cells with increased nuclear size	Serum glucose levels (mg/dl)
0–6	135–145
4–6	146–155
5–9	156–165

is a neutrophil chemotactic defect. To overcome this, a positive feed back mechanism acts, resulting in an increased inflammatory component [10–13].

Table 7 Comparison of number of microbial colonies with serum glucose levels

No. of microbial colonies	Serum glucose levels (mg/dl)
10–35	135–145
30–39	146–155
32–43	156–165

Fig. 2 Speckled Periodic Acid Schiff staining pattern in an individual cell ($\times 100$) **Fig. 3** Diffuse Periodic Acid Schiff staining pattern in an individual cell ($\times 100$) **Fig. 4** Lymphocytic infiltration of buccal squames (PAS stain, $\times 100$) **Fig. 5** Microbial colony of cocci and bacilli (PAS stain, $\times 100$) **Fig. 6** Fungal hyphae (PAS stain, $\times 100$) **Fig. 7** Increase in nuclear size (PAS stain, $\times 100$) **Fig. 8** Binucleation (PAS stain, $\times 100$)

Poor glycaemic control in patients makes the oral cavity more vulnerable to microbial infections [14, 15]. In this study too, an increase in the number of bacterial micro organisms (Figs. 5, 6) infesting individual cells was observed in patients with higher glycaemic status; $r = 0.284$ and $P = 0.046$. Only one case of candidiasis was reported.

Of the cellular features noted, the number of cells showing increase in nuclear size (Fig. 7) was found to be higher in patients with high serum glucose levels; $r = 0.3915$ and $P = 0.0049$. Other findings like karyolysis, karyorrhexis and binucleation (Fig. 8) were also noted. Studies in cytomorphometry have yielded similar results. These changes have been attributed to the increased cellular age of diabetics. Atherosclerosis in diabetes leads to ischemia which in turn causes a decline in cell turnover and thus a greater concentration of aged cells [10].

The overall process of aging in an individual is greatly affected by genetic factors, diet, social conditions and age related diseases, such as diabetes. In addition, there is good evidence that cellular alterations induced by aging are an important component of aging in an organism. Cellular aging is a progressive decline in the proliferative capacity and life span of cells; the effects of continuous exposure to exogenous factors cause the progressive accumulation of cellular and molecular damage. Morphological alterations in aging cells include irregular and abnormally lobed nuclei and pleomorphism [16–18].

The smears of the control group revealed a minimum of 5 and a maximum of 18 PAS positive cells in patients with corresponding blood glucose levels of 60–115 mg/dl. Comparatively the PAS staining intensity of the buccal squames in the control group was lighter than those of the study group. No significant findings other than a few micro organisms were seen.

Conclusion

The frequent use of invasive techniques in diabetic patients is losing viability due to variations in blood glucose levels and in the disease itself. Recent advances in quantitative techniques have refined the potential role of cytology with an appraisal of its value in oral diagnosis. Noteworthy changes in the mucosa of diabetics, will give the clinician a more accurate image of what happens in diabetes. Identifying these changes can also help in early diagnosis and screening of diabetic patients. The future scope of this study lies in assessing a larger sample size, evaluating and comparing cellular glycogen expression in early diagnosed

cases and treated cases of type II diabetes. Further research must be done to investigate various cytomorphological changes taking place as the disease progresses. Thus, oral cytological changes observed in patients with diabetes mellitus could be associated to cellular metabolic disturbances. A cytological profile assessment in diabetic individuals would be a simple method to evaluate glycaemic status and treatment efficacy.

References

1. Mohan V, Sandeep S (2007) Epidemiology of type 2 diabetics: Indian scenario. *Indian J Med Res* 125:217–230
2. Ramachandran A, Snehalatha C, Viswanathan V (2002) Burden of Type 2 diabetes and its complications—the Indian scenario. *Curr Sci* 83(12):1471–1475
3. Rickles NH (1972) Oral exfoliative cytology: an adjunct to biopsy. *CA Cancer J Clin* 22:163–171
4. Alberti S, Cesar TS (2003) Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. *JOPM* 32:538–545
5. Kishi K (1975) Cytochemical study of exfoliated cells of oral mucosa. *Acta Med Okayama* 29:103–109
6. Powell RN (1969) Gingival tissue physiology in vitro. *J Periodontol Res* 4:36–41
7. Porter K, Lefkowitz W (1965) Glycogen in developing and young rat oral epithelium. *JDR* 44:954–961
8. Kuzuya T (2002) Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract* 51(1):65–85
9. Olokoba AB, Obateru OA, Olokoba LB (2012) Type 2 diabetes mellitus: a review of current trends. *Oman Med J* 27(4):269
10. Muruganandhan J, Sivakumar G, Sujatha G, Srinivasa P, Ramasamy A (2011) Diabetes mellitus and oral cytology—simplifying management. *J Orofacial Sci* 3(2):61–65
11. Weiss MD, Weinmann JP, Meyer J (1959) Degree of keratinization and glycogen content in the uninflamed and inflamed gingiva. *J Periodont* 30:208–218
12. Ben-Aryeh H, Serouya R, Kanter Y, Szargel R, Laufer D (1993) Oral health and salivary composition in diabetic patients. *J Diabetes Complicat* 7(Suppl 1):57–62
13. Suvarna M, Anuradha C, Kumar KK, Sekhar PC, Chandra KLP, Reddy BVR (2012) Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. *J Dr. NTR Univ Health Sci* 1:33–3710
14. Trott JR (1957) The presence and distribution of glycogen in the gingiva. *Aust Dent J* 2:283–294
15. Li X, Kolltveit KM, Tronstad L, Olsen I (2000) Systemic diseases caused by oral infections. *Clin Microbiol Rev* 13(Suppl 4):547–558
16. Roe JN, Smoller BR (1998) Review of bloodless glucose measurement. *Crit Rev Ther Drug Carrier Syst* 15(Suppl 3):199–241
17. Kumar V, Cotran RS, Robbins SL (2004) Robbins basic pathology, 7th edn. Saunders, Philadelphia, pp 42–1198
18. Davidson SS, Haslett C (2005) Davidson principles and practice of medicine, 19th edn. Churchill Livingstone, Edinburgh