



Cytomorphometric Assessment of Buccal Mucosa Cells and Blood Sugar Status in Diabetic Patients in Zahedan (2019)

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Abstract

Background: Diabetes mellitus is one of the major global health threats. Diabetes can cause adverse cytopathological changes in cells and predispose them to pathological lesions. The present study aimed to investigate the cytopathological changes of oral mucosal cells in type 1 and 2 diabetes patients and its relationship with blood sugar status.

Methods: This study descriptive-analytical was performed on 40 type-1 diabetes patients, 40 type-2 diabetic patients, and 20 non-diabetic individuals (control group) with simple sampling in Zahedan (2019). Their buccal mucosa was sampled by a cytobrush and the microscope slides were prepared with Papanicolaou staining. The nuclear and cytoplasmic area and cytoplasmic-nuclear ratio were calculated. Furthermore, the relationship of hemoglobin A1C and fasting blood sugar with these parameters were also examined. Data was analyzed with one-way-ANOVA, Kruskal-Wallis, Post Hoc Tukey, Mann-Whitney, Pearson correlation and Spearman correlation tests. In this regard, the statistical software SPSS (version 21) was used and a p-value <0.05 was considered statistically significant.

Results: Based on the findings, only the nuclear area was significantly larger in type 1 and type 2 diabetes patients, compared to the control group (p<0.001 and p=0.010), respectively. Moreover, the comparison of cytomorphometric changes between type 1 and type 2 diabetes patients did not show a significant difference. In addition, the hemoglobin A1C levels were merely associated with the cytoplasmic area in type 2 diabetes patients (p=0.011), while fasting blood sugar levels were not associated with any of the parameters in type 1 and type 2 diabetes patients (p>0.050).

Conclusion: Diabetes, as an independent factor, can cause cytomorphometric changes in the buccal mucosal cells of type 1 and type 2 diabetes patients. It seems that the type of diabetes does not affect these changes. hemoglobin A1C levels were correlated with cytoplasmic area in type 2 diabetes patients.

Keywords: Diabetes mellitus, Cytology, Glycemic Index, Papanicolaou Test

Conflicts of Interest: None declared

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Introduction

Diabetes mellitus (DM) is a heterogeneous metabolic disorder that leads to chronic hyperglycemia due to impaired insulin secretion, impaired insulin effect, or both

(1). Various forms of diabetes can be diagnosed by tests, including fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), and hemoglobin A1C (HbA1C). Ac-

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↑What is “already known” in this topic:

Based on the literature review, only a few studies have compared the cytopathological changes of oral mucosa in type 1 and type 2 diabetes patients. Moreover, the relationship between blood sugar control status and cytopathological changes in oral mucosa in type 1 diabetes patients has not been studied yet.

→What this article adds:

Diabetes, as an independent factor, can cause cytomorphometric changes in the buccal mucosal cells of type 1 and type 2 diabetes patients. It seems that the type of diabetes does not affect these changes. HbA1C levels were correlated with cytoplasmic area in type 2 diabetes patients.

According to the latest categorization by the American Diabetes Association in 2020, the types of diabetes are type 1 diabetes, type 2 diabetes, gestational diabetes mellitus, and certain types of diabetes for other reasons, such as monogenic diabetes syndromes, pancreatic exocrine diseases, and drug-induced diabetes (2). Some of the oral symptoms of diabetes include periodontitis, dental caries, periapical abscess, dry mouth, burning mouth syndrome, oral mucosal lesions, taste disorders, and oral cancer (3, 4).

Diabetes can increase the size of the nucleus and reduce the cytoplasmic-nuclear ratio, which can predispose cells to malignant changes. On the other hand, the review of the related literature has shown that hyperglycemia and DM are significantly associated with squamous cell carcinoma of the head and neck, and the provision of anti-cancer strategies in diabetics can prevent head and neck cancers (5).

Cytology is a method of description, measurement, and evaluation of cell characteristics, such as cell size, nucleus size, cytoplasmic-nuclear ratio, and aneuploid or diploid nuclei (6). Cytological examination of the buccal mucosa cells can be used as an early method for the detection of cell changes (7).

Agrawal et al. investigated the relationship between fasting blood sugar (FBS) and cytomorphometric values of buccal mucosal cells in diabetic patients with type 2 diabetes. According to their findings, there was a significant difference between the type 2 diabetes patients and non-diabetic subjects regarding the mean of the nuclear area and the cytoplasmic-nuclear ratio, which had a significant relationship with FBS. The results of the above-mentioned study also showed that the cytoplasmic area was not significantly different between diabetics and non-diabetic individuals and was also not associated with FBS levels. Therefore, it was found that type 2 diabetes has a significant effect on the morphology of oral mucosal cells and that cytopathological examination of cells can be used as a beneficial diagnostic test (7).

Moreover, in a study performed by Seifi et al., which was conducted in Iran, the two-lobed or multi-lobed nuclei, Karyorrhexis, and cytoplasmic vacuolation were significantly higher in diabetes patients compared to non-diabetic individuals. Furthermore, there was a significant decrease in the nuclear and cytoplasmic size and an increase in the nuclear to cytoplasm ratio in the buccal and tongue mucosa of the diabetes patients, compared to the non-diabetic participants. On the other hand, there was no significant difference in the quantitative cytometric characteristics of type 1 and type 2 diabetes patients (6).

Based on the literature review, only a few studies have compared the cytopathological changes of oral mucosa in type 1 and type 2 diabetes patients (6, 8, 9). Moreover, the relationship between blood sugar control status and cytopathological changes in oral mucosa in type 1 diabetes patients has not been studied yet. Therefore, the present study aimed to investigate the cytopathology of buccal mucosal cells in type 1 and type 2 diabetes patients and its association with the status of blood sugar control to accurately determine the role of blood sugar in cytopathologi-

cal changes.

Methods

Participants

The Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran, approved this study under the project No. 9253 (IR.ZAUMS.REC.1398.010). The present descriptive-analytical study was performed on 80 subjects with type 1 diabetes (n=40) and type 2 diabetes (n=40) who were referred to the Diabetes Center of Bu Ali Hospital in Zahedan University of Medical Sciences, Iran in summer 2019. Patients were 20-50 years old and had diabetes for at least one year. Twenty healthy individuals were selected as control groups who were matched in terms of age and gender. The initial diagnosis criteria for patients with diabetes, according to the latest classification of the American Diabetes Association, was an FBS of ≥ 126 and HbA1C of ≥ 6.5 (2). On the other hand, the exclusion criteria consisted of 1) poor oral hygiene, 2) nutrition disorders, especially anemia, 3) habits to tobacco and alcohol consumption, 4) oral lesions, 5) gingivitis and periodontitis, 6) other systemic diseases, such as infection, immunodeficiency, autoimmune conditions, malignancy, and chronic complications of diabetes (e.g., retinopathy, nephropathy, and renal failure, and 7) usage of a specific drug unrelated to diabetes (6, 10). An internal medicine specialist and an oral and maxillofacial pathologist systematically confirmed the presence of inclusion criteria and the absence of exclusion criteria.

Afterward, the purpose of the study and its benefits were explained to the eligible participants and reassured that the study was completely safe and that their information would not be disclosed. Then written informed consent was obtained. Moreover, the demographic characteristics of the subjects, such as age, gender, duration of diabetes (less than 10 years, and more than 10 years), FBS and HbA1C levels (according to the latest blood test data reported in the last month) were recorded in the related form.

Sampling

Before simple sampling, the subjects were first asked to rinse their mouths with water to remove food particles and debris. Afterward, the buccal mucosa was dried with gauze to completely remove the debris and excess saliva. Subsequently, a senior dental student sampled from the buccal mucosa with a gentle, non-bleeding scratch motion by using a cytobrush (Radteb darmam, Iran) moistened with normal saline. Then disjunct buccal mucosal cells were placed in the center of a dry, clean glass slide and spread over a larger area so that the cells did not accumulate on top of each other (6). Next, the prepared smears were fixed in Carnoy Fixative for 30-35 min. Finally, the slides were stained using the Papanicolaou staining (Padtanteb, Iran) technique and examined with an optical microscope (Labomed, US) at 400 \times magnification (8).

Cytomorphometric measurement

In total, 50 non-wrinkled cells with well-defined cellular limits were selected from each smear in a step-wise man-

ner. In this procedure, in order not to count a cell twice, the field of view was moved from left to right and then down. Afterward, the cell area (CA) was drawn by a digitizer cursor on the images obtained with the camera, and its area was calculated in square micrometers in the Motic Image Plus 3 software. Moreover, the nuclear area (NA) was drawn by a digitizer cursor and its area was calculated as a square micrometer. The cytoplasmic area (CA) was obtained through the (Cell Area – Nuclear Area=CA) formula. In addition, the cytoplasmic to the nuclear ratio (CNR) was obtained by dividing the cytoplasm area by the nuclear area.

Statistical analysis

In order to compare cytological variables (NA, CA, and CNR) in three groups of type 1 and 2 diabetes patients and non-diabetic subjects, regarding the normal or abnormal distribution of the data, one-way-ANOVA, Kruskal-Wallis test, Post Hoc Tukey test, and Mann-Whitney tests were used. Besides, to compare cytological variables in diabetic patients with a duration of less and more than 10 years, T-test and Mann-Whitney tests were used, respectively. In addition, to investigate the correlation between cytology variables with FBS and HbA1C levels, Pearson correlation and Spearman correlation were used based on the data distribution. In this regard, the statistical software SPSS (version 21) was used and a p-value of $P < 0.05$ was considered statistically significant.

Results

Participant characteristics: In total, three groups of type 1 diabetes patients (n=40), type 2 diabetes patients (n=40),

and non-diabetic subjects (n=20) with the mean ages of 30.05 ± 8.84 (20-53 years), 43.93 ± 6.54 (25-51 years), and 23.85 ± 1.93 years old were examined in this study. It should be mentioned that in all of the groups, 50% of the participants were male.

The mean values of FBS and HbA1C were 203.35 ± 75.58 milligrams per deciliter (mg/dL) and $9.01 \pm 2.57\%$ in type 1 diabetes patients. In type 2 diabetes patients, the mean values of FBS and HbA1C were 202.30 ± 81.13 mg/dL and $8.79 \pm 2.19\%$. The mean values of FBS and HbA1C were 84.45 ± 7.42 mg/dL and $4.99 \pm 0.29\%$ in non-diabetic persons.

Comparison of cytological variables: Comparison of the NA ($p < 0.001$) and CA ($p < 0.001$) and the CNR ($p < 0.001$) in the three groups of type 1 and type 2 diabetes patients and control revealed significant differences. Based on the two-by-two comparison of the groups, the NA was significantly larger in types 1 and 2 diabetes patients, compared to the control group (Post Hoc Tukey test, $p < 0.001$ and $p = 0.010$, respectively). Moreover, the CA was significantly smaller in types 1 and 2 diabetes patients in comparison to the control group (Mann-Whitney test, $p < 0.001$). Furthermore, the CNR was significantly less in types 1 and 2 diabetes patients than in the control group (Post Hoc Tukey test, $p < 0.001$). However, a comparison of the NA, the CA, and the CNR did not show a significant difference between types 1 and 2 diabetes patients (Table 1).

As is showed in Table 2, a comparison of the NA and CA and the CNR between the two groups of types 1 and 2 diabetes patients did not show a significant difference based on the diabetes duration of less or more than 10 years.

Table 1. Comparison of cytological variables in types 1 and 2 diabetes patients and control group

Variable	Group	Number	Average (μm^2)	Standard deviation	P value
Nuclear area	Type 1 diabetes	40	6372.55	1525.17	<0.001*
	Type 2 diabetes	40	5732.83	1441.02	
	Control	20	4607.55	765.71	
Cytoplasmic area	Type 1 diabetes	40	189550.33	49318.45	<0.001**
	Type 2 diabetes	40	193784	59002.44	
	Control	20	320730.50	22866.62	
Cytoplasm to nuclear ratio	Type 1 diabetes	40	30.88	8.32	<0.001*
	Type 2 diabetes	40	35.14	11.13	
	Control	20	71.03	9.89	

* ANOVA test

** Kruskal-Wallis test

Table 2. Cytological variables in types 1 and 2 diabetes patients with a duration of less and more than ten years

Variable	Group	Average (μm^2)	Standard deviation	P value
Nuclear area	Type 1 diabetes with a duration of less than ten years	6629.55	1214.74	0.293*
	Type 1 diabetes with a duration of more than ten years	6115.55	1777.65	
	Type 2 diabetes with a duration of less than ten years	5878.30	1604.27	
	Type 2 diabetes with a duration of more than ten years	5587.35	1282.23	
Cytoplasmic area	Type 1 diabetes with a duration of less than ten years	194668.35	59852.11	0.947**
	Type 1 diabetes with a duration of more than ten years	184432.30	36813.23	
	Type 2 diabetes with a duration of less than ten years	212680.40	68032.36	
	Type 2 diabetes with a duration of more than ten years	174887.60	42019.81	
Cytoplasm to nuclear ratio	Type 1 diabetes with a duration of less than ten years	29.52	7.23	0.309*
	Type 1 diabetes with a duration of more than ten years	32.23	9.26	
	Type 2 diabetes with a duration of less than ten years	37.48	11.79	
	Type 2 diabetes with a duration of more than ten years	32.80	10.18	

* T-test

** Mann-Whitney test

Correlation of cytological variables with blood sugar status

As is shown in Table 3, in type 1 diabetes patients, NA, CA, and CNA were not correlated with FBS and HbA1C. However, in type 2 diabetes patients, the CA area had a positive correlation with HbA1C (p=0.011), while it did not correlate with FBS. Moreover, in type 2 diabetes, the NA and CNR were not correlated with FBS and HbA1C. In the control group, only the CA had a positive correlation with the mean FBS (p=0.012). In addition, in type 2 diabetes patients with a duration of more than 10 years, the NA was positively correlated with FBS, and in type 2 diabetes patients with a duration of fewer than 10 years, the CA had a positive correlation with HbA1C (p=0.018 and p=0.011, respectively).

Discussion

This study aimed to investigate the cytopathology of

buccal mucosal cells in type 1 and type 2 diabetes patients and its association with the status of blood sugar control. Based on the findings of the present study, the NA of types 1 and 2 diabetes patients was significantly larger than that of non-diabetic participants. This result was in line with the findings of the majority of the studies performed on this subject (7, 11-13). However, Seifi et al. and Abdolsamadi et al. found that the nuclear size in type 1 diabetic patients was smaller than the control group, which is inconsistent with the present study (6, 9). It should be noted that there are several reasons for the large size of the nucleus in diabetic patients that are mentioned in the following. Firstly, in diabetic patients, increased glycation of proteins, fats, and nucleic acids leads to excessive accumulation of products of glycation in the walls of large vessels as well as the basement membrane of small vessels. This leads to a narrowing of vessels lumen, reduction of blood flow to the damaged tissue, reduction

Table 3. Correlation status of cytological variables with FBS and HbA1C in types 1 and 2 diabetes patients with a duration of less and more than ten years and control group

Cytomorphometric variables	Group	Glycemic variables	Correlation coefficient	P value	
Nuclear area	Type 1 diabetes total	FBS	0.032	0.843*	
		HbA1C	0.062	0.704**	
	Type 1 diabetes with a duration of less than ten years	FBS	-0.129	0.588*	
		HbA1C	-0.169	0.477**	
	Type 1 diabetes with a duration of more than ten years	FBS	0.262	0.264*	
		HbA1C	0.294	0.208**	
	Type 2 diabetes total	FBS	0.245	0.128*	
		HbA1C	0.163	0.315**	
	Type 2 diabetes with a duration of less than ten years	FBS	0.092	0.70*	
		HbA1C	0.200	0.397**	
	Type 2 diabetes with a duration of more than ten years	FBS	0.524	0.018*	
		HbA1C	0.236	0.317**	
Control	FBS	0.307	0.188**		
	HbA1C	0.094	0.694**		
Cytoplasmic area	Type 1 diabetes total	FBS	-0.106	0.515**	
		HbA1C	-0.132	0.416**	
	Type 1 diabetes with a duration of less than ten years	FBS	-0.288	0.218**	
		HbA1C	-0.514	0.54**	
	Type 1 diabetes with a duration of more than ten years	FBS	0.056	0.816**	
		HbA1C	0.389	0.09**	
	Type 2 diabetes total	FBS	0.053	0.746**	
		HbA1C	0.398	0.011**	
	Type 2 diabetes with a duration of less than ten years	FBS	0.363	0.116**	
		HbA1C	0.558	0.011**	
	Type 2 diabetes with a duration of more than ten years	FBS	0.229	0.332**	
		HbA1C	0.377	0.101**	
	Control	FBS	0.550	0.012**	
		HbA1C	0.227	0.336**	
	Cytoplasm to nuclear ratio	Type 1 diabetes total	FBS	-0.140	0.39*
			HbA1C	-0.223	0.168**
		Type 1 diabetes with a duration of less than ten years	FBS	-0.261	0.266*
			HbA1C	-0.424	0.063**
Type 1 diabetes with a duration of more than ten years		FBS	-0.206	0.383*	
		HbA1C	-0.065	0.785**	
Type 2 diabetes total		FBS	-0.092	0.574**	
		HbA1C	0.193	0.232**	
Type 2 diabetes with a duration of less than ten years		FBS	0.334	0.151*	
		HbA1C	0.280	0.231**	
Type 2 diabetes with a duration of more than ten years		FBS	-0.426	0.061*	
		HbA1C	0.108	0.649**	
Control		FBS	-0.011	0.962**	
		HbA1C	0.060	0.801**	

* Pearson correlation ** Spearman correlation

of turnover, and ultimately a delay in the process of epithelial keratinization. Delay in the process of differentiating epithelial cells leads to an increase in the number of cells with large nuclei (7, 13, 14). Secondly, due to ischemia and atherosclerosis secondary to diabetes, the epithelial cells of the oral mucosa age faster, resulting in the accumulation of incomplete proteins within the cell, a decrease of the turnover, and an increase of the nuclear size (6). Thirdly, diabetics suffer from dehydration due to reduced salivary flow (dry mouth), which can lead to mucosal atrophy. When the smear is removed from the atrophic oral mucosa, it is possible to observe non-keratinized cells of the basal and parabasal layers, which are smaller in size but have a relatively larger nucleus. Therefore, it seems like the nuclear size has increased (10, 13). On the other hand, dry mouth leads to an increase in oral mucosa trauma, which leads to cell loss. Basal cells replace lost cells by increasing the proportion of cells that are actively dividing and have large and prominent nuclei (15, 16). Fourthly, diabetic patients are prone to chronic oral inflammatory diseases, such as candidiasis, stomatitis, gingivitis, periodontitis, and lichen planus. This can lead to an increase in the size of the nucleus and a decrease in the size of the cytoplasm, especially in young cells (8, 17, 18). However, it should be noted that none of the diabetic patients who participated in this study had oral diseases or lesions.

In the present study, the CA in types 1 and 2 diabetes patients was significantly smaller than non-diabetic subjects ($p=0$), which were consistent with the results of a study performed by Seifi et al. and Abdolsamadi et al. (6, 9). Prasad et al. and Sahu et al. revealed that the CA in diabetic patients was smaller compared to non-diabetic individuals; however, this difference was not significant (13, 18). The results of the present study were also inconsistent with those of the studies conducted by Hallikerimath et al. and Agrawal et al. since they found that the CA was larger in diabetic patients in comparison to non-diabetic subjects (7, 14). Cytoplasmic size decreases due to a variety of reasons. First, the relative deficiency of insulin in diabetes patients prevents glucose uptake by epithelial cells that need to grow, and thereby the size of cytoplasm decreases (16). Moreover, ischemia is one of the vascular changes secondary to diabetes that causes epithelial cells not to have enough oxygenation and be contracted (19). On the other hand, these vascular lesions in diabetic patients lead to precocious aging of the epithelial cells of the mouth which reduces the production of protein and nucleic acid, the number of cellular organelles, and eventually the cytoplasm size (6). Furthermore, in diabetic patients, glycation of the extracellular matrix proteoglycans is facilitated, cell bonds are altered, and cell size is reduced (19). Besides, dry mouth and dehydration are common in diabetic patients (6, 19); therefore, glucose is not easily excreted through the cell membrane holes into the cell, and the osmotic pressure in the extracellular fluid increases, leading to the osmotic passage of water out of the cell. This causes the cell membrane to shrink and the cytoplasmic size to decrease (20).

The CNR of types 1 and 2 diabetes patients was signifi-

cantly less than non-diabetic individuals ($p=0$), which is consistent with the results of other studies (7, 13, 14). Sahay et al. emphasized the importance of CNR and considered it a proper parameter that shows the dramatic changes that occur in the cell (15).

Results of the present study also showed that NA, CA, and CNR of types 1 and 2 diabetes patients did not have a significant difference, which was consistent with the findings of a study performed by Seifi et al. (6). Mirescu et al. also did not find a significant difference regarding the cellular changes between patients with type 1 and type 2 diabetes (8). However, Abdolsamadi et al. found that the NA and CNR in type 1 diabetes patients were significantly smaller than those of type 2 diabetes patients. Nevertheless, they did not find a significant difference in the CA between type 1 and type 2 diabetes (9).

Based on the results of the present study, the NA, CA, and CNR did not differ significantly between the two groups of types 1 and 2 diabetes patients, based on the duration of less or more than 10 years. Given that no study has been conducted on the relationship between cytomorphometric variables in diabetic patients and the duration of diabetes, it was not possible to compare the results of the present study with those of other studies.

The results of the present study revealed that the NA had an insignificant relationship with the FBS and HbA1C in the control group and type 2 diabetes patients. This result was in line with the findings of a study performed by Lamichhane et al. (10). The present study also showed that in type 2 diabetes patients with a duration of more than 10 years, the NA was significantly correlated with FBS ($p=0.02$) which is similar to the findings of other studies (14, 20, 21). Moreover, the results of this study revealed that CA had an insignificant positive relationship with FBS and HbA1C in the control group and type 2 diabetes patients. This result was consistent with those of the studies performed by Agrawal et al. and Hallikerimath et al. (7, 14).

Another finding of the present study was that the CA had a significant positive correlation with FBS in the groups of control ($p=0$) and with HbA1C in type 2 diabetes patients with a duration of fewer than 10 years ($p=0$). Lamichhane et al. also noted a significant positive correlation between blood glucose levels and cytoplasm size in type 2 diabetes patients, similar to the present study (10). Due to the central role of glucose in metabolic processes, the increase of the glucose level directly provides the conditions for cell growth. A growing active cell is characterized by a large and clear nucleus in a large cytoplasm (13, 15).

Based on the results of the present study, FBS had a negative correlation with the CNR in the groups of control and type 2 diabetes patients. However, Agrawal et al. and Hallikerimath et al., in their study, found a significant positive correlation between FBS and CNR (7, 14). Moreover, the present study revealed that CNR had an insignificant negative correlation with HbA1C. Nevertheless, Karthik et al. study found this negative correlation to be significant (16).

This study also revealed that in type 1 diabetes patients,

the FBS and HbA1C had an insignificant positive correlation with the NA and an insignificant negative relationship with the CA and the CNR. According to the review of the related literature, no study has examined the relationship between cytormorphometric variables and blood sugar status in type 1 diabetes patients.

Various studies had similarities and differences in their results with the present study. Differences in results can be attributed to differences in the status blood sugar control, duration of illness, age and number of participants, type of cytormorphometric measurement software, magnification of the used microscope for the evaluation of the smear, and lack of a specific method for the cytormorphometric evaluation of the samples (6).

Given the results of this study showed that diabetes could increase cytormorphometric changes, it is suggested to evaluate the simultaneous effect of diabetes and other factors affecting cytormorphometric changes such as smoking in future studies.

Conclusion

In conclusion, based on the findings of the present study, there is a significant difference between cytormorphometric changes in types 1 and 2 diabetes patients and the control group. Therefore, on the one hand, the results show that oral cytology can be used as a cheap, fast, simple, painless, and non-invasive method for microscopic evaluation of cytormorphometric changes in the oral epithelial cells of diabetes patients. On the other hand, in addition to age, gender, smoking habits, and nutritional deficiencies, diabetes is an independent factor in the development of cytormorphometric changes in the buccal mucosal cells of types 1 and 2 diabetes patients.

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Ethical approval

This study was approved by the Ethics in Research Committee of Zahedan University of Medical Sciences, ethic number: IR.ZAUMS.REC.1398.010.

Conflict of Interests

The authors declare that they have no competing interests.

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