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Original Article

Comparison of serum and salivary folate in oral squamous cell carcinoma patients

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Abstract

Background: Folate is needed for sustaining the natural function of DNA methylation and nucleotide synthesis. Problems in the metabolism folate can cause abnormality in cell activity and cell proliferation. The aim of this study was to compare the folate in serum and saliva of patients with oral squamous cell carcinoma (SCC) and healthy subjects.

Methods: In this cross-sectional study, 30 patients with oral SCC referred to the ENT department and 30 healthy individuals were studied. 2 cc saliva and 5 cc venous blood were taken from participants and were evaluated with a human folate ELISIA kit. Independent *t* test and Pearson correlation test were performed using SPSS 17.

Results: Serum folate in patients with oral SCC (8.18 ± 4.37 ng/mL) were significantly lower than control group (10.61 ± 5.79 ng/mL) ($P = 0.005$). Salivary folate was significantly lower in patients with SCC (1.13 ± 1.32 ng/mL) than in control group (2.84 ± 4.40 ng/mL) ($P = 0.029$).

Conclusion: Since the levels of serum and salivary folate in patients with oral SCC were significantly lower than that of healthy individuals, low folate levels may be associated with oral SCC.

Keywords: Biomarkers, Tumor, Folic acid, Saliva, Squamous cell carcinoma of head and neck

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Introduction

Oral squamous cell carcinoma (SCC) is a major global health problem. This cancer is the sixth most common malignancy in men and the fifteenth in women.[1] Despite some progress in treating these cancers, the prognosis remains poor, and in the last four decades, there has been little improvement in the 5-year survival rate.[2] In many countries, the incidence and mortality of oral SCC has been steady or increasing over the past decades. Mortality rates can be reduced by early detection and appropriate treatment.[3] Folic acid is a water-soluble vitamin that provides a single-carbon group for DNA duplication and prevents tumorigenic processes.[4] Folate deficiency causes DNA damage and disturbs the methylation of some specific genes, such as P16 and, most, likely P15.[5] Any Problem in the metabolism of folate can cause abnormal cell activity and proliferation due to folate's essential role in sustaining normal function of DNA methylation and nucleotide synthesis.[6] A Meta-analysis showed that that folate intake may protect against oral cancer.[7] Also, A meta-analysis study of 35,758 patients concluded that increased serum homocysteine levels and folate deficiency are associated with elevated risk of cancer.[8]

A study examining the role of folate as a prognostic factor in the development of laryngeal SCC showed that serum folate levels in patients with cancer were significantly lower than in controls.[9]

Most studies concerning the relationship between folate levels and oral cancers have been conducted on serum folate levels. Considering that saliva testing is more convenient than serum testing, the aim of this study was to compare the serum and salivary folate between patients with oral SCC and controls.

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Methods

Patient selection

In this cross-sectional study, 30 patients with oral SCCs were included by convenience sampling from Tabriz Imam Reza Hospital. The 30 control group participants were selected among healthy individuals from the oral medicine department of the faculty of dentistry for dental treatment. The sex and age of the controls were matched to the case patients. To determine the sample size, the study by Sridharan et al[1] was used. considering $\alpha = 0.05$ and power of 80%, the number of 25 samples in each group was determined. To increase the validity of the study, 20% was added to this number, and 30 samples were selected in each group.

In both groups, participants aged 20 to 70 were included in the study. Patients using any medication that can alter folate levels, such as folic acid supplementation and oral contraceptives and estrogens, and also smokers, alcohol consumers, and people with chronic renal disease and malnutrition were excluded. All procedures were in accordance with the Declaration of Helsinki of 1975, as revised in 2008. The study was explained to the patients and detailed written informed consent was attained from the participants.

In the case group, the diagnosis of oral SCC was confirmed by a biopsy and histopathological study. The patients with Oral SCC were all newly diagnosed and had not yet received any treatment.

Salivary and serum sampling

Saliva sampling was done from 9–11 am and patients were asked to refrain from eating and drinking 90 minutes before giving samples. Two cc saliva and 5 cc venous blood were taken from patients, and the collected saliva specimens were centrifuged at 4000 g for 10 minutes at 4 °C. Then, folate levels were evaluated with a human FOLAT ELISIA kit (Monobind Inc., Lake Forest, United States). A free complete blood count test was performed for the healthy controls to encourage them to participate.

Statistical analysis

A skew and elongation test was performed to assess the normality of data. An independent *t* test was performed for comparing the folate levels of the case and control groups. Pearson correlation test was used to study the correlation between serum and saliva. Statistical analysis was done using SPSS 17. *P*value less than 0.05 was considered significant.

Results

The study sample size was 60. Of the participants, 21 (70%) were female and 9 (30%) were male in each group. The mean age in the case group was 49.02 ± 12.09 and 46.36 ± 12.94 in control group. There was not any statistically significant difference in the ages of study groups ($P = 0.315$).

All patients in the case group were in stage one of the tumor node metastasis (TNM) classification system.

Serum folate levels in the SCC group were significantly ($P = 0.005$) lower than healthy participants and salivary folate levels in the SCC patients were significantly ($P = 0.029$) lower than the healthy participants (Table 1).

Serum folate levels in the case ($rp = 0.135$, $P = 0.006$) and control ($rp = 0.133$, $P = 0.003$) groups were correlated with salivary folate levels.

In the participants of both groups, serum and salivary folate levels were significantly higher in females (Table 2).

In the patients with oral cancer (case group) the serum and salivary folate levels decreased with increasing age, but this was not significant ($P = 0.06$ and $P = 0.20$, respectively) (Table 3). Also, there was no significant difference between the serum and salivary folate levels regarding the location of the cancer ($P = 0.41$ and $P = 0.32$, respectively) (Table 3).

Discussion

Clinical diagnosis of SCC is only possible in advanced stages; therefore, early detection is of high importance.[10] Due to the effect of folate in DNA methylation and its relation with cancers, this study compared the folate levels in patients with oral SCC with healthy participants. The oral cavity is the first location for nutrient and carcinogen exposure; therefore, appreciation of the relationship between folate and oral cancer may provide a better understanding of the effect of folate in other cancers.

The results of this study pointed out that serum folate

Table 1. Comparison of serum and salivary folate levels in study groups

	Case (n = 30)	Control (n = 30)	*P* value*
Serum folate level (ng/mL)	8.18 ± 4.37	10.61 ± 5.89	0.005
Salivary folate level (ng/mL)	1.13 ± 1.32	2.84 ± 4.40	0.029

**P* values are based on independent *t* test.

Table 2. Comparison of serum and salivary folate levels based on gender in study groups

Gender	Case		Control	
	Serum folate level (ng/mL)	Salivary folate level (ng/mL)	Serum folate level (ng/mL)	Salivary folate level (ng/mL)
Male	7.11 ± 2.03	0.9 ± 0.21	9.1 ± 3.1	2.1 ± 0.17
Female	8.67 ± 1.80	1.22 ± 0.34	11.29 ± 2.87	3.13 ± 1.1
P value*	0.045	0.02	0.037	0.041

**P* values are based on independent *t* test.

Table 3. Comparison of serum and salivary folate levels based on Age and location of the lesions in case group

Variables	Sub categories	No. (%)	Serum folate level (ng/mL)	*P*-value*	Salivary folate level (ng/mL)	*P* value*
Age (y)	40–50	14 (46.7%)	8.92 ± 2.43	0.06	1.15 ± 0.20	0.20
	50–60	11 (36.6%)	8 ± 1.09		1.11 ± 0.10	
	60–70	5 (16.7%)	6.6 ± 0.89		0.99 ± 0.19	
Site	Tongue	8 (26.6%)	9.12 ± 2.69	0.41	1.21 ± 0.61	0.32
	Floor of the mouth	6 (20%)	8 ± 1.26		1.12 ± 0.59	
	Gingiva	6 (20%)	7.8 ± 2.16		0.98 ± 0.21	
	Hard palate	5 (16.7%)	8.16 ± 0.98		1.14 ± 0.32	
	Lip	5 (16.7%)	7.4 ± 2.19		0.91 ± 0.12	

* *P* values are based on one-way ANOVA.

levels were significantly lower in SCC group than in healthy participants. Salivary folate levels were lower in SCC patients than in controls. In the present study, there was a significant and direct correlation between salivary and serum folate levels in healthy participants and patients with SCC. A study by Eleftheriadou et al[11] in 2006 found that serum folate levels were significantly lower in cancer patients compared to healthy subjects, which is consistent with the results of the present study. In a study by Tastekin et al,[12] the serum folate levels were lower in patients with lung cancer than in healthy subjects, and a significant and inverse relationship between the stage of lung cancer and serum folate levels was found.

In line with the present study, in the study of Pelucchi et al,[13] serum folate levels in oral SCC patients were lower than in healthy subjects. Almadori et al[14] in 2004, showed folate levels in patients with leukoplakia were lower than in healthy participants, which is similar to the findings of the present study. Erugula et al[15] concluded that the serum folate level is a useful biomarker for onset and progression of the disease. A study by Gorgulu et al[16] in 2010 found that serum folate levels in Laryngeal SCC patients were lower than in healthy subjects. A study by Fan et al. [17] exhibited that inadequate folate intake or folate deficiency in serum may increase the risk of head and neck cancers, the oral cavity, and the pharynx.

The present study also examined salivary folate levels in patients with SCC, which was significantly lower than it was in healthy individuals. In the literature search, no study was found on the subject of salivary folate levels in oral cancer patients.

In Patients with oral SCC, the folate levels were significantly greater in females. Similar to a study by Cohen et al,[18] the results of the present study indicated that folate levels were higher in females in healthy controls as well. Therefore, it seems that this difference is present in general and is not related to oral SCC.

In the present study, it was found that there was not any significant difference among the folate levels of the three age groups. This is in accordance with the results of a study by Lee et al[19] although some studies have shown that folate levels decrease with increasing age.[20] This difference can be due to the different populations of these studies.

Strengths and Limitations

The lack of enough patients to categorize them into different TNM stages was the limitation of the study. Therefore, due to the advantages of using saliva instead of serum, it is suggested that further studies be conducted on folate levels of saliva of patients with different stages of oral SCC, especially cases with metastasis. Investigation of saliva folate levels in smokers with SCC can be useful.

Conclusion

It is likely that low folate levels are associated with oral SCC, and more studies are needed to explore the role of folate levels in early detection and prevention of oral cancer and the role of folate supplementation on the malignant transformation of potentially malignant oral disorders. Also, due to the significant relationship between folate's salivary and serum levels, it is feasible to use saliva samples instead of serum to measure folate levels as a non-invasive method. further prospective studies can bring further insights into the cause-and-effect relationship between folate levels and oral SCC.

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Authors' Contribution

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Competing Interests
The authors declare that they do not have any competing interests

Data Availability Statement
The data for this research is available upon request from the corresponding author.

Ethical Approval
The Ethics Committee of Tabriz University of Medical Sciences has approved this article (IR.TBZMED.REC.1397.779).

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