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Original Article

Comparison of salivary and serum homocysteine levels in oral squamous cell carcinoma

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Abstract

Background: No factor alone has been defined as the cause of oral squamous cell carcinoma (SCC). Identification of biochemical markers involved in SCC metabolic reactions is important in SCC diagnosis. Homocysteine is an amino acid whose altered levels have been observed in various malignancies. This study aimed to assess the serum and salivary levels of homocysteine in patients with SCC and compare these data with those of healthy individuals.

Methods: In this case-control analytical study, 21 patients with oral SCC and 21 healthy subjects were studied. Salivary and serum samples were obtained, and homocysteine levels were evaluated with an HCY ELISA Kit. Independent *t*-test and Pearson correlation coefficient were used, and statistical analysis was done using SPSS 17. A *P* value < 0. 05 was considered significant.

Results: The level of serum homocysteine was 3.71 ± 2.5 in the patient group and 2.01 ± 2.11 in the control group ($P = 0.008$). The salivary homocysteine levels were 3.12 ± 1.66 in the case group and 2.93 ± 1.71 in control group ($P = 0.782$).

Conclusion: Homocysteine levels in the serum might be a good marker for diagnosis of oral SCC; however, more research is needed on using salivary homocysteine levels as a diagnostic indicator.

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

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Introduction

More than 90% of malignancies in oral cavity are squamous cell carcinomas (SCC).[1] The disease may be asymptomatic in the early stages. Therefore, patients will not seek treatment until they notice the presence of a mass, pain, paresthesia, ulcers, and unexplained bleeding. When symptoms appear, the lesion is often at an advanced stage. The 5-year survival rate of oral squamous cell carcinoma (SCC) is less than 50%,[2] despite the advances in surgery, radiotherapy, and chemotherapy, this rate has not increased. Considering that the incidence and prevalence of oral SCC is increasing, its early diagnosis and appropriate and timely treatment are vital and may meaningfully reduce morbidity and mortality. Identifying biomarkers involved in the malignancy is the first step in early detection of oral SCC.[3] Clinical examinations and biopsy are standard methods for identifying lesions, but these cannot detect the malignancies in early stages.[4] Therefore, research is shifting toward non-invasive methods, such as measurement of biomarkers, for early diagnosis. Amino acids and proteins derived from malignant cells are found in the serum and saliva of patients. The use of saliva instead of serum has advantages such as non-invasiveness, ease of collection, also low cost.[5]

One of the biological markers for detecting oral SCC is homocysteine, an amino acid that is produced from another amino acid called methionine.[6] Cysteine is a source of sulphur and is part of the metabolism of various elements, such as iron, zinc, and copper, in the body. It also acts as an antioxidant. If homocysteine cannot convert to cysteine or enter the methionine cycle, its levels increase.[7,8] Homocysteine is an intermediary metabolite in the process of methionine metabolism. It is metabolized by two distinct pathways, namely the remethylation pathway that facilitates the regeneration of methionine, and the trans-sulphuration pathway that firstly transforms homocysteine to cysteine, then to taurine. The homocysteine occupies a critical point in the metabolic pathway given its direct and indirect impact on methyl and sulphur group metabolism. DNA methylation is a pivotal determinant in regulating gene expression, chromosomal modifications, and aberrations.[9] The precise mechanism underlying the potential contribution of elevated levels

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of homocysteine to the pathogenesis of cancer remains elusive. Numerous in vitro investigations have posited various hypotheses. These include hyperproliferation, elevated mutagenesis, aberrant genomic and locus-specific methylation, anomalous apoptosis, DNA damage, and DNA repair. The most widely accepted hypothesis is the promoter hypermethylation of main tumor suppressor genes.[10] Therefore, this amino acid can be used to detect early potentially malignant disorders.[11]

Jaganath et al in 2016 evaluated plasma homocysteine levels in patients with oral SCC and oral submucosal fibrosis (OSMF) and showed that plasma homocysteine levels were higher in oral SCC and OSMF patients than in healthy subjects. Thus, they reported that plasma homocysteine levels can be a marker for early diagnosis of these diseases.[12]

Narang's research team in 2014 also concluded that the level of plasma homocysteine in all patients with OSMF increased regardless of sex and age.[13]

Given that clinical diagnosis of SCC often occurs at an advanced stage of the disease, in most cases, prognosis is very low at this stage. Therefore, early diagnosis can be of great importance. Most research has focused on the plasma levels of homocysteine, and few studies have examined its salivary levels; hence, this study aimed to compare the serum and salivary levels of this amino acid between patients with oral SCC and healthy people.

Methods

Twenty-one patients with SCCs were included by convenience sampling method from Imam Reza hospital of Tabriz University of Medical Sciences, in this case-control analytical study. The control group participants were selected among healthy individuals who had come to the oral medicine department of the faculty of dentistry for dental treatment. The control group were selected to match the gender proportions of the case group. Informed consent was attained from all participants.

The sample size was determined by the results of a study by Erugula et al.[14] Considering $\alpha = 0.05$ and 80% power, the number of samples in each group was calculated as 17. To increase the validity of the study, 20% was added to this number and 21 samples were selected for each group.

In both groups, participants aged 20 to 70 were included. In the case group the oral SCC was confirmed by biopsy and histopathological assessment. The patients with oral SCC were all newly diagnosed and had not yet received any treatment.

People who had received vitamin B12 in the previous six months, those with renal insufficiency, those with diseases that affect salivary homocysteine, including diabetes, Alzheimer's and Parkinson's disease, and osteoporosis, those with coronary artery disorders and venous thrombosis, and smokers were excluded from the study.[15,16] None of the patients were alcohol consumers.

Venous blood (5 cc) and salivary samples (2 cc) were collected from case and control group participants. Sampling was done between 9 and 11 am. To collect the samples, patients were instructed to refrain from consumption of food and drinking 90 minutes before saliva sampling. In order to determine the level of homocysteine, salivary samples were first centrifuged and frozen at -80°C until testing. Samples were analyzed with a Human Homocysteine (HCY) ELISA Kit. The serum and saliva homocysteine levels of each participant as well as their age, gender, and medical history were inserted into a data collection form.

A skewness and elongation tests were performed, to determine the normality of data. Independent *t* test was used for comparing the homocysteine levels of the study groups, due to the normality of data. Pearson correlation was used to study the correlation between serum and saliva samples. SPSS 17 was used for statistical analysis. The *P* value < 0.05 was considered to be significant.

Results

In both groups there were 12 males and 9 females. The mean age of patients was 67.6 ± 4.5 and 64.8 ± 3.9 in the case and control groups, respectively.

From the 21 patients in case group, 19 were in stage 1 and two were in stage 2 of the tumor node metastasis (TNM) classification system.

The comparison of the mean levels of homocysteine in study groups is shown in Table 1. In the patient group, the serum homocysteine levels were 3.71 ± 2.5 ng/mL, which is significantly ($P = 0.008$) higher than the serum homocysteine levels in the control group (2.01 ± 2.11 ng/mL). There was no significant difference ($P = 0.782$) between the salivary levels of homocysteine in the case (3.12 ± 1.66 ng/mL) and control (2.93 ± 1.71 ng/mL) groups.

Pearson correlation coefficient, showed that there was not any correlation between salivary and serum levels of homocysteine in the case group ($P = 0.726$) or the control ($P = 0.943$) group.

Discussion

Late detection, poor tumor response to chemotherapy and radiotherapy, and lack of reliable biomarkers for early diagnosis are some of the most common problems in oral malignancies.[17] Therefore, considering that early detection is the key to better treatment, efforts to benefit from reliable biochemical tests in early diagnosis of disease are very significant. One of the biological markers

Table 1. The mean levels of homocysteine in the study groups

Variable	Case	Control	*P* value*
Homocysteine in serum (ng/mL)	3.71 ± 2.5	2.01 ± 2.11	0.008
Homocysteine in saliva (ng/mL)	3.12 ± 1.66	2.93 ± 1.71	0.782

* *P* value based on independent *t* test.

for detecting oral SCC is homocysteine. Homocysteine represents an intermediary metabolite within the context of methionine's metabolic pathway. The methionine cycle represents a significant metabolic pathway linked to the carcinogenesis of oral SCC.[14]

Based on the findings, serum homocysteine levels in patients with SCC are significantly higher in comparison with healthy subjects. One of the reasons for the increase in the level of homocysteine in the patient group might be the lack of vitamin B12 and folic acid, which act as co-factors and co-substrates in homocysteine metabolism.[18,19] This finding is in accordance with the results of the study by Almadori et al, who found significant differences between homocysteine levels of patients with carcinoma, and both smoking and non-smoking controls.[20] Also, a study by Erugula et al yielded similar results.[14] However, a study by Gorgulu et al showed no significant difference between the SCC and the control group.[21] The difference in results might be due to the fact that the patients in Gorgulu and colleagues' study had laryngeal SCC, but the patients in the present study had oral SCC.

A study by Weinstein et al showed there was not any association between serum homocysteine and the risk of oral cancer and this means there is not any association between folate and oral cancer.[10]

Homocysteine levels have been studied in other cancers as well. A Meta-analysis was conducted by Shiao et al to investigate the association between homocysteine and the risk of developing colorectal cancer. The study demonstrated a positive correlation between high serum homocysteine and susceptibility towards the development of colorectal cancer and adenomatous polyps.[22]

In the current study, it was also shown that homocysteine levels in saliva were higher in SCC group than in the controls, though, the difference was not significant. Previous studies have evaluated homocysteine levels only in the serum, and to our knowledge, this is the only study to investigate homocysteine levels both in serum and saliva. Therefore, a comparison between the results of salivary homocysteine levels was not available.

Strengths and Limitations

The limitation of this study was the lack of enough patients to categorize them according to different TNM stages. Also, this study was a case-control study, and further prospective studies can bring further insights into the findings of this study.

Conclusion

Based on the results of this study, serum homocysteine levels might be a good marker for diagnosis of SCC. However, more research is needed on using salivary homocysteine levels for early diagnosis of SCC. Therefore, due to advantages of using saliva instead of serum, it is suggested that further studies be conducted on homocysteine levels of saliva of patients in different stages of oral SCC. Investigation of saliva homocysteine levels in smokers with squamous cell carcinoma can be useful.

Authors' Contribution

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Competing Interests

None.

Data Availability Statement

The data of this study are available from the corresponding author upon request.

Ethical Approval

The Ethics Committee of Tabriz University of Medical Sciences approved the present study (IR.TBZMED.REC.1396.1302).

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