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Original Article

Assessment of biochemical factors in blood serum of patients with oral squamous cell carcinoma

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ABSTRACT

Background: Recently, the role of biochemical factors in the etiology of oral squamous cell carcinoma (OSCC) has attracted some attention. Serum levels of biochemical factors may change in cancer patients. This study aimed to assess the serum level of folate, Vitamin B12, homocysteine, iron, copper, and selenium in patients with OSCC.

Materials and Methods: This descriptive analytical study was conducted on 30 primary OSCC patients (15 males and 15 females) presenting to Imam Khomeini Cancer Institute, who had not yet undergone treatment. Blood samples were taken and serum levels of folate, Vitamin B12, homocysteine, iron, copper, and selenium were measured. Serum levels of micronutrients in patients with different tumor sizes were analyzed by one-way ANOVA. Serum levels of micronutrients were compared among groups with and without metastasis and lymph node involvement using Student's *t*-test ($P < 0.05$).

Results: Serum levels of B12, folic acid, homocysteine, copper, iron, and selenium were 232.5 ± 102.68, 8.66 ± 4.06, 18.87 ± 8.81, 96.0 ± 22.64, 55.27 ± 40.58, and 92.47 ± 18.83 ng/mL, respectively. Relatively similar values were measured in patients with different tumor sizes with and without lymph node involvement and presence or absence distant metastasis. However, the serum level of folic acid in OSCC patients without lymph node involvement was significantly higher than that in OSCC patients with lymph node involvement ($P < 0.05$).

Conclusion: Despite some variations, serum levels of micronutrients in OSCC patients were within the normal limits. Considering the variations in serum level of copper in OSCC patients, it may be used as a diagnostic marker. However, further studies are warranted in this respect.

Key Words: Copper, folic acid, homocysteine, squamous cell carcinoma of head and neck, Vitamin B 12

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INTRODUCTION

Squamous cell carcinoma (SCC) is a malignant neoplasm of epithelial cells and is the most common malignancy in the oral cavity accounting for 90% of all oral cancers.[1,2]

Recently, advances have been made toward understanding the etiology and underlying molecular mechanisms of oral SCC (OSCC).[3] A combination of intrinsic and extrinsic factors is required for malignant

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transformation of cells.[4] Diet and intake of nutrients may also play a role in this respect. Recently, the role of serum levels of some minerals and vitamins including zinc, selenium, Vitamins B6, E, C, and A, niacin, folate, and B12 in the etiology of SCC has attracted some attention.[5] Among these, folate, B12, and homocysteine have a more prominent role because folate deficiency leads to DNA destruction and impaired methylation of some specific genes such as P16 and most probably P15, which inhibit tumor growth.[6] Furthermore, B12 deficiency impairs folate storage and results in impaired stability of the genes and destruction of DNA strands.[7]

Homocysteine is an intermediate metabolite of methionine metabolism and is metabolized into methionine or cysteine. Metabolism of homocysteine depends on Vitamin B6, Vitamin B12, and folic acid cofactors, which result in homocysteine conversion or its remethylation and conversion to methionine.[8] Increased serum level of homocysteine is associated with oxidative stress.[9]

Several factors such as genetic variations in methylene hydrofolate reductase, continuous use of Vitamin B12 or B complex and dietary folic acid can affect the serum level of homocysteine.[10]

Folate and Vitamin B12 deficiency are among the important parameters that increase the serum level of homocysteine.[11] One important strategy recommended by the International Institute of Cancer Prevention to prevent the occurrence of OSCC is eating an abundance of fruits and vegetables because insufficient consumption of fruits and vegetables results in folate deficiency.[12] Folate is involved in DNA metabolism and synthesis of purine and pyrimidine bases as a methyl group donor; thus, folate deficiency impairs repair and methylation of some genes.[13]

Some researchers believe that high levels of folic acid can be responsible for epigenetic variability (effective setup for gene expression modification), and thus, by inducing a change in gene expression, it inhibits the expression of tumor suppressor genes or activates proto-oncogenes.[14] Considering the role of folate as a coenzyme in different biological phases of synthesis, repair and methylation of DNA, sound mitotic activity of cells depends on sufficient use of this vitamin, and long-term folate deficiency can predispose the individuals to neoplasms.[15]

Epidemiologic studies evaluated the dietary intake of folate and Vitamin B12 and their serum levels in individuals and assessed their association with risk of some cancers such as prostate, stomach, lung, colorectal, and kidney cancers. However, no definite conclusion was drawn regarding the possible association of high or low serum levels of homocysteine with cancer.[16,17] Copper as a rare earth element participates in the structure of some proteins such as plasma ceruloplasmin, vascular endothelial growth factor, and transcuprein; the serum levels of copper increase in some cancer patients.[18] Evidence shows that cancer cells have very high concentrations of copper. This element plays an important role in tumor growth and angiogenesis via copper-dependent amine oxidase pathway.[19]

Selenium is a critical element for human health. The most important route of selenium intake is via the consumption of meet and grains.[20,21] Selenium has a protective effect against carcinogens and inhibits the progression of tumor in its early inflammatory stage.[22] It prevents the invasion of tumoral cells and inhibits angiogenesis.[23,24]

Studies on the correlation of OSCC with serum levels of folate, Vitamin B12, homocysteine, and rare earth elements such as iron, copper, and selenium are scarce.

For example, Gorgulu *et al*., in 2010, reported that serum levels of Vitamin B12 and folate were significantly lower in SCC patients than in healthy controls.[9] Almadori *et al*., in 2005, showed that reduction in serum folate increases the risk of SCC of the head and neck.[25] Aune *et al*., in 2011, reported that risk of oral cancers decreased by an increase in dietary intake of folate.[17] Thus, considering the significance of OSCC and absence of a comprehensive study in this respect, this study aimed to assess the relationship of serum levels of folate, homocysteine, Vitamin B12, iron, copper, and selenium with OSCC via serological analyses.

MATERIALS AND METHODS

This descriptive analytical study was conducted on patients with OSCC with no previous treatment or tumor recurrence presenting to Imam Khomeini Cancer Institute. Written informed consent was obtained from patients and the study protocol was approved by the ethics committee of Qazvin University of Medical Sciences (approval ethical committee number: 7928). First, 20 mL blood samples were obtained from patients and transferred

to the laboratory within 30 min. Age, sex, type of diet, place of residence, smoking status, alcohol consumption, and the site of oral involvement were all recorded. Next, serum levels of iron, copper, selenium, folate, Vitamin B12, and homocysteine were measured and staging of cancer was done by a surgeon. Serum levels of biochemical factors in patients with different clinical stages of OSCC were compared. The data were analyzed using SPSS software (Version 20, Chicago, IL, USA). Independent *t*-test was applied to compare serum levels of biochemical factors with their normal range. Archival data was used for assigning TNM staging.[1] Furthermore, the Pearson's correlation test was used to assess the correlation of serum levels of biochemical factors with patient's age. Chi-square test was applied to assess the relationship of qualitative demographic factors with serum level of biochemical factors ($P < 0.05$).

RESULTS

Thirty OSCC patients with no previous treatment or tumor recurrence were evaluated in Imam Khomeini Cancer Institute; out of which, 15 (50%) were male and 15 (50%) were female. The mean age (±standard deviation) of patients was 66.33 ± 10.05 years (range, 47–81 years). Assessment of history of systemic diseases revealed that 8 (26.6%) had hypertension, 2 (6.7%) had diabetes, 2 (6.7%) had hyperlipidemia, 2 (6.7%) had cardiovascular diseases, 2 (6.7%) had kidney stones, 2 (6.7%) had prostate disease, 4 (13.3%) had renal insufficiency, and 2 (6.7%) had urethral cancer. Four patients (13.3%) had positive history of cigarette smoking, but none had a history of alcohol consumption.

Based on the results, the serum levels of biochemical factors were 155.27 ± 40.58 ng/mL for iron, 92.47 ± 18.83 ng/mL for selenium, and 96.0 ± 22.64 ng/mL for copper [Figure 1]. The mean serum levels of Vitamin B12, folic acid, and homocysteine were 232.5 ± 102.68 ng/mL, 8.66 ± 4.06 ng/mL, and 18.87 ± 8.81 ng/mL, respectively [Table 1].

Serum levels of iron in patients with T1, T2, T3, and T4 tumor sizes were 44.33 ± 28.92, 56.93 ± 48.15, 76.25 ± 40.67, and 24.7 ± 28.71 ng/mL, respectively; no significant difference was noted in this respect (one-way ANOVA, $P = 0.53$). Serum levels of selenium in T1, T2, T3, and T4 tumor sizes were75.6 ± 17.08, 98.32 ± 18.49, 97.23 ± 17.16, and 90.75 ± 25.1 ng/mL, respectively. The difference in this respect was not significant either (one-way ANOVA, $P = 0.39$). Serum levels of copper in T1, T2, T3, and T4 tumor sizes were 72.0 ± 24.02, 110.83 ± 13.93, 93.5 ± 17.71, and 92.5 ± 31.82, respectively; the difference in this respect was not significant either (one-way ANOVA, $P = 0.09$).

Serum levels of Vitamin B12 in T1, T2, T3, and T4 tumor sizes were 156.67 ± 19.73, 243.83 ± 103.34, 259.5 ± 140.6, and 284.0 ± 0 ng/mL, respectively; the difference in this respect was not significant either (one-way ANOVA, $P = 0.58$). Serum levels of folic acid in T1, T2, T3, and T4 tumor sizes were 7.8 ± 4.67, 9.98 ± 5.03, 6.45 ± 2.14, and 10.4 ± 2.83 ng/mL, respectively; the difference in this respect was not significant either (one-way ANOVA, $P = 0.56$). Serum levels of homocysteine in T1, T2, T3, and T4 tumor sizes were 17.0 ± 6.25, 19.83 ± 11.29, 18.5 ± 9.88, and 19.57.78 ng/mL, respectively; the difference in

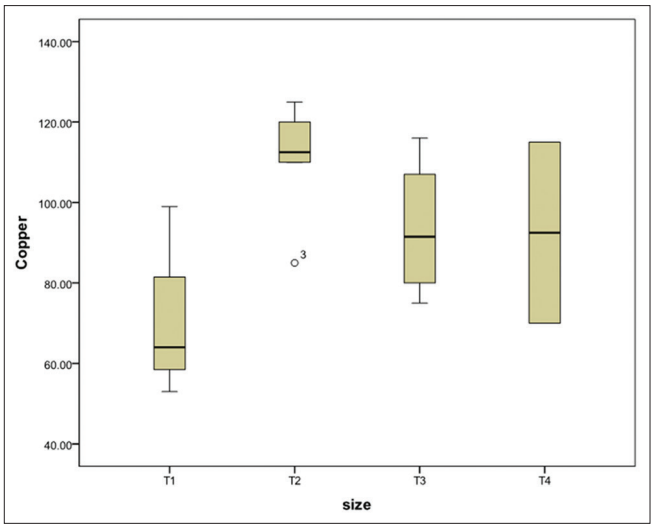

Figure 1: Serum levels of copper in OSCC with different tumor Size. OSCC: Oral squamous cell carcinoma.

Table 1: The mean±standard deviation of serum levels of biochemical factors in oral squamous cell carcinoma patients

Metabolite	Mean±SD (ng/mL)	Minimum (ng/mL)	Maximum (ng/mL)
Iron	55.2±40.5	4.4	145.0
Selenium	92.4±18.8	56.0	131.0
Copper	96.0±22.6	53.0	96.0
Vitamin B12	232.5±102.6	108.0	447.0
Folic acid	8.6±4.6	2.5	18.5
Homocysteine	18.8±8.8	8.0	37.0

SD: Standard deviation

this respect was not significant either (one-way ANOVA, $P = 0.98$).

The serum level of iron was 55.31 ± 43.07 ng/mL in patients without and 55.0 ± 28.28 in patients with distant metastasis with no significant difference (Student's *t*-test, $P = 0.99$). Moreover, the serum level of selenium was 90.24 ± 16.64 ng/mL and 107.0 ± 33.94 ng/mL in OSCC patients without and with distant metastasis, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.26$).

The serum levels of copper were 92.69 ± 22.35 ng/mL and 117.5 ± 10.6 ng/mL in OSCC patients without and with distant metastasis, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.16$). The serum levels of Vitamin B12 were 215.58 ± 89.18 ng/mL and 334.0 ± 159.81 ng/mL in OSCC patients without and with distant metastasis, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.14$). The serum levels of folic acid were 8.11 ± 3.19 ng/mL and 192.0 ± 26.21 ng/nL in OSCC patients without and with distant metastasis, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.46$).

The serum levels of iron were 59.28 ± 43.47 and 39.2 ± 33.5 ng/mL in OSCC patients without and with lymph node involvement, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.46$). The serum levels of selenium were 89.76 ± 17.32 and 103.33 ± 24.69 ng/mL in OSCC patients without and with lymph node involvement, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.28$). The serum levels of copper were 93.0 ± 24.31 and 108.0 ± 8.19 ng/mL in OSCC patients without and with lymph node involvement, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.32$). The serum levels of folic acid were 9.67 ± 3.85 and 4.63 ± 1.87 ng/mL in OSCC patients without and with lymph node involvement, respectively; the difference in this regard was statistically significant and serum level of folic acid was significantly higher in OSCC patients without lymph node involvement (Student's *t*-test, $P < 0.05$). The serum levels of homocysteine were 17.33 ± 7.85 and 25.0 ± 11.53 ng/mL in OSCC patients without and with lymph node involvement, respectively, with no statistically significant difference (Student's *t*-test, $P = 0.19$).

DISCUSSION

Based on the results, the serum levels of Vitamin B12, folic acid, and homocysteine were found to be 232.5 ± 102.68 ng/mL, 8.66 ± 4.06 ng/mL, and 18.87 ± 8.81 ng/mL, respectively, which seem to be within the normal range despite some slight variations. The normal ranges are 2.7–17 ng/mL for folate,[25] 140–960 ng/mL for Vitamin B12,[9] and 31.0 ng/mL for homocysteine.[8]

Almadori *et al*., in 2005, showed that reduction in serum folate increases the risk of SCC of the head and neck,[25] which is in contrast to our observations with regard to OSCC. They also showed increased serum levels of homocysteine in SCC patients compared to healthy controls and a reduction in serum level of folate in patients with leukoplakia.[25] On the contrary, our results showed that serum levels of homocysteine were within the normal limits in OSCC patients. Gorgulu *et al*., in 2010, reported that serum levels of Vitamin B12 and folate were significantly lower in SCC patients than in healthy controls.[7] In contrast, in our study, serum levels of Vitamin B12 and folic acid in OSCC patients were within the normal limits; this controversy in the results of the two studies may be due to not having a control group or small sample size in our study. On the other hand, it should be noted that the study by Gorgulu *et al*. was conducted on patients with SCC of the larynx, while our study evaluated OSCC patients.

Aune *et al*., in 2011, reported that risk of colon, rectal, laryngeal, and oral cancers decreased by an increase in dietary intake of folate, which is in contrast to our study; however, the role of confounders in this respect should not be overlooked.[16]

Rezaeiia *et al*., in 2011, showed that dietary intake of folate was not correlated to the risk of breast cancer in women.[26] Some previous studies have reported low dietary folate, low serum level of folate, and high serum level of homocysteine in patients with colorectal carcinomas.[27,28] Some other studies pointed to an inverse correlation between dietary folate intake and serum level of folate with the risk of esophageal cancer;[25,29] however, all the afore-mentioned studies focused on cancers other than OSCC.

The mean serum level of copper was found to be 96.0 ± 22.64 ng/mL in patients with OSCC, which indicates an increase compared to the normal serum level of copper that is 70–150 ng/mL; this finding is

in line with the results of previous studies pointing to increased serum levels of copper in patients with urethral cancer,[30] breast cancer,[31] colorectal cancer,[32] and gastrointestinal cancer.[33] Copper as a rare element participates in the formulation of some proteins such as plasma ceruloplasmin, vascular endothelial growth factor and transcopernin; the serum levels of these three factors along with the serum level of copper increase in some patients with malignancies.[18] Evidence shows that cancer cells have very high concentrations of copper since they have the ability to absorb it from the nonceruloplasmin part of the plasma. This element plays an important role in tumor growth and angiogenesis via copper-dependent amine oxidase pathway.[19] Furthermore, it has been documented that copper chelators can serve as tumor inhibitors.[34] A previous study tested tetrathiomoly as a chelator of dietary copper in different types of cancers and indicated that it stopped tumor growth and no new tumors developed in the following years.[35] Copper ion participates in oxidation and reduction reactions and plays an important role in production of free oxygen metabolites.[36] Free radicals can bond to cell components and result in peroxidation of fat, oxidation of proteins and destruction of nucleic acids.[31] Free radicals play a role in the initiation and progression of carcinogenesis and development of cancer. Thus, increased serum levels of copper can increase the susceptibility to oral cancer since it increases the oxidation reactions.

Swain and Gopal in 2011 revealed ascending serum levels of copper in patients with leukoplakia to SCC, which is in line with the findings of other studies.[37] Khanna and Karjodkar in 2006 also demonstrated ascending serum levels of copper in patients susceptible to cancer.[38] Mazdak *et al*., in 2010, reported a significant increase in serum level of copper in urethral cancer patients compared to controls, which is in agreement with our findings and those of previous studies.[30]

The serum level of iron was found to be 55.27 ± 40.58 ng/mL in patients with OSCC in our study. The normal serum level of iron is 12–150 ng/mL in healthy women and 12–300 ng/mL in healthy men. Khanna and Karjodkar in 2006 showed that serum levels of iron decreased in cancer patients.[38] Mazdak *et al*., in 2010, also reported significant reduction in serum level of iron in patients with urethral cancer compared to healthy controls.[30] Similarly, Hossain *et al*., in 2007, noticed that low serum levels of iron increased the risk of oral malignancies.[39]

Despite many strengths of the current study such as adequate sample size, precise sampling, and measurement of serum levels of biochemical factors, it had some limitations; for instance, cross-sectional data were used, which is associated with possible bias. Despite the descriptive design of the current study, some statistical comparisons were made with regard to tumor size, lymph node involvement, and distant metastasis and their correlations with the serum levels of folate, Vitamin B12 and homocysteine were evaluated; but no significant differences were noted in this respect and almost similar serum levels were noted in different tumor sizes irrespective of presence/absence of distant metastasis or lymph node involvement. However, the serum level of folic acid was significantly higher in OSCC patients without lymph node involvement compared to those with lymph node involvement (with the mean values of 9.67 and 4.63 ng/mL, respectively; $P < 0.05$).

The current results must be interpreted with caution because serum levels of folate, Vitamin B12, homocysteine, and other micronutrients may not reflect the long-term effects of nutritional habits; thus, we cannot offer advices to correct the pattern of dietary intake of these factors based on the current findings. Moreover, daily variations in serum levels of biomarkers (as seen for serum iron) may affect the study results (despite using fasting blood samples to control for the effect of circadian changes). On the other hand, the serum levels of micronutrients were measured after the patients were diagnosed with cancer and these changes could have been related to changes in diet in the recent days. Last but not least, we did not have a cancer-free control group in our study and the obtained values were compared with the normal ranges reported in the literature and this issue may affect the accuracy of the results as well. Furthermore, there are a preponderance of systematic reviews which have various results, including Keshani *et al*., that suggested that higher serum levels of copper and zinc in a primary screening of OSCC in suspected patients.[40] Similarly, this study also recommends serum levels of copper in primary diagnosis of patients with OSCC.

CONCLUSION

Considering the changes in serum levels of copper in patients with OSCC compared to healthy individuals,

it may be used to enhance early detection of oral cancerous lesions.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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