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Evaluation of the Roles of Serum Vitamin B_{12}, Folate and Homocysteine Levels in Laryngeal Squamous Cell Carcinoma

O Gorgulu[1], T Selcuk[1], S Ozdemir[1], Ç Sayar[1], Y Beyazit[2] and Y Akbas[1]

[1]Department of Otolaryngology, Adana Numune Research and Training Hospital, Adana, Turkey; [2]Department of Gastroenterology, Turkiye Yuksek Ihtisas Training and Research Hospital, Ankara, Turkey

Metabolic changes in head and neck carcinogenesis are often non-specifically correlated with carcinomas. The study of metabolic disorders can improve the understanding of tumourigenesis at the cellular level. This study was designed to evaluate the role of serum homocysteine, folate, and vitamin B_{12} levels in the pathogenesis of laryngeal squamous cell cancer (LSCC) by measuring serum levels in 60 consecutive untreated patients with LSCC and 60 controls (30 smokers and 30 non-smokers). Compared with smoker and non-smoker control groups, significantly lower levels of vitamin B_{12} were found in patients with LSCC. Folate levels in patients with LSCC were also significantly lower than in the smoker and non-smoker control groups. There were no significant differences in the homocysteine levels between these three groups. Metabolic alterations in vitamin B_{12} and folate levels, particularly hypofolataemia, could be associated with the development of LSCC, although further research is required to confirm their roles definitively.

KEY WORDS: Laryngeal carcinoma; Vitamin supplements; Folate; Vitamin B_{12}; Homocysteine

Introduction

Laryngeal cancer constitutes 25% of head and neck cancers, a great majority of which are squamous cell carcinomas.[1,2] Smoking is the most important risk factor for laryngeal squamous cell carcinoma (LSCC) development and, in association with alcohol consumption, it particularly increases the risk of supraglottic tumours.[3,4] Other aetiological factors involved in LSCC development are poor nutrition, mineral deficiencies and radiation exposure, although there is also an association between LSCC and gastro-oesophageal or laryngopharyngeal reflux disease.[4 – 6] Metabolic alterations due to poor nutrition in head and neck carcinogenesis are often non-specifically correlated with carcinoma. These changes have been studied less extensively but could play a major role in tumour development and also as indicators of disease progression.[7]

The development of secondary tumours is probably related to molecular abnormalities as well as to metabolic alterations that might promote (or derive from) neoplastic progression,[2,4] and these aspects are important in identifying new targets for therapy. The

basic biochemical function of vitamin B_{12} is to support the synthesis of DNA for cell division and reproduction by regenerating folate levels following folate consumption.[8] Vitamin B_{12} and folate both play a role in converting homocysteine to methionine.[9,10] The primary function of the folate molecule is to be the carrier of methyl groups for DNA methylation and DNA synthesis.[7,10,11] Alterations in these pathways interfere with DNA synthesis, repair and methylation, which may promote carcinogenesis.[11] Folate deficiency is almost always secondary to insufficient dietary intake and is associated with an elevation of homocysteine levels: folate supplementation reduces hyperhomocysteinaemia.[7] Homocysteine is an intermediate metabolite of methionine metabolism and is metabolized into methionine or cysteine.[7] One of these metabolic cycles leads to the production of *S*-adenosyl methionine, which is considered to be the main methyl donor in the human body.[12]

The aim of the present study was to evaluate the role of serum homocysteine, folate and vitamin B_{12} levels in the pathogenesis of LSCC.

Patients and methods

STUDY POPULATION

This study was carried out between January 2006 and April 2009 on newly diagnosed, untreated patients with LSCC who were admitted to the Otolaryngology Department of the Adana Numune Research and Training Hospital (Adana, Turkey); tumours were staged according to the tumour node metastasis (TNM) classification system.

Data from patients with LSCC were compared with data from two healthy control groups, comprising subjects from the same geographical area as the patients. One control group included participants who were current smokers; the second control group included participants who had never smoked. Among the LSCC patient group, those who had stopped smoking ≤ 5 years before their diagnosis of malignancy were considered as smokers. In all patients and control subjects, smoking frequency was determined by the number of packs smoked per year. Daily alcohol intake was also assessed for all patients and control subjects, and was measured in terms of g/day or glasses/day. Heavy alcohol intake (> 35 g/day or ≥ 4 glasses/day) is a risk factor for head and neck carcinoma, and for impairment of folate absorption and metabolism,[8] therefore participants with a heavy alcohol intake were excluded. Participants with nutritional deficiency, renal or hepatic failure, those receiving drugs capable of affecting homocysteine levels, and those taking vitamin B_{12} and folic acid supplements, were also excluded from the study.

The study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Adana Numune Research and Training Hospital. Written informed consent was obtained from each participant.

LABORATORY ANALYSES

Fasting blood samples were collected from each participant. The sample tube was immediately placed on ice and centrifuged within 1 h. Prepared serum was stored at between –20 and –25 °C. Serum levels of homocysteine, folate and vitamin B_{12} were measured within 2 weeks of collection.

Homocysteine was measured using an automated AxSYM® system (Abbott Laboratories [Diagnostics Division], Abbott Park, IL, USA), which is based on fluorescence polarization immunoassay technology. Vitamin B_{12} was measured using an Elecsys® 2010 device (Roche, Hitachi High

Technologies Corp., Tokyo, Japan) with an electrochemiluminescence immunoassay immunological test. Folate was measured quantitatively using an Elecsy® 2010 device (Roche, Hitachi High Technologies Corp.) with a binding method immunological test.

STATISTICAL ANALYSES

Data were analysed using the SPSS® statistical package, version 12.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Because numerical variables in independent groups could not be provided as a condition to comply with normal distribution for χ^2-test analysis, comparisons of two groups were analysed by the Mann–Whitney *U*-test and comparisons of three groups by the Kruskal–Wallis test. In the *post-hoc* tests, the α-significance level was applied with the Bonferroni correction and accepted as < 0.05. Linear regression analysis was used in determining influential factors.

Results

Demographic and clinical characteristics for the patients and control groups included in this study are shown in Table 1. The patients with LSCC were all newly diagnosed and had not yet received treatment. Seventeen patients had stage I tumours, two had stage II tumours, 15 had stage III tumours and 26 had stage IV tumours, according to TNM classification.

Alcohol was consumed by 16.7% of the patients with LSCC, but by only 6.7% of subjects in the smoker control group. None of the patients in the non-smoker group had a history of alcohol consumption, however the proportion of patients consuming alcohol was significantly higher in the LSCC group than in either of the control groups (P = 0.036). The frequency of smoking among patients with LSCC was 93.3% (P < 0.001 versus the smoker control group). A mean ± SD of 46.3 ± 21.5 packs/year were smoked by

TABLE 1:
Demographic and clinical characteristics of patients with laryngeal squamous cell carcinoma (LSCC) and control subjects

		Controls	
Variable	**LSCC patients *n* = 60**	**Smokers *n* = 30**	**Non-smokers *n* = 30**
Age, years	59.9 ± 10.8	48.7 ± 8.0	51.7 ± 9.0
Smoking status			
Smoker[a]	56 (93.3)[d]	30 (100)	–
Non-smoker	4 (6.7)	–	30 (100)
Alcohol consumption[b]			
Drinkers	10 (16.7)[e]	4 (6.7)	0
Non-drinkers	50 (83.3)	56 (93.3)	30 (100)
LSCC stage[c]			
I – II	19 (31.7)	–	–
III	15 (25.0)	–	–
IV	26 (43.3)	–	–

Data presented as mean ± SD or *n* (%) of patients.
[a]Patients who had stopped smoking ≤ 5 years prior to LSCC diagnosis were considered as smokers.
[b]Alcohol intake for all patients and control subjects, ≤ 35 g/day or < 4 glasses/day.
[c]Tumour staging according to tumour node metastasis classification.
[d]P < 0.001 versus smoker control group.
[e]P = 0.036 versus the smoker and non-smoker control groups (Pearson's χ^2-test).

LSCC patients, whereas a mean ± SD of 23.5 ± 10.1 packs/year were smoked by participants in the smoker control group.

Table 2 presents the results of the biochemical analyses. Serum levels of vitamin B_{12} and folate in patients with LSCC were significantly lower than those in the smoker and non-smoker control groups ($P < 0.05$, all comparisons). There was no significant difference with respect to serum vitamin B_{12} or folate levels between the smoker and non-smoker control groups. There was no significant difference between any of the three groups with respect to serum homocysteine levels.

No significant difference was found with respect to serum vitamin B_{12}, folic acid and homocysteine levels between alcohol-using and non-alcohol-using LSCC patients (data not shown). In addition, no significant differences were detected among patients with LSCC stage I – II, stage III and stage IV disease, with respect to serum vitamin B_{12}, folic acid and homocysteine levels (data not shown).

When data from the LSCC and healthy control groups were combined and stratified according to smokers and non-smokers, there was a significant difference in mean ± SD levels of vitamin B_{12} between smokers (219.2 ± 119.0 pg/ml) and non-smokers (292.9 ± 112.1 pg/ml) ($P < 0.001$). However, no statistically significant difference in plasma levels of folate or homocysteine were noted between smokers and non-smokers when LSCC patients and healthy control groups were stratified in this manner.

Discussion

Despite medical and surgical advances, the prognosis of patients with LSCC has not changed substantially during the last two decades. Thus, to clarify the pathogenesis of LSCC, molecular alterations have been studied extensively.[7] It is also known that carcinogenesis can be associated with metabolic disturbances that might promote or derive from neoplastic progression,[13] therefore the study of metabolic disturbances can lead to a better understanding of the biological characteristics of tumours and of the tumour-patient relationship.[14]

The methionine cycle is an extensively studied metabolic process in the carcinogenesis of head and neck squamous cell carcinoma.[12] Serum levels of homocysteine, vitamin B_{12} and folic acid were measured in the present study, as these are compounds from the methionine cycle. Alterations in the methionine cycle have been reported in several human malignancies. For

TABLE 2:
Comparison of serum vitamin B_{12}, folate and homocysteine levels in patients with laryngeal squamous cell carcinoma (LSCC) and in control subjects

		Controls		
	LSCC patients $n = 60$	Smokers $n = 30$	Non-smokers $n = 30$	Statistical significance[a]
Vitamin B_{12} (pg/ml)	206.8 ± 123.6	254.4 ± 114.5	292.3 ± 104.2	$P < 0.05$
Folate (ng/ml)	5.8 ± 3.0	7.0 ± 2.4	7.1 ± 2.1	$P < 0.05$
Homocysteine (µmol/l)	11.5 ± 9.0	9.7 ± 4.7	8.7 ± 3.1	NS

Data presented as mean ± SD.
[a]Comparison of LSCC patients versus the smoker and non-smoker control groups (Pearson's χ^2-test). NS, not statistically significant ($P > 0.05$).
Normal ranges: vitamin B_{12}, 211 – 946 pg/ml; folate, 3.1 – 17.5 ng/ml; homocysteine, 3.33 – 20.41 µmol/l.

example, methionine cycle disruptions causing a reduction of intracellular *S*-adenosyl methionine can alter cytosine methylation in DNA, leading to inappropriate activation of proto-oncogenes and induction of malignant transformation.[7 – 9]

Maintenance of an adequate folate status from dietary sources and/or by synthetic folic acid supplementation has been associated with a protective effect and a decreased incidence of a variety of human malignancies.[12] Although hypofolataemia does not have an active, initiating role in head and neck carcinogenesis and other malignancies, it does increase the rate of tumour progression by having a synergistic influence with genetic and environmental factors, and by weakening the cell's defence mechanisms against mutagens.[7] Epidemiological data suggest an inverse association between LSCC development and the consumption of fruit and vegetables.[7] The protective effects of dietary fruit and vegetables may be due, at least in part, to the presence of folic acid.[13] Published studies report the use of diet and/or synthetic sources of folic acid, which supplied sufficient folate levels to reduce the incidence of several human malignancies, thereby providing protective effects.[15,16] It has been shown that having a high dietary folate intake protects heavy smokers from developing squamous cell carcinoma of the lungs.[16] Furthermore, folate and vitamin B_{12} supplementation were shown to induce the regression of bronchial squamous metaplasia and breast carcinoma.[16,17]

Various studies have described insufficient folate intake, low serum folate levels and high serum homocysteine levels in patients with colorectal carcinoma.[18 – 22] Similarly, Almadori *et al.*[7] reported low serum folate and high serum homocysteine levels in patients with head and neck squamous cell carcinoma. Moreover, it has been reported that vitamin B_{12} and folate deficiencies can cause chromosomal damage in buccal mucosa in tobacco users.[23,24] In light of these reports, it could be proposed that hypofolataemia and vitamin B_{12} deficiency might be risk factors for LCSS, but cannot be used as markers for tumour progression in these patients. Thus, these deficiencies could be used in the early diagnosis of LSCC and to inform patient treatment.

In conclusion, adding folate and vitamin B_{12} to the diets of patients with LSCC may help to protect them from local recurrences and from secondary tumour development, which are major direct causes of death. Such dietary supplementation, especially in people at high risk of LCSS (e.g. smokers), may also help to induce the regression of pre-cancerous lesions. Longer follow-up studies, involving larger patient groups, are needed to determine the definitive role of these metabolic disturbances in the pathogenesis of LSCC.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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Author's address for correspondence
Dr Yavuz Beyazit
Department of Gastroenterology, Turkiye Yuksek Ihtisas Training and Research Hospital,
4 Kizilay Street, TR-06100 Ankara, Turkey.
E-mail: yavuzbeyaz@yahoo.com