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Association between Squamous Cell Carcinoma of the Head and Neck and Serum Folate and Homocysteine

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Abstract. *The aim of this study was to evaluate the serum levels of folate and homocysteine in patients with squamous cell carcinoma of the head and neck (SCCHN) and to correlate them with the clinical stage. An attempt was made to associate the results with the effects of smoking. Materials and Methods: Serum levels of folate and homocysteine were measured in 150 patients with histologically-proven SCCHN before any treatment and in 150 healthy volunteers (77 smokers and 73 non-smokers). Results: The study indicated a positive correlation between hyperhomocysteinemia and hypofolatemia and the presence of SCCHN. Folate deficiency and high levels of homocysteine were noted in a large number of healthy smokers. Conclusion: If metabolic disorders of the methionine cycle are confirmed as risk factors for SCCHN, folate as a dietary supplement might play a role in chemoprevention and the post-treatment care of SCCHN patients.*

Head and neck cancer, mainly squamous cell carcinoma of the oral cavity, pharynx and larynx, is a common human malignancy that worldwide affects about 500,000 patients annually. Despite the improvements in surgical techniques and in chemo- and radiotherapies, the overall 5-year survival rate for patients with head and neck cancer is among the lowest of the major tumor types (1). The lack of progress in head and neck oncology emphasizes the need to identify the molecular markers associated with the initiation and biological behavior of individual tumors. The patient will benefit from this diagnostic and prognostic information regarding the clinical course and outcome. In an attempt to improve the treatment of squamous cell carcinoma of the head and neck (SCCHN), molecular abnormalities have been widely studied. Gene alterations were assessed as risk factors (2), although metabolic disorders, particularly biochemical alterations, have not been extensively evaluated, despite occurring frequently in cancer. The identification of their role in the carcinogenesis of SCCHN has not been fully discussed.

The most well-studied metabolic process associated with carcinogenesis is the methionine cycle. Metabolism of the amino acid methionine in the synthesis of many proteins affects several biochemical pathways involving the production of nutrients, which are essential for the optimal functioning of the cardiovascular, skeletal and nervous systems. Homocysteine is an intermediate product of methionine metabolism and is itself metabolized following two pathways: the re-methylation pathway, which regenerates methionine, and the trans-sulphuration pathway, which initially converts homocysteine into cysteine and finally into taurine. In essence, the intermediate metabolite homocysteine is located at a critical metabolic crossroad and, therefore, both directly and indirectly, affects all methyl and sulphur group metabolism occurring in the body. Folate plays an essential role in one-carbon transfer involving the re-methylation of homocysteine to methionine, which is a precursor of S-adenosylmethionine, the primary methyl donor group for most biological methylations. DNA methylation is an important factor in gene expression, chromosomal modifications and aberrations (3).

The mechanism by which folate and homocysteine levels promote carcinogenesis is not clear at present. Several animal and *in vitro* studies (4-10) have proposed various hypotheses including hyperproliferation, increased mutagenesis, aberrant genomic and site-specific methylation, abnormal apoptosis, DNA damage and impaired DNA repair (11-16). Currently, the most popular hypothesis is the promoter hyper-methylation of key tumor suppressor genes (17).

Several epidemiological studies suggested that adequate folate intake may be important for the prevention of breast

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cancer, particularly among women who consume relatively large amounts of alcohol (18), and also lung cancer (19, 20). Elevated homocysteine levels were confirmed as an independent risk factor for colorectal cancer (8, 21) and increased levels were also found in hematological cancers (22, 23). Very few reports have investigated the folate and homocysteine status in SCCHN patients (24-26).

The aim of this study was to investigate whether hyperhomocysteinemia and hypofolatemia are involved in the pathogenesis of SCCHN. This was a prospective study into the association between plasma folate and homocysteine, including patients with SCCHN and healthy subjects, smokers and non-smokers

Materials and Methods

Patients. One hundred and forty-nine patients suffering from primary SCCHN were included in this study. The diagnosis was confirmed by histological biopsy findings. The tumor extent, nodal involvement and distant metastases were assessed by a detailed physical examination and imaging investigation. All patients were staged according to the International Union against Cancer (UICC) TNM classification system (27). In addition, 150 healthy subjects, from the same geographic area, without evidence of neoplasm, comprised the reference group. The gender- and age-matched control group was divided in two subgroups according to their smoking habits. The first included 143 smokers (97 men, 46 women, 52 subjects under 60 years, 41 between 60 and 70 years and 50 over 70 years old).and the second one, 156 non-smokers (108 men, 48 women, 71 subjects under 60 years, 43 between 60 and 70 and 42 up to70 years old). Alcohol consumption was not systematic in these populations. All the patients and all the healthy volunteers had normal renal function and had not received folate as a supplement during the year prior to our study.

Informed consent was given by all the patients and healthy subjects according to a protocol of the Ethical Committee of the University of Athens, Greece.

Methods. Serum samples were collected from each patient before any treatment and from each healthy control subject. All the samples were separated and the sera were frozen and stored at –80˚C until testing. Following the manufacturer's instructions, the serum folate levels were measured using the chemiluminescent microparticle immmunoassay (CMIA) technology referred to as Chemiflex (Abbott Diagnostics, Abbott Park, IL, USA), detected by the Architect immunoassay optical system. Homocysteine was measured with a fluorescence polarization immunoassay kit (Abbott Diagnostics, Wiesbaden, Germany) on a AxSYM Analyzer

Statistical analysis. The homocysteine and folate serum levels were compared between healthy volunteers and SCCHN patients using the ANOVA test.

Results

The mean folate level was determined to be 5.32±1.88 ng/ml in the SCCHN patients, 5.95±2.9 ng/ml in the smoking controls and 8.75±1.83 ng/ml in the non-smoking controls, with a statistically significant difference between the SCCHN patients and smokers ($p<0.001$) and between the SCCHN patients and non-smokers ($p<0.001$). Similarly, the mean homocysteine levels were 9.9±2.72 in SCCHN patients, 8.43±2.8 ng/ml in the smoking controls and 5.92±1.80 ng/ml in the non- smoking controls, with a statistically significant

Table I. *The serum levels for folate and homocysteine in SCCHN patients according to various clinical aspects of the disease.*

Classification	Number	Folate level* (ng/ml)	Homocysteine level* (ng/ml)
All	149	5.32±1.80	9.9±2.70
Gender			
Male	101	5.11±8.40	9.89±2.87
Female	48	5.71±.9.0	9.89±3.90
Smoking			
Yes	131	5.44±.8.80	9.85±2.90
No	18	5.00±1.80	9.99±2.65
Age			
<60	68	5.21±0.77	9.83±0.12
>60	32	5.21±0.99	9.00±.2.11
>70	49	5.53±1.91	10.18±2.48
Site			
Supraglottic	16	4.56±1.96	9.60±2.28
Glottic	47	5.15±1.86	10.5±2.74
Subglottic	5	5.24±1.77	9.56±3.36
Oral	28	5.6±1.77	8.79±3.13
Nasopharynx	48	5.57±1.79	10.00±2.48
Parotid	5	5.22±3.19	9.6±1.77
T			
T1	45	5.39±1.82	10.29±2.35
T2	50	5.55±1.88	9.6±2.97
T3	45	5.00±1.95	10.00±2.88
T4	2	4.87±1.82	8.58±2.48
N			
N0	78	5.28±1.87	10.1±2.56
N1	54	5.68±1.78	9.7±3.14
N2	17	4.27±1.83	9.50±2.78
M			
M0	133	5.36±1.80	9.86±2.64
M1	16	4.87±2.31	10.2±3.21
G			
G1	62	5.38±1.78	9.56±2.66
G2	58	4.88±1.84	9.79±3.44
G3	29	6.00±2.14	10.80±-2.15
Stage			
I	63	5.37±1.92	10.16±2.54
II	42	5.38±1.69	9.60±2.97
III	31	5.39±1.88	9.70±2.82
IV	13	4.59±2.33	9.82±2.9
Drinking			
Yes	50	5.28±1.65	9.79±2.74
No	99	5.33±1.91	9.94±2.71

*Mean value± standard deviation

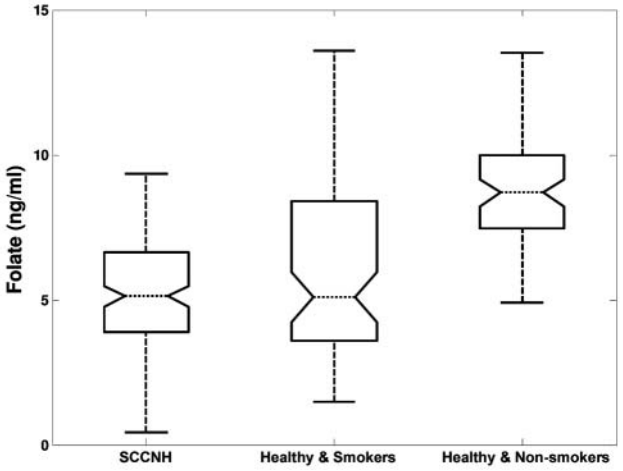

Figure 1. *Serum levels of folate in the patient group and in the control groups.*

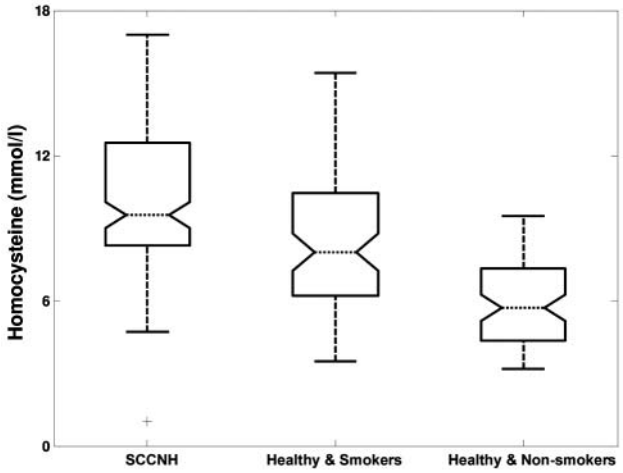

Figure 2. *Serum levels of homocysteine in the patient group and in the control groups.*

difference between the SCCHN patients and smokers ($p<0.001$) and between the SCCHN patients and non-smokers ($p<0.001$). The results indicated that the presence of primary SCCHN had a positive correlation, both with a high level of homocysteine and with a low level of folate. In healthy smokers, the levels of homocysteine appeared to be higher, while the folate levels appeared to be lower in comparison to the healthy non-smokers.

Figures 1 and 2 depict the ANOVA analysis results of the above data. The relative *p* values indicated that statistically significant differences existed between the groups ($p<0.001$).

However, no statistically significant differences were observed among the different categorical variables, such as clinical stage, T stage, N stage, age, histological degree of differentiation and tumor site in SCCHN patients, for either the homocysteine or folate levels (Table I). Further, no statistically significant differences in serum levels of homocysteine and folate were found between the healthy smokers and non-smokers.

Discussion

In the current literature, the evaluation of hyperhomocysteinemia and hypofolatemia, as risk factors in SCCHN, is contradictory. One-carbon metabolism factors have been associated with the risk of several malignancies (21, 28-30). Some researchers reported a significant inverse association between folate deficiency and SCCHN (7, 24-26, 31). On the other hand, similar case-control studies conducted in the U.S.A. have not found consistent associations (32-34).

We found a positive relation between SCCHN and both high levels of homocysteine and low levels of folate. Nevertheless, no correlations were found between the homocysteine or folate levels and other parameters such as tumor stage, age, gender and site. Thus, hypofolatemia and hyperhomocysteinemia cannot be considered to be markers of tumor progression. Almadori *et al.* (26) reported no significant differences between the levels of folate and homocysteine in leukoplakia and SCCHN, indicating that they are of no value in early diagnosis. It should be noted that the above findings may also be attributed to the possible presence of oxidative stress, since long-lasting immune activation accounts for hypofolatemia and hyperhomocysteinemia, due to an increased need for vitamins (35, 36).

It is well known that the majority of patients with SCCHN are smokers. Consequently, in our study, folate deficiency and hyperhomocysteinemia were observed in a large number of healthy smokers, confirming previous results (37, 38).

It should be noted that several studies have suggested that folate may play a role in the chemoprevention and treatment of cancer (5, 10, 34, 39). If hypofolatemia and hyperhomocysteinemia are confirmed as risk factors for SCCHN, folate could be used as a dietary supplement in the chemoprevention of head and neck cancer and in the post-treatment care of SCCHN patients.

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