Serum levels of folic acid and vitamin B12 in basal cell carcinoma patients: a case-control study

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Received: 29 April 2020
Accepted: 28 November 2020

Background: Basal cell carcinoma (BCC) is the most common form of malignancy in white populations. It has been hypothesized that dietary factors may play a role in BCC development. In this study, serum levels of folic acid and vitamin B12 were evaluated in patients with BCC to investigate the potential role of these vitamins in BCC development.

Methods: Forty-five BCC patients and 45 age- and sex-matched healthy controls were enrolled in the study. Patients with a history of radiotherapy in the affected area were excluded. All participants completed a questionnaire including demographic characteristics, and blood samples were taken to evaluate serum levels of folic acid and vitamin B12.

Results: There were no significant differences in serum levels of vitamin B12 and folic acid between healthy controls and BCC patients. Serum levels of vitamin B12 were significantly higher in female patients than male patients.

Conclusion: It seems that BCC is not related to serum levels of vitamin B12 and folic acid; however, this issue should be studied with larger sample sizes.

Keywords: Basal cell carcinoma, vitamin B12, folic acid

Iran J Dermatol 2021; 24: 315-319

DOI: 10.22034/ijd.2020.229147.1083

INTRODUCTION

Non-melanoma skin cancers (NMSCs), including squamous cell carcinoma (SCC) and Basal cell carcinoma (BCC), are the most malignancies in white populations [1]. Despite the low mortality rate, NMSCs present medical, social, and economic problems because of their high prevalence and associated morbidity [2]. BCC represents 80% of newly diagnosed tumors in Caucasians and Australian populations [3]. The main established risk factor for BCC is intermittent ultraviolet exposure. Other risk factors include fair skin, red hair, and a tendency to burn rather than tan [4].

Some studies have evaluated the relationship between trace elements and dermatologic disorders, such as the association between iron profile and melasma [5,6]. Dietary factors have also been hypothesized to play a role in BCC development. Animal studies suggest that selenium, vitamin C and E, and β-carotenes may help protect the skin against oxidative damage [7]. Moreover, several studies have suggested a positive association between fat intake and BCC, an inconsistent association for retinol, and little relation between β-carotenes and NMSC development. Other studies have reported a weak association between vitamin E, vitamin C, and selenium levels and BCC development [8].

Other nutrients such as folate (vitamin B9) may also influence BCC development [9]. Several studies have investigated the association between folate, vitamin B12, and chronic inflammatory skin disorders such as psoriasis [8]. Still, the role of folate in BCC development is not fully elucidated. Two

prospective studies showed a direct association between folate intake and BCC [9,11]. One possible explanation for this association might be that rapidly proliferating cells require a high folate level to continue DNA synthesis [10]. In 2013, a meta-analysis showed no association between folic acid supplementation and NMSCs [13]. However, a case-control study showed a lower mean serum folic acid level in BCC patients than healthy controls [14].

To the best of our knowledge, few studies have investigated the roles of folic acid and vitamin B12 in BCC. In the present study, we focused on the serum levels of vitamin B12 and folic acid in BCC patients and compared them with healthy controls.

PARTICIPANTS AND METHODS

We conducted a case-control observational study in Razi Hospital, Tehran, Iran, from 2016 to 2017. Forty-five patients of either sex with BCC and 45 age- and sex-matched healthy controls were enrolled in the study. The patients were collected from the Tumor Clinic of Razi Hospital, and the diagnosis of BCC was confirmed by skin biopsy. The controls were selected among healthy people referring to general dermatology clinics for cosmetic reasons and had no history of malignancy or premalignant lesions. All participants gave informed consent and were questioned regarding age, gender, height, weight, history of sun exposure, smoking, and past history of skin cancer. Also, we collected data on lesion-related variables such as anatomical sites of the lesions, age of disease onset, and histological subtypes of BCC. Exclusion criteria were taking methotrexate or vitamin supplementation (because vitamin B12 and folate are highly related to the dietary regimen), significant renal or liver diseases, recent malignancy, and history of tumor field radiation. After filling of informed consent forms, blood samples were taken from all patients and controls to determine serum folic acid and vitamin B12 levels. The serum levels of vitamin B12 and folic acid were measured using standard laboratory protocols. Folate status was assessed by measuring serum folic acid according to the WHO folate cut-off points reported in 2012; a serum folate level of > 20 ng/ml is considered elevated, 6-20 ng/ml is normal, < 6 ng/ml is considered deficient. Then, the obtained results were statistically analyzed using IBM SPSS statistics version 23. The independent sample t-test and chi-squared test were used to compare case and control groups' data. P-values < 0.05 were considered statistically significant. Correlation between variables was evaluated with logistic regression. The Mann-Whitney U test was applied to compare the differences between healthy controls and patients as well as patients with different genders. Association between numerical variables was evaluated with the Spearman rho test.

RESULTS

In total, 90 individuals (54.4% male and 45.6% female) participated in this study. Comparing baseline characteristics of two groups showed no significant differences in gender, mean age, smoking, and body mass index (Table 1).

The most common anatomical sites of BCC cases were the nose (42.2%) and the scalp (17.7%). Overall, 91.1% of BCC lesions were nodular, and 4.4% were superficial. Of the 45 BCC patients, 33.3% were housewives, 22.2% were farmers, and 15.6% were workers. Also, 77.8% of patients and 22.2% of healthy controls had a history of prolonged sun exposure, indicating a significant difference across the two groups ($P < 0.001$). The history of radiation was negative in all patients as our excluding criterion. The mean age of disease onset was 64.88 years, and the mean age at disease diagnosis was 65.7 years. Table 2 compares serum folic acid levels in patient and control groups according to the WHO cut-off points. The mean folic acid level in the BCC and control groups was 15.02 ± 4.95 and 15.56 ± 4.26 ng/ml, respectively, with no significant

Table 1. The clinical characteristics of the study subjects

Subjects	Group 1 (controls)	Group 2 (BCC)	*P*
Male gender (%)	23 (51.11%)	26 (57.77%)	0.672
Age, mean ± SD (years)	54.29 ± 11.4	68.1 ± 14.2	0.122
BMI, mean ± SD (kg/m^2)	26.25	25.8	0.237
Smoking (smoker)	28.9%	28.9%	1

Table 2. Comparison of serum folic acid levels in patients and controls according to the WHO cut-off points

Folic acid level	Group 1 (controls)	Group 2 (BCC)	*P*
<6	11.1 %	22.3 %	0.182
6-20	57.8 %	40 %	
>20	31.1 %	37 %	

Table 3. Correlation of gender with serum levels of folic acid and vitamin B12 in the BCC group (Mann-Whitney U test)

	Female, mean rank	Male, mean rank	*P*
Vitamin B12	29.87	17.98	0.003
Folic acid	23.74	22.46	0.741

difference. Moreover, the mean vitamin B12 level in the pointed subgroups was 378.04 ± 261.46 and 333.47 ± 162.455 pg/mL, respectively, with no significant difference ($P = 0.334$). Serum level of vitamin B12 was significantly higher in female patients than male patients, but there was no correlation between serum level of folic acid and gender (Table 3). Also, we found no correlation between serum levels of folic acid or vitamin B12 and age, BMI, smoking, anatomical site of lesions, and history of prolonged sun exposure.

DISCUSSION

BCC is one of the most common skin malignancies in the white population and is increasing in prevalence in the Iranian population. According to clinical observations, predisposing factors of BCC include age, gender, occupation, and environmental factors. Ultraviolet radiation (UVR) is an important risk factor for BCC development. UVR in the pathogenesis of BCC can be explained in two ways: direct mutagenesis of DNA (especially in tumor suppressor genes) and immune suppression. UVR absorbed by DNA can break bonds in DNA. In addition, UVR can damage DNA through reactive oxidative species (ROS) formation [15,16].

As oxidative stress plays a vital role in developing BCC, antioxidant activity may be crucial to prevent this cancer [17,18]. Skin protection against UVR is provided through melanin and antioxidants. Because of the limited antioxidant capacity of the skin, chronic UV light exposure leads to oxidative damage, possibly resulting in early skin aging and NMSCs. Skin protective antioxidants include enzymatic antioxidants such as catalase and non-enzymatic low molecular weight antioxidants such as vitamin E and vitamin C [15]. The human body cannot synthesize antioxidants such as vitamin C and many others and should be taken by the diet. There are currently controversial data on the use of exogenous antioxidants to prevent skin cancers [8]. In this regard, in this case-control study, we evaluated the probable association between BCC and serum folic acid and vitamin B12 levels as antioxidant agents [14,15].

Folic acid is the most stable form of folate and is available as a supplement. The active form of folic acid (5-methylenetetrahydrofolate) plays a key role in the synthesis and methylation of DNA and in amino acid metabolism. It is also involved in cell division in tissues, especially in the hematopoietic system, gastrointestinal tract epithelia, and fetal tissues [16]. Epidemiological studies suggest an increased risk of breast, colon, lung, pancreas, esophagus, stomach, cervix, prostate, and ovarian cancers with a lower level of folate [21,22]. Vitamin B12 (cobalamin) is an essential nutrient that plays a crucial role in cell division. Several studies have shown an association between high levels of vitamin B12 and malignancy [23,24]. These findings may imply the effects of high plasma levels of vitamin B12 on the progression of pre-neoplastic cells and undiagnosed neoplastic lesions rather than tumor induction. On the other hand, high vitamin B12 status can lead to decreased folate levels and subsequent impaired DNA methylation [25].

In our study, in most BCC patients, the primary anatomical sites of lesions were the nose and scalp, which agrees with the literature [26]. Notably, 77.8% of our patients had a history of prolonged sun exposure, which indicated that UV radiation is the most important environmental risk factor in BCC development.

Serum levels of vitamin B12 were higher in females than males; this may be because females are more likely than males to take vitamin supplements. However, Ala Qatatsheh [27] showed no correlation between gender and serum vitamin B12 levels.

Normal serum folic acid level is essential in DNA methylation, which plays a key role in maintaining genome integrity. It is hypothesized that lower levels of folate are associated with the development of some cancers. In this regard, Laing *et al.* [28] showed the role of DNA hypomethylation in the development of skin SCCs. Similarly, Malgorzata Drobnicka-Steppien *et al.* [16] showed lower folic acid levels in the serum of BCC patients compared to healthy controls (median 9.6 µg/l vs. 16.5 µg/l; respectively).

In contrast, an experimental study showed that folic acid is photo-reactive and generates DNA modifications when irradiated with UVA (360 nm) in

the presence of DNA under cell-free conditions [29]. In the literature, a direct association has been suggested between folate intake and BCC development [12,13]. In this regard, in a cohort study of more than 43000 men by Van Dam *et al.*, a 21% increased risk of BCC was shown for the third quintile of folate consumption compared with the lowest quintile [9]. They found that folate supplementation was associated with a 22% increased risk of BCC, but only if folate intake exceeded 600 µg/d. Similarly, Fung *et al.* [11] showed a 10% increased risk of BCC with 400 µg/d of folate supplementation compared with no intake. However, Vollset *et al.* [13] published a meta-analysis that showed no association between folic acid supplementation and NMSCs. Similarly, our study showed no significant relationship between the serum levels of folic acid and vitamin B12 and BCC.

This study had some limitations. Firstly, according to WHO cut-off points, all folic acid levels above 20 ng/ml were placed in the same category, so no differentiation was possible. Finally, although individuals with a history of supplement intake were excluded, our study did not consider the dietary factors that can influence the serum levels.

CONCLUSION

Our results did not confirm the involvement of folic acid and vitamin B12 in BCC development. Further studies are needed to evaluate the role of vitamin B12 and folic acid in the development of NMSCs.

Conflicts of Interest: None declared.

Ethical Issues

All participants gave informed consent. The Ethics Committee of Tehran University of Medical Sciences approved this study.

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