Pergamon

Oral Oncol, Eur J Cancer, Vol. 32B, No. 2, pp. 120–122, 1996

Printed in Great Britain
0964–1955/96 $15.00+0.00

0964–1955(95)00076–3

Serum Vitamins' Status in Oral Leucoplakias—a Preliminary Study

G. Ramaswamy,[1] V.R. Rao,[1] S.V. Kumaraswamy[2] and N. Anantha[3]

[1]Department of Biochemistry; [2]Department of Oral Surgery; and [3]Department of Radiotherapy, Kidwai Memorial Institute of Oncology, Hosur Road, Bangalore 560 029, India

Vitamins, such as A, β carotene, C, E, B_{12} and folate, are the micronutrients with the strongest evidence of having a link to cancer prevention and control. Deficiency of these vitamins at the dietary, systemic or mucosal level will interact with tobacco use and increase the risk of oral precancerous lesions. The objective of this study was to (1) establish the baseline circulating levels of these vitamins in our normal population with and without tobacco use and (2) compare these levels with the values obtained in cases of oral leucoplakias. 50 normal controls with 25 each in chewers and non-chewers, matched for age and sex, were selected. 50 cases of oral leucoplakias (clinically detectable white patches) from the field constituted the study group. Simultaneous measurement of serum vitamin B_{12} and folate were carried out by radioassay. The other serum vitamins were estimated spectrophotometrically. Except for serum vitamin E, all the other serum vitamin levels were significantly decreased in oral leucoplakias compared to the controls. Cancer chemopreventive agents acting as inhibitors of both initiation and promotion, as analysed in our population, is promising for further intervention trials.

Keywords: vitamins, oral leucoplakia, tobacco

Oral Oncol, Eur J Cancer, Vol. 32B, No. 2, pp. 120–122, 1996.

INTRODUCTION

Epidemiological and experimental data have suggested that some micronutrients, including various carotenoids, retinoids and 2-tocopherol may have chemopreventive activity against certain types of cancers [1–5]. Oral cancer and preneoplastic oral leucoplakia (whitish patches that cannot be removed by scraping) occur in buccal mucosal cells. It has been shown that intake of β carotene, 2-tocopherol (vitamin E) and retinol (vitamin A), or its analogues causes regression of oral leucoplakia, thus preventing its progression to cancer [6–10]. In India, tobacco and/or betel use is accepted as the most important risk factor for oral precancers and oral cancer [11, 12]. Smokeless tobacco users are at high risk of precancerous lesions which transform to neoplasms at a rate of 3–5% per year [13]. There is a growing body of evidence which supports the association between low systemic levels of folate and/or vitamin B_{12} and an increased risk of cancer and precancer in epithelial tissues [14, 15]. A positive association may also have importance in supporting and expanding the clinical work of Heimburger and collaborators [16] who have suggested that the treatment of epithelial precancers with B_{12} and folate supplements prevents their transformation into neoplasms. Our objective was to establish the baseline circulating levels of these micronutrients in our normal population with and without tobacco chewing and to compare these levels with the values obtained in cases of oral leucoplakias, to evaluate the possibility of clinical supplementation of vitamins in oral leucoplakias.

Correspondence to G. Ramaswamy.
Received 6. Mar. 1995; provisionally accepted 18 Apr. 1995; revised manuscript received 30 Oct. 1995.

MATERIALS AND METHODS

50 normal controls with 25 each in chewers and non-chewers, matched for age and sex were selected. 50 cases of oral leucoplakia (clinically detectable white patches) from the field constituted the study group.

Blood was drawn from each subject, after an overnight fast, by venipuncture into a foil wrapped tube. The clot was allowed to retract in a dimly lit room. The serum was separated and stored in 0.50 ml aliquots at −70°C until analysis. The entire analysis of vitamins was carried out in dim light.

Vitamin A and β carotene were estimated spectrophotometrically using trifluoroacetic acid as the chromogenic agent [17]. Retinyl acetate and β carotene used as standards were obtained from Sigma Chemical Co., St. Louis, Missouri,

U.S.A. Petroleum ether, ethanol and chloroform used as solvents were of AR grade. Vitamin E was estimated as described by Baker and Frank [18]. Tocopherol from Sigma was used as a standard, dipyridyl and ferric chloride were of AR BDH grade.

Vitamin B_{12} and total folic acid were estimated by the radioassay method using dual count solid phase no boil reagents (Diagnostic Products Corporation, Louisiana, U.S.A.). Co^{57} vitamin B_{12} and I^{125} folic acid were used as tracers. The reproducibility (CV up to 7%) and recovery of these assays (90–95%) were satisfactory. Statistical analyses was carried out using Student's *t* test.

RESULTS

Figure 1 shows the mean serum levels of antioxidant vitamins A, β carotene, C and E in normal non-chewers, normal chewers and leucoplakias. A significant decrease in the serum levels of vitamin A, β carotene and C, except vitamin E were found in chewers compared to normal non-chewers ($P<0.001$, 0.05, 0.001, respectively). The serum vitamin levels, except vitamin E, were significantly low in oral leucoplakias compared to the normal controls.

Table 1 shows the mean serum levels of folate and vitamin B_{12} in normal non-chewers, normal chewers and oral leucoplakias. A significant decrease in the serum levels of these vitamins are observed in oral leucoplakias compared to the normal controls.

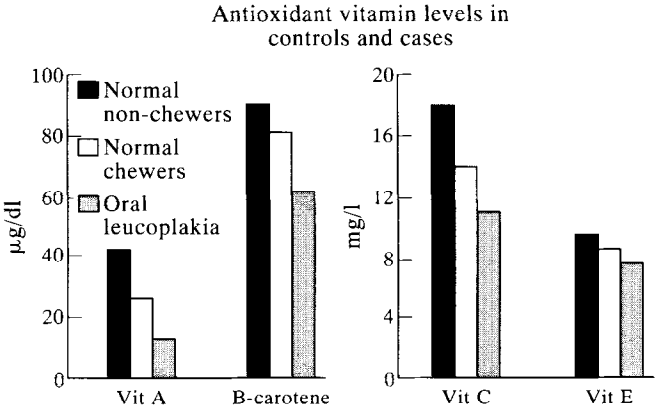

Fig. 1.

Table 1. Serum folate and B_{12} levels in controls and oral leucoplakias (mean ± S.E.M.)

Group	Folate (nmol/l)	Vitamin B_{12} (pmol/l)
1. Controls		
(A) Non-chewers $n=25$	30.4 ± 0.90	306.4 ± 58.3
(B) Chewers $n=25$	13.4 ± 0.91	162.0 ± 21.9
2. (C) Oral leucoplakias $n=50$	8.4 ± 0.86	125.8 ± 12.4

Significance was as follows:

Folate	B_{12}
A versus B $P<0.001$	A versus B $P<0.01$
B versus C $P<0.01$	B versus C $P<0.01$
C versus A $P<0.001$	C versus A $P<0.001$

DISCUSSION

Recently, efforts in cancer prevention have focussed on the importance of dietary intake of vitamins and micronutrients. Many micronutrients occur in the same foods and their intake is highly intercorrelated. It is difficult to separate the effect of one from another. Any observed effect may disappear in analysis when adjustments are made for other correlated nutrients.

Green leaf vegetables and fruits are excellent sources of folate β carotene and ascorbate, whereas foods of animal origin are good secondary sources of folate and the only dietary source of cobalamin. The literature has described the benefits of folate [19–21], cobalamin [20], carotene [22–25] and ascorbate [26, 27] in reducing the risk of cancer or oral precancer in epithelial tissues. These nutrients are likely to take the active role in the risk reduction effect. The blood concentration of carotene, ascorbate and folate have been shown to be lower in tobacco smokers than in non-smokers [28–30] and the buccal mucosal cells of tobacco smokers were shown to have a decreased concentration of folate [31]. Our study shows significantly very low levels of serum vitamins A, β carotene, C, folate and B_{12} in tobacco chewers than in non-chewers. Although, it cannot be suggested that the chewing of tobacco has the same effect on the body as smoking tobacco, some of the carcinogenic substances present in tobacco smoke are also present and released in the chewing of the tobacco quid. Carotene and ascorbate can function as antioxidants [32, 33], protecting the epithelium against cellular and genetic damage from free radicals generated from the tobacco quid. Ascorbate prevents the formation of carcinogens [32], whereas β carotene is an intracellular antioxidant functioning as a blocking agent [34]. Carotene may also be important as a retinol precursor. Folate and cobalamin act as cancer suppressors. Sufficient quantities of folate and cobalamin are required for the adequate functioning of a cellular repair mechanism to control damage from cytotoxic and genotoxic manifestation, thus inhibiting neoplastic transformation [35]. The chemopreventive actions of these nutrients are linked. Folate is crucial in the metabolic pathways of DNA synthesis, and methyl cobalamin is required as a coenzyme for methionine synthase, an enzyme involved in a critical C-1 transfer in the folate metabolism. A deficiency of cobalamin which slows or shuts down the methyl transfer, causes the body's folate to accumulate as 5 methyl tetrahydrofolate, unable to be demethylated to tetrahydrofolate, and returned for use in other essential folate pathways. Tetrahydrofolate is required in a pathway that produces the DNA precursor material deoxythymidine monophosphate from deoxyuridine monophosphate. In a state of folate or cobalamin deficiency, deoxythymidine monophosphate may not be present in sufficient quantity, and nucleotide biosynthesis can be slowed or halted [36]. Folate sensitive fragile sites exist in human chromosomes [37–39].

The chemopreventive importance of blood or tissue folate and cobalamin concentration is not as obvious as that of the antioxidant micronutrients, but in rural south India, folate or cobalamin might be the critical chemopreventive micronutrients. Several investigators noted that the Indian diet, particularly in the poor and in strict vegetarians, was lacking in

folate and cobalamin and that Indian populations have lower blood concentrations of these micronutrients than a Western population [40–44].

The lack of certain micronutrient rich foods in the diets of South Indian tobacco/betel quid chewers may be associated with increased risk of precancerous lesions. The subclinical deficiency of certain chemopreventive micronutrients, carotene, ascorbate, folate and cobalamin, available only in marginal quantities in the diets, may be a factor for the increased risk. Supplementation trials to determine the effectiveness of these micronutrients as a weapon to reduce the high incidence of oral premalignancy is an obvious next step.

1. Moon TE, Micozzi Ms (Eds). *Nutrition and Cancer Prevention. Investigating the Role of Micronutrients*. New York, Marcel Dekker, 1989.
2. Garewal HS. Potential role of β carotene in prevention of oral cancer. *Am J Clin Nutr* 1991, **53**, 294–297.
3. Gensler HL, Magdaleno M. Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by ultraviolet irradiation. *Nutr Cancer* 1991, **25**, 97–106.
4. Ziegler RG. A review of the epidemiologic evidence that carotenoids reduce the risks of cancer. *J Nutr* 1989, **119**, 116–122.
5. Betram JS, Kolonel LN, Meyskens FL, Jr. Rationale and strategies for chemoprevention of cancer in humans. *Cancer Res* 1987, **47**, 3012–3031.
6. Koch HF. Biochemical treatment of precancerous oral lesions: the effectiveness of various analogues of retinoic acid. *J Maxillofac Surg* 1987, **6**, 59–63.
7. Shah JP, Strong EW, DeCosse JJ. Effect of retinoids on oral leukoplakia. *Am J Surg* 1983, **146**, 466–470.
8. Stich HF, Mathew B, Sankaranarayanan R, Nair MK. Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of β carotene or vitamin A. *Am J Clin Nutr* 1991, **53** (suppl), 2983–3048.
9. Garewal HS, Meyskens FL, Jr, Killen D. Response of oral leukoplakia to β carotene. *J Clin Oncol* 1990, **8**, 1715–1720.
10. Benner SE, Winn RJ, Lippman SM, *et al.* Regression of oral leukoplakia with 2 tocopherol; a community clinical oncology program chemoprevention study. *J Natl Cancer Inst* 1993, **85**, 44–47.
11. Nandakumar A, Thimmasetty KT, Sreerameredy TC, *et al.* A population based case-control investigation on cancers of the oral cavity in Bangalore, India. *Br J Cancer* 1990, **62**, 847.
12. Sankaranarayanan R. Oral cancer in India: an epidemiologic and clinical review. *Oral Surg Oral Med Oral Path* 1990, **69**(3), 325.
13. Christen AG. The case against smokeless tobacco: five facts for the health professional to consider. *JADA* 1980, **101**, 464.
14. Krumdieck CL, Eto J. Role of vitamin B_{12} and folate in carcinogenesis. *Adv Exp Med Biol* 1986, **206**, 313.
15. Sankaranarayanan R, Duffy SW, Day, *et al.* A case control investigation of cancer of the oral cavity, tongue and floor of the mouth in southern India. *Int J Cancer* 1989, **44**, 617.
16. Heimburger DC, Alexander CB, Birch R, *et al.* Improvement in bronchial squamous metaplasia in smokers treated with folate and vitamin B_{12}. *JAMA* 1988, **289**(10), 1525.
17. Neeld JB, Pearson WN. Macro methods for the determination of serum vitamin A using trifluoroacetic acid. *J Nutr* 1963, **79**, 454–462.
18. Baker H, Frank O. Determination of serum tocopherol. *Clinical Vitaminology*. New York, Wiley, 1968, 172.
19. Butterworth CE. New concepts in nutrition and cancer. Implication of folic acid. *J Dent Child* 1981, **48**, 300–303.
20. Heimburger DC, Alexander CB, Brich R, *et al.* Improvement in bronchial squamous metaplasia in smokers treated with folate and vitamin B_{12}. *J Am Med Assoc* 1988, **289**, 1525–1530.
21. Butterworth CE, Hatch KD, Macaluso M, *et al.* Folate deficiency and cervical dysplasia. *J Am Assoc* 1992, **267**, 528–533.
22. Peto R, Buckley JD, Sporn S. Can dietary beta carotene materially reduce human cancer rates. *Nature* 1981, **290**, 201–208.
23. Ziegler RC. A review of epidemiological evidence that carotenoids reduce the risk of cancer. *J Nutr* 1989, **119**, 116–122.
24. Ziegler RC. Epidemiologic studies of vitamins and cancer of the lung, esophagus and cervix. *Adv Exp Med Biol* 1986, **296**, 11–26.
25. Ibrahim J, Jafarey NA, Zuberi SJ. Plasma vitamin A and carotene levels in squamous cell carcinoma of the oral cavity and oropharynx. *Clin Oncol* 1977, **3**, 203–207.
26. Mclaughlin JK, Gridley G, Block G, *et al.* Dietary factors in oral and pharyngeal cancer. *JNCI* 1988, **80**, 1237–1243.
27. Winn DM, Ziegler RG, Pickle LW, *et al.* Diet in the etiology of oral and pharyngeal cancer among women from the Southern US. *Cancer Res* 1984, **44**, 216–222.
28. Witter FR, Blake DA, Baumgardner R, Mellits ED, Niebyl JRR. Folate, carotene and smoking. *Am J Obstet Gynecol* 1982, **144**, 857–861.
29. Nakagawa Y, Chida K, Imatoh N, *et al.* Serum folic acid levels and antipyrine clearance rates in smokers and non-smokers. *Drug Alcohol Dependence* 1983, **11**, 210–217.
30. Chow CK, Thasker RR, Changcit C, *et al.* Lower levels of vitamin C and carotenes in plasma of cigarette smokers. *J Am Coll Nutr* 1986, **5**, 305–312.
31. Piyathilake CJ, Hine RJ, Dasanayake AP, *et al.* Effect of smoking on folate levels in buccal mucosal cells. *Int J Cancer* 1992, **52**, 1–4.
32. Burton GW. Antioxidant action of carotenoids. *J Nutr* 1989, **119**, 109–111.
33. Kyrtopoulos SA. Ascorbic acid and the formation of N-nitroso compounds: possible role of ascorbic acid in cancer prevention. *Am J Clin Nutr* 1987, **45**, 1349–1350.
34. Wattenberg LW. Chemoprevention of cancer. *Cancer Res* 1985, **45**, 1–18.
35. Krumdieck CL, Eto I. Role of vitamin B_{12} and folate in carcinogenesis. *Adv Exp Med Biol* 1986, **206**, 313–330.
36. Zittoun J, Copper BA (Eds). *Folates and Cobalamins*. New York, Springer, 1989.
37. Krumdieck CL, Howard Peebles PN. On the nature of folic-acid-sensitive fragile sites in human chromosomes: an hypothesis. *Am J Med Genet* 1983, **16**, 23–28.
38. Chem A, Reidy JA, Annest JL, Welty TK, Zhou HG. Increased chromosome fragility as a consequence of blood folate levels, smoking status and coffee consumption. *Environ Mol Mutagen* 1989, **13**, 319–324.
39. Yunis JJ, Hoffman WR. Nuclear enzymes, fragile sites and cancer. *J Gerontol* 1989, **44**, 37–44.
40. Iyengar L. Folic acid requirements of Indian pregnant women. *Am J Obstet Gynecol* 1971, **111**, 13–16.
41. Kumar S, Ghosh K, Das KC. Serum vitamin B_{12} levels in an Indian population: an evaluation of three assay methods. *Med Lab Sci* 1989, **46**, 120–126.
42. Baker SJ, Mathan VI. Evidence regarding the minimum daily requirement of dietary vitamin B_{12}. *Am J Clin Nutr* 1981, **34**, 2423–2433.
43. Dastur DK, Quadros EV, Wadia NH, Desai MM, Bharucha EP. Effects of vegetarianism and smoking on vitamin B_{12}, thiocynate and folate levels in the blood of normal subjects. *Br Med J* 1972, **3**, 260–263.
44. Yusuf J, Mathan VI, Baker SJ. Iron, folate and vitamin B_{12} nutrition in pregnancy. A study of 1000 women from Southern India. *Bull WHO* 1973, **48**, 15–22.