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Blood iron, glutathione, and micronutrient levels and the risk of oral cancer and premalignancy

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Abstract

The relationship between serological levels of iron, vitamins A, B2, C, E, zinc, thiamin, and glutathione (GSH) and the risk of oral cavity cancer was examined in a hospital-based case-control study. The case group included 65 patients with incident histologically-confirmed oral cancer and 13 patients with oral premalignancies, and the control group included 85 sex- and age-matched subjects without cancer attending the hospital dental clinic. Compared to the lowest tertiles, significant decreased risks were observed for the highest tertile of free iron (odds ratio [OR] = 0.3, 95% CI: 0.1,0.6) and transferrin saturation (iron/total iron binding capacity (TIBC) × 100) (OR= 0.4, 95% CI: 0.2,0.9). The OR for TIBC, which measures the concentration of the iron delivery protein transferrin and is increased in iron-deficiency, was 3.2 (95% CI: 1.3,8.1). These associations were stronger in never-smokers than in ever smokers. While the levels of the iron storage protein ferritin was higher in cases, this may be attributed to disease-related inflammation or comorbidity. Significant associations of the endogenous antioxidant GSH (OR = 0.4, 95% CI: 0.1,0.9) and GSH reductase activity coefficient (indicative of riboflavin deficiency) OR = 1.6, 95% CI: 1.3,3.7) with oral cancer risk were also observed. In premalignant cases, serum iron levels were 16% higher in controls (P<0.05). These findings suggest that mild iron deficiency, as indicated by low levels free iron and transferrin and high levels of TIBC, as well as low levels of the major cellular antioxidant GSH are associated with increased risk of oral cancer.

Keywords

oral cancer; micronutrients; iron; glutathione; antioxidants

Introduction

The annual incidence of oral and pharyngeal cancer is approximately 75,000 in the U.S. [1] and 615,000 worldwide [2]. The major risk factors for oral squamous cell carcinoma are cigarette smoking and chronic alcohol consumption [3–6] and low dietary intake of fruits [7, 8].

The role of nutrition is not well understood, and one aspect of diet that has not been widely studied is iron metabolism. Iron is an essential nutrient, and iron deficiency is a very common form of malnutrition worldwide. The possibility that low iron intake might cause

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oral cancer came from observations of a high incidence rate of alimentary tract cancer among Swedish women in the early 20th century [9]. Many of these women presented with Plummer-Vinson (Patterson-Kelly) syndrome [10], which is characterized by nutritional deficiencies including iron deficiency. The introduction of iron supplementation in Swedish foods led to a subsequent decline in Swedish oral cancer incidence rates [11].

There have been a limited number of epidemiologic studies of iron and oral cancer risk, but three studies found a trend in the odds ratios (ORs) with decreasing dietary intake of iron [12–14], and one found a nonsignificant association between oral premalignancy and low iron intake in Indian women [15]. In experimental studies of 4-nitroquinoline-N-oxide (4-NQO) exposed laboratory rats, the incidence of oral tumors was significantly elevated in iron deficient animals [16] although these differences were not confirmed in subsequent studies [17]. Despite these findings, the role of low iron in oral cancer is not well understood.

The mechanism(s) by which iron deficiency may induce oral cancer may involve the induction of oxidative stress through the production of free radicals and reactive oxygen species that are potentially able to induce cellular injury [18]. Oxidative stress is often defined as the disturbance of equilibrium of prooxidant and antioxidant systems in favor of oxidation leading to direct damage to cellular molecules such as DNA [19, 20]. Increased levels of oxidative stress have been associated with iron deficiency anemia in numerous studies and is thought to contribute to the pathogenesis of patients with this disorder [21–23]. Oxidative stress has also been linked to the development of oral cancer [24–27] and diets high in antioxidant/nutrients have been associated with reduced risk for oral cancer whereas increased risk has been associated with low fruit intake [28] and low intake and serum levels of β-carotene, selenium, vitamin A, α-tocopherol, ascorbic acid and lycopene [29, 30] In chemoprevention trials of oral premalignancies and cancer, several of these micronutrient antioxidants exhibited antiproliferative effects but data on recurrence is more limited [31].

Glutathione (GSH) is the major intracellular antioxidant that is likely playing an important role in protection against cancer development [32]. Aside from its antioxidant activities, GSH is also responsible for the detoxification of many carcinogens through Phase II conjugation, and the maintenance of immune function by regulating mitogenic response and lymphocytic proliferation [33, 34]. Low blood GSH levels have been observed in oral cancer patients [35, 36], although the relationship between blood GSH and oral cancer is unknown. An inverse association between increased dietary intake of GSH from fruits and vegetables and risk for oral cancer indicates that GSH may have an important protective effect [37].

The current study was conducted to determine whether blood iron levels, glutathione, and selected serum micronutrient/antioxidant levels are associated with the risk of oral cancer. We also examined the micronutrient levels in 13 subjects with oral leukoplakia or keratosis, premalignant conditions that may predispose individuals to oral cancer.

Since only a small percent of ingested iron is absorbed, dietary intake is not necessarily related to physiological levels [38] and the use of biological markers is critical. In the assessment of body iron status, we have included both functional and storage forms. Most iron in the body is bound to hemoglobin, which reflects the level of functional iron. About 30% is stored as ferritin and a small percent is in the form of transferrin. The medical laboratory tests used most frequently to assess iron status include serum-free (unbound) iron, total iron binding capacity (TIBC) which indirectly measures the extent to which transferrin, the major iron-binding site in the serum, can be saturated, and transferrin saturation which is the ratio of serum iron to TIBC. The liver increases production of transferrin in response to iron deficiency, and low serum iron and high TIBC together are indicative of iron deficiency and possibly anemia. Their ratios are also used as a diagnostic tool for persons with

symptoms of iron overload (e.g. hemochromatosis). The levels of TIBC can be affected by other factors such as malnutrition and inflammation. Ferritin is the major iron storage protein and its levels reflect the levels of iron in the liver, spleen and bone marrow. Decreased ferritin levels are indicative of iron deficiency, whereas increased levels can be indicative of iron overload, liver disease, infection, inflammation and other disorders.

Methods

A case-control study of oral cancer (International Classification of Diseases [9th revision] codes 141, 143–145, 148–149) was conducted in collaboration with the Head and Neck Service of the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York, NY. Eligible cases were men and women with newly diagnosed histopathologically confirmed primary cancer of the oral cavity, excluding carcinomas of the lip, salivary gland, and nasopharynx. A two-stage stratified sampling scheme was developed. The first phase was based on enrolling all eligible participants, and the second phase included nonsmokers only to increase the sample size for this subgroup. Healthy controls were subjects without cancer who were recruited from the MSKCC Dental Service, which specializes in maxillofacial surgery. Control subjects were frequency matched to cases by age (within 10 years), sex, and month of interview. All cases and controls were interviewed using a structured questionnaire that contained questions on smoking history, alcohol consumption and occupation. Subjects signed a consent form that was approved by the Institutional Review Boards of MSKCC and the American Health Foundation. Approximately 90% of both cases and controls who were approached agreed to participate.

After the interview, the subjects were brought to a phlebotomy station for venipuncture. Fasting venous blood samples were obtained from an antecubital vein into two 10 ml Trace Metal Vacutainer tubes containing heparin as the anticoagulant (Becton Dickinson) using a sterile 19 gauge needle. A sample of 0.5 ml of the whole blood was taken for GSH assay and the remainder was centrifuged to separate plasma, erythrocytes and buffy coat. Erythrocytes were washed in saline 3x. Plasma and erythrocyte samples were then aliquoted for each assay and all samples were immediately frozen and stored at −70°C.

Blood Analyses

Serum iron was assayed spectrophtometrically by complexing ferrozine chromagen and measuring absorbance at 526 nm [39]. Serum TIBC was determined with an autoanalyzer by the method of Cook [40]. Serum transferrin saturation was calculated as serum iron/TIBC × 100. Serum ferritin was determined by a two-site immunoradiometric assay [41]. Hemoglobin levels were measured in red cells and whole blood by the ferricyanide-cyanide method as an indicator of anemia [42]. Plasma concentrations of retinol, alpha-tocopherol, and beta-carotene were determined simultaneously by high-performance liquid chromatography [43]. Riboflavin status was assessed in red cells by measuring the in vitro activation of erythrocyte glutathione reductase activity by flavin adenine dinucleotide (FAD), and its levels are expressed as an activity coefficient (activity in the presence of added FAD/activity without added FAD) [44]. Thiamin status was assessed in red cells by measuring the *in vitro* activation of erythrocyte transketolase activity by thiamin pyrophosphate and expressed as an activity coefficient (activity in the presence of added thiamin pyrophosphate/activity without added thiamin pyrophosphate) [45]. Red blood cell folate content was determined using a radioimmunoassay (Diagnostic Products, Los Angeles). The concentrations of zinc in erythrocytes and plasma were measured by flameless atomic absorption spectrophotometry using a Model 460 atomic absorption spectrophotometer equipped with an HGA-400 graphite furnace (Perkin-Elmer Corp. Norwalk Ct) [46].

The ascorbic acid concentration of perchloric acid extracts of plasma was determined by HPLC with electrochemical detection [44]. Whole blood glutathione levels were determined by first deproteinating whole blood with 4-volumes of ice-cold 5% metaphosphoric acid (MPA). After centrifugation at 13,000 × g for 2 min, glutathione content of supernatants were analyzed using an HPLC method with dual electrochemical detection [47]. Plasma cotinine levels were measured to validate smoking status (current vs. nonsmoking) using a modified radioimmunoassay [48].

Statistical analysis

All analyses were performed using SAS statistical software (version 9.1, Cary, NC). The blood micronutrient values were compared by smoking status, age and other potential confounders using Student's t-test. The relationship between the blood iron measurements was determined by correlation coefficients. Odds ratios (OR) and 95 percent confidence intervals (95% CI) were calculated to determine the risk of oral cancer associated with tertiles of serum biomarkers. The effects of smoking were determined by classifying ever smokers as having smoked at least one cigarette per day for ≥ 1 year. Never smoking was defined as never having smoked, or smoking cigarettes for < 1 year. Former smoking was defined as having quit at least one month prior to the interview. The Cochran-Mantel-Haenszel test was conducted to determine the homogeneity of the odds ratios between smokers. Logistic regression models were used to calculate adjusted odds ratios and 95% CI. There were too few premalignant cases to conduct meaningful relative risk estimates, and therefore the mean levels of biomarkers were compared between these cases and the entire control group using t-tests.

Results

The study included 65 oral cancer cases. The most frequent site of the tumor was the tongue (50%), followed by the floor of the mouth (18%), gingiva (14%) and other areas (18%). The case group also included 13 subjects with oral premalignancy, of which 9 (69%) had lesions located on the tongue. Nine were diagnosed with keratosis and 4 with leukoplakia. Eighty-five subjects participated as controls. About 2/3rd of all cases and controls were men, and the mean age was 58 in all oral cancer cases, 60 in the premalignancy cases and 54 in controls (Table 1). Seventeen cancer cases (26%) and 37 controls (44%) were never smokers. The reported smoking status (current vs. not current) was confirmed as 100% accurate using cotinine levels as the validation standard. Fourteen (82%) of the never smoking cases had tongue cancer, who accounted for all of the cases <40 yr. of age. The mean age of all male never-smoking cases (41.3 ± 19.0) was substantially lower than female never-smokers (63.7 ± 15.0) and in all ever-smokers (61.6 ± 7.81).

When blood iron levels were classified according to clinically normal values, 3 controls and 12 cases had serum iron levels that fell below normal values (60–170 μg/dl). Nine controls and 1 case had clinically elevated levels. Five cases and controls fell below normal TIBC levels (<240–450), and 15 cases and 17 controls had elevated TIBC levels. About 25% of all subjects had clinically defined iron deficiency based on cut-off values of transferrin saturation (<15%).

Eight controls and 12 cases had greater than normal ferritin levels (e.g. >12–300 ng/dl). Hemoglobin levels were within normal ranges for all subjects and did not differ between cases and controls.

Since serum ferritin increases with age, we examined the biomarkers of iron metabolism by age in cases and controls (Table 2). No significant age differences were observed for the iron biomarkers except for ferritin levels, which were significantly increased in both men

and women greater than age 50 yr. Among oral cancer cases, mean ferritin levels did not vary significantly by the site of the tumor (Table 3).

Cigarette smoking status (current vs. not currently smoking) was not related to mean levels of iron measurements and most micronutrients (data not shown). Ascorbic acid levels were 20% lower and plasma zinc levels were 11% lower in smokers compared to non-smokers ($P<0.05$) whereas, glutathione reductase activity coefficients (a measure of riboflavin status) were 12% higher and plasma zinc levels were 13% higher in smokers compared to non-smokers ($P<0.05$).

The levels of iron biomarkers, GSH and other micronutrients in cases and controls were compared between never-smokers and ever-smokers (Table 4). In never-smokers, mean free iron levels were 45% higher in controls than in cases ($P<0.002$), however, no significant differences were observed in smokers. Higher mean transferrin saturation levels were found in controls vs. cases in both never-smokers and smokers, but the difference was greater in never-smokers (55%) than in smokers (28%). Mean ferritin levels were 51% higher in cases than controls among never-smokers ($P<0.05$) but were not significantly different among ever-smokers. In never-smokers, mean levels of vitamin A, α-tocopherol and ascorbic acid were increased by 33%, 17% and 63%, respectively, in cases than in controls, while no differences were observed in ever-smokers.

Table 5 shows the adjusted odds ratios for oral cancer associated with tertiles of blood iron biomarker levels. Despite differences in mean levels of various measures between cases and controls, the risk associated with the upper tertiles of exposure did not vary significantly by smoking status. After controlling for smoking, age and other factors, the risk was significantly decreased in subjects with the highest tertile of both free iron and transferrin saturation, compared to their lowest tertiles, respectively. In contrast, the highest levels of ferritin and TIBC were associated with a significantly increased risk of oral cancer. While tests for homogeneity by ever-smoking status were not significant, the association with the highest tertile was stronger in never-smokers (free iron; OR=0.12; 95% CI 0.02, 0.71) and (transferrin saturation; OR=0.13; 95% CI 0.02, 0.82). The risk for the highest tertile of ferritin in never-smokers was 5.8 (95% CI 1.2, 28). When blood iron levels were grouped into clinical diagnostic categories, a significant dose response relationship was also observed between increasing risk for oral cancer and decreasing levels of iron (low [<60μg/dl]: OR [CI]=8.25 [1.72–39.6]; low normal [60–110 μg/dl]: OR [CI]=2.38 [0.84–7.76]; high normal [111–160 μg/dl]: OR [CI]=1.42 [0.41–4.84]; high [>160 μg/dl]: reference group).

Table 6 shows the risk of oral cancer associated with blood levels of antioxidant micronutrients. Significantly decreased risk was observed for the highest tertile of blood glutathione (OR=0.4; 95% CI 0.1, 0.9). Glutathione levels were not correlated with any of the four iron measurements. A significantly increased risk was observed for individuals with glutathione reductase activity coefficient >1.29 (indicative of riboflavin deficiency, a condition characterized by lesions of the skin, digestive tract surface, or nervous disorders) (OR=3.0; 95% CI 1.3, 7.0). No associations were observed for other micronutrients.

Iron and other micronutrient values were determined for 13 cases with preneoplastic lesions. Mean serum iron levels were 16% higher, and riboflavin activity coefficients were 14% higher in controls compared to cases. RBC Zinc levels were 13% higher in cases than in controls (Table 7, $P<0.05$).

Discussion

In this hospital-based case-control population, increased risk for oral cancer was associated with markers of low body iron stores, but not anemia, as well as low levels of the major

endogenous antioxidant GSH. These findings may result form enhanced levels of oxidative stress associated with either decreased iron status or GSH levels.

There are several possible limitations that need to be considered in this study. First, the concentrations of the serum nutrients reflect recent food intake and not long-term dietary patterns. This may limit the ability to make inferences regarding diet, although biochemical measures are not subject to recall accuracy and biases that have been well documented in food frequency assessments. Secondly, diurnal variation in blood biomarker levels, as have been observed for serum iron and transferrin saturation levels may impact these results, although fasting blood samples were obtained to help control for circadian fluctuations. In addition, data suggest that iron determinations restricted to a specific time of day does not improve the reliability of iron test results [49] Thirdly, biomarkers levels in cases were measured post-diagnostically and may reflect recent changes in dietary habits. Our questionnaire data found that subjects did not change their dietary habits as a result of their cancer diagnosis. Further, the levels of numerous diet related micronutrients did not differ between cases and controls. The development of cancer or its treatment might affect the serum levels of blood nutrients including iron measurements. To help control for treatment effects, the case patients in this study were enrolled post-surgically but prior to chemo-and radiation therapy. While it is possible that the development of oral cancer alters the regulation of iron metabolism and affects the serum levels of iron, mean levels of serum iron were also lower in patients with precancerous oral lesions.

Our findings are consistent with the high rates and risk of oral cancer in Swedish women during the 1950's that was linked with iron deficiency conditions that characterize Plummer-Vinson syndrome [9] and with more recent case-control studies that found an increased risk of oral cancer associated with decreasing iron intake [12–14]. Since transferrin saturation levels depend upon serum iron, a similar trend in risk was observed with this measure. Similarly, elevated levels of TIBC (>460 $\mu g/dl$), which are indicative of decreased iron levels, were significantly associated with an increased risk. The associations between biomarkers of iron status and risk appeared to be strongest for never-smokers.

In contrast to serum iron, ferritin levels were higher in cases and controls. Although a low ferritin level is a diagnostic criteria for iron deficiency, high ferritin levels can occur in response to numerous factors including infection, inflammation and chronic disease [50]. Thus, ferritin levels can be increased, even in the presence of an iron deficiency condition [51]. Ferritin levels are known to be increased in patients with oral disease [52, 53], breast cancer, Hodgkin's disease, and surgical tissue injury [54–57].

The findings on iron biomarkers do not appear to be due to confounding or selection bias. The higher iron levels in women vs. men and the lack of an effect of age on transferrin saturation in this study are consistent with previous reports [58, 59]. The lowered levels of vitamin C in smokers are also consistent with other data [60]. In reviewing the age-distribution of the male cases, the younger age of never smokers is also consistent with reports of increased incidence of tongue cancer in nonsmoking young men [61, 62]. The levels of TIBC were somewhat higher here than in other studies [41, 63, 64]. Consequently, the mean transferrin saturation percent in controls (25.8%) was lower than that reported for normal healthy subjects. Arithmetic mean values in other studies ranged from about 31–35% [41, 64]. The geometric mean of 23.8% was slightly lower than that reported for controls in an epidemiologic study of colon cancer (25.8%)[63].

Increasing blood glutathione levels were significantly associated with a decreased risk for oral cancer in our data. One study found an association between increased dietary intake of GSH from fruits and vegetables and decreased risk for oral cancer (OR=0.5) [65]. As the most

abundant antioxidant in nearly all cells and tissues including the oral epithelium, glutathione is the first line of defense against oxidative stress and other cellular insults [66]. Glutathione conjugation is a major pathway for the detoxification of carcinogens including polycyclic aromatic hydrocarbons thought to be involved in the development of aerodigestive tract cancers [67]. Consequently, glutathione is thought to play an important protective role against numerous diseases including cancer [68]. A direct role for glutathione in the inhibition of oral carcinogenesis was suggested by the finding that oral administration of glutathione was associated with a reduction in dimethylbenz[a]anthracene-induced cheek pouch tumors in the hamster [69, 70], and numerous laboratory findings found that GSH depletion increases the incidence of experimentally-induced oral cancer.

The finding that riboflavin deficiency, as measured by glutathione reductase activity coefficient, is associated with increased risk for oral cancer is consistent with previous findings [12, 71]. It is of interest that many of the clinical symptoms of riboflavin deficiency are similar to those observed in iron deficiency [72–74]. Further, riboflavin status can markedly influence iron economy due to the specific role of flavins in the mobilization of iron from its intracellular carrier protein, ferritin [75, 76]. Riboflavin deficiency can also impair the gastrointestinal absorption of iron [77]. Feeding mice a diet deficient in riboflavin causes morphological alterations in skin and upper alimentary tract epithelium which are similar to those observed in patients suffering from Plummer-Vinson disease [78]. Riboflavin deficiency was also associated with enhanced development of dimethylbenanthracene-induced skin tumors in mice [79] an effect that may be related to enhanced carcinogen activating capacity [80].

Previous studies have generally observed that vitamin C intake protects against the development of oral cancer [29]. We observed no significant association with serum vitamin C levels after adjustment for smoking. It is possible that we did not detect an association because the effects of vitamin C seem to be dependent upon the levels and combinations of other micronutrients.

There have been few nested case-control studies of prediagnostic serum antioxidant micronutrient levels and oral cancer risk. Although such studies have had small numbers of cases, the results have been somewhat consistent with studies of dietary intake of micronutrients and cancer risk. In the Iowa Women's Health Study cohort, of 33 women who developed oral, pharyngeal or esophageal cancer [81], the combined higher intakes of carotene and vitamins C and E were associated with lower risks of these cancers. High serum levels of beta-carotene were protective against oral precancer in Japanese men but not in Japanese women [82]. In a nested case-control study of 28 cases with oral or pharyngeal cancer and 112 matched controls, dose-related increased risks were found with lower levels of all individual serum carotenoids, and especially beta-carotene [83]. In contrast, the risks were significantly elevated with increasing serum levels of gamma-tocopherol and selenium. A nested study of 16 oral/pharyngeal cases in Japanese men found reduced risks associated with serum alpha-carotene and beta-carotene.

Oxidative stress may be the critical factor that links low body iron stores, riboflavin status and GSH levels with increased risk for oral cancer. Oxidative stress has been associated with iron deficiency in numerous studies [21–23] and is thought to be an important contributor to oral carcinogenesis [24–27]. As described above, GSH represents the major intracellular antioxidant and maintenance of its levels is dependant upon riboflavin, which, through its metabolite FAD, serves as a key co-factor for the enzyme, glutathione reductase, responsible for maintaining GSH in its reduced state [84]. The finding that other antioxidant/nutrient levels were not associated with oral cancer risk may be due to their dependency on diet. While GSH levels can be related to diet, regulation of their levels *in vivo* are dependant on a

complex endogenous regulatory system involving numerous metabolic pathways [85]. Our previous results indicate that blood GSH levels are an accurate biomarker of body status that are not influenced by acute changes in diet or environmental exposures [86–88].

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Table 1

Study Subject Characteristics

	Controls		Cancer Cases		Pre-cancer Cases	
	N	%	N	%	N	%
All	85	(100)	65	(100)	13	(100)
Sex						
Male	51	60.0	44	67.7	6	46.2
Female	34	40.0	21	32.3	7	53.8
Age						
<35	10	11.8	4	6.2	0	0
35–44	12	14.1	4	6.2	1	7.7
45–54	20	23.5	14	21.5	3	23.1
55–64	20	23.5	20	30.8	4	30.8
≥65	23	27.1	23	35.4	5	38.5
Smoking Status						
Never	37	43.5	17	26.2	5	38.5
Former	32	37.7	40	61.5	4	30.8
Current	16	18.8	8	12.3	4	30.8
Alcohol (oz./day)						
0	40	47.1	21	32.3	8	61.5
<1	22	25.8	18	27.7	3	23.1
1–4	10	11.8	6	9.2	1	7.7
4–7	13	15.3	20	30.8	1	7.7
Site of Lesion						
Palate			6	9.2	1	7.7
Pharynx			1	1.5		
Tongue			34	52.3	9	69.2
Floor of mouth			10	15.4	1	7.7
Gingiva			9	13.9	1	7.7
Retromolar trigone			2	3.1		
Buccal mucosa			3	4.6	1	7.7

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Table 2

Levels of iron stores by age and sex in oral cancer cases and controls

	Serum Iron *(μg/dl)*	**Transferrin saturation *(%)***	**TIBC *(μg/dl)***	**Ferritin *(ng/ml)***	**Hemoglobin *(g/dl)***
Men					
All ages	110 ± 42.2	22.7 ± 9.04	399 ± 89.4	188 ± 178	15.4 ± 1.72
<50	115 ± 42.2	22.9 ± 8.9	417 ± 91.3	134 ± 103 *	15.5 ± 1.43
>50	107 ± 42.2	22.5 ± 9.2	390 ± 87.8	218 ± 204	15.4 ± 1.86
Women					
All ages	102 ± 36.5	22.8 ± 11.4	376 ± 93.4	131 ± 120	13.0 ± 1.65
<50	101 ± 41.9	23.3 ± 10.8	383 ± 105	51.8 ± 41.3≠	13.2 ± 1.32
>50	102 ± 35.4	27.7 ± 11.7	373 ± 91.0	151 ± 126	12.9 ± 1.75

Values are mean ± SD

*P<0.02.

≠P<0.001.

Table 3

Mean level and standard deviation of iron measurements in cases by most common oral cancer site

Site	Serum Iron *(μg/dl)*	Transferrin saturation *(%)*	TIBC *(μg/dl)*	Ferritin *(ng/ml)*
Palate	85.1 ± 17	17.0 ± 6.2	382 ± 70	304 ± 175
Tongue	95.3 ± 42	18.5 ± 8.0	412 ± 85	201 ± 151
Floor of mouth	110 ± 34	22.1 ± 8.7	432 ± 93	319 ± 279
Gingiva	98.1 ± 41	19.8 ± 8.6	388 ± 71	121 ± 117

Table 4

Mean levels of iron stores and other micronutrients in cases and controls by smoking status

	Never-Smokers			Ever-Smokers		
	Control N=37	**Case N=17**	**P- value**	**Control N=48**	**Case N=48**	**P- value**
Serum iron *(μg/dl)*	121 ± 40.8	83.2 ± 33.5	0.002	113 ± 37.1	101 ± 38.0	NS
Ferritin *(ng/ml)*	102 ± 92.0	154 ± 102	0.04	182 ± 188	229 ± 194	NS
TIBC *(μg/dl)*	381 ± 102	414 ± 78.4	NS	383 ± 97.0	408 ± 81.3	NS
Transferrin Saturation *(%)*	26.6 ± 10.9	17.2 ± 6.65	0.005	25.3 ± 10.7	19.7 ± 8.13	0.007
Hemoglobin *(g/dl)*	14.0 ± 2.31	14.3 ± 1.92	NS	15.2 ± 2.15	14.6 ± 1.76	NS
Vitamin A *(mg/dl)*	34.4 ± 16.6	45.8 ± 12.7	0.02	42.0 ± 18.6	39.4 ± 18.7	NS
Vitamin C *(mg/l)*	16.6 ± 4.31	19.4 ± 6.47	0.06	16.1 ± 6.81	17.3 ± 6.06	NS
Vitamin E *(mg/dl)*	464 ± 179	757 ± 436	0.002	633 ± 351	627 ± 318	NS
Folate *(ng/ml)*	381 ±111	363 ± 101	NS	369 ± 116	414 ± 114	0.05
RBC Zinc *(μg/g Hb)*	17.4 ± 2.87	18.3 ± 3.46	NS	18.4 ± 3.08	18.0 ± 3.26	NS
Plasma Zinc *(μg/l)*	689 ± 190	706 ± 160	NS	751 ± 226	694 ± 150	NS
Riboflavin *(AC)*	1.28 ± 0.180	1.20 ± 0.154	NS	1.22 ± 0.133	1.24 ± 0.171	NS
Thiamin *(AC)*	1.10 ± 0.126	1.08 ± 0.105	NS	1.05 ± 0.071	1.08 ± 0.082	NS
Glutathione *(mM)*	5.93 ± 1.14	5.35 ± 0.892	0.07	5.60 ± 1.14	5.26 ± 1.03	NS

Values are mean ± SD

Table 5

Odds ratio for oral cancer associated with iron measurements

	Cases N (%)	Controls N (%)	OR[1]	95% CI
Serum Iron *(μg/dl)*				
Lower (≤69.0)	29 (44.6)	19 (22.6)	1.0	1.0
Middle (69.1–91.0)	21 (32.3)	28 (33.3)	0.4	0.2,0.9
Highest (>91.0)	15(23.1)	37 (44.0)	0.3	0.1,0.6
Transferrin saturation *(%)*				
Lower (<17.1)	28 (48.3)	16 (22.2)	1.0	
Middle (17.1–24.7)	16 (27.6)	26 (36.1)	0.5	0.2,1.1
Highest (>24.7)	14 (24.1)	30 (41.7)	0.4	0.2,0.9
Ferritin *(ng/ml)*				
Lowest (<72.0)	12 (24.0)	25 (41.0)	1.0	1.0
Middle (72.1–154)	12 (24.0)	23 (37.7)	0.8	0.4,2.1
Highest (>154)	26 (52.0)	13 (21.3)	3.1	1.3,7.4
TIBC *(μg/dl)*				
Lowest (<345)	13 (22.4)	28 (38.9)	1.0	
Middle (346–424)	20 (34.5)	24 (33.3)	1.9	0.7, 4.7
Highest (>424)	25 (43.1)	20 (27.8)	3.2	1.3,8.1

[1]Adjusted for age, sex and smoking status.

Table 6

Odds ratio for oral cancer associated with tertiles of blood micronutrient values

Vitamins	Cases N (%)	Controls N (%)	OR	95% CI
Vitamin A *(mg/dl)*				
Lowest (<32.0)	16 (25.4)	28 (35.9)	1.0	
Middle (32.0–48.0)	23 (36.5)	26 (33.3)	2.1	0.9,5.0
Upper (>48.0)	24 (38.1)	24 (30.8)	1.8	0.8, 4.1
Vitamin C *(mg/l)*				
Lowest (<14.0)	20 (30.8)	30 (35.3)	1.0	
Middle (14.0–18.8)	22 (33.8)	31 (36.5)	1.1	0.4,2.6
Upper (>18.8)	23 (35.4)	24 (28.2)	1.4	0.6,3.4
Vitamin E *(mg/dl)*				
Lowest (<453)	15 (25.0)	31 (39.2)	1.0	
Middle (453–646)	20 (33.3)	26 (32.9)	1.4	0.6, 3.1
Upper (>646)	25 (41.7)	22 (27.8)	1.9	0.8, 4.3
Plasma Zinc *(μg/l)*				
Lowest (<611)	21 (32.3)	29 (34.9)	1.0	
Middle (611–778)	24 (36.9)	25 (30.1)	1.5	0.7,3.5
Highest (>778)	20 (30.8)	29 (34.9)	1.1	0.5,2.6
Erythrocyte Zinc *(μg/g Hb)*				
Lowest (<29.1)	21 (32.8)	28 (35.4)	1.0	
Middle (29.1–34.0)	19 (29.7)	30 (38.0)	0.9	0.4,2.0
Highest (>34.0)	24 (37.5)	21 (26.6)	1.6	0.7,3.8
Riboflavin *(AC)*				
Lowest (<1.14)	16 (26.7)	30 (41.7)	1.0	
Middle (1.14–1.29)	19 (31.7)	23 (31.9)	1.6	0.7,3.8
Highest (>1.29)	25 (41.7)	19 (26.4)	3.0	1.3,7.0
Thiamin status *(AC)*				
Lowest (<1.02)	22 (34.4)	28 (36.4)	1.0	
Middle (1.02–1.09)	19 (29.7)	25 (32.5)	1.2	0.5,2.8
Highest (>1.09)	23 (35.9)	24 (31.2)	1.6	0.7,3.7
Glutathione *(mmol/g Hb)*				
Lower (<4.94)	24 (40.7)	19 (27.5)	1.0	
Middle (4.94–5.91)	21 (35.6)	21 (30.4)	0.7	0.3,1.7
Highest (>5.91)	14 (23.7)	29 (42.0)	0.4	0.1,0.9

Adjusted for age, sex and smoking status.

Table 7

Mean levels of iron stores and other micronutrients in 13 oral premalignancies

	Case N=13	Control N=85	P-value
Serum iron *(μg/dl)*	99.5 ± 24.6	116 ± 38.7	0.033
Ferritin *(ng/ml)*	97.8 ± 52.5	149 ± 160	0.048
TIBC *(μg/dl)*	340 ± 78.8	382 ± 98.5	NS
Transferrin Saturation *(%)*	21.8 ± 9.55	25.8 ± 10.7	NS
Vitamin A *(mg/dl)*	34.8 ± 14.5	38.6 ± 18.1	NS
Vitamin C *(mg/l)*	19.6 ± 6.36	16.3 ± 5.83	NS
Vitamin E *(mg/dl)*	635 ± 344	560 ± 300	NS
Folate *(ng/ml)*	359 ± 115	374 ± 113	NS
RBC Zinc *(μg/g Hb)*	35.6 ± 6.25	31.4 ± 5.82	0.022
Plasma Zinc *(μg/l)*	757 ± 192	724 ± 212	NS
Riboflavin *(AC)*	1.10 ± 0.082	1.25 ± 0.157	0.002
Thiamin *(AC)*	1.09 ± 0.096	1.07 ± 0.100	NS
Glutathione *(mmol/g Hb)*	5.77 ± 1.54	5.30 ± 0.964	NS

Values are mean ± SEM