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A Case Study of Nutrient Intervention of Oral Precancerous Lesions in India

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Tobacco chewing and/or smoking are strongly related to several cancers, mainly of the upper aerodigestive tract. Several studies on diet and cancer links suggest that micronutrients, particularly antioxidant vitamins and minerals, are risk modifiers of cancers of epithelial origin. This study looks at the impact of micronutrients such as vitamin A, riboflavin, zinc and selenium as intervention agents in subjects with and without precancerous lesions in a high risk group (reverse smokers of chutta-rolled tobacco leaf). Reverse smokers from four villages were enrolled in the study. 150 subjects were supplemented with four nutrients, namely vitamin A, riboflavin, zinc and selenium in the form of a capsule twice a week for 1 year. 148 controls received a placebo capsule containing lactose for the same period. Clinical history and anthropometric data were collected from all the subjects and a clinical photograph of the palate was taken. Micronutrients were estimated in random blood collected from a sub-sample before and after the study. Micronutrients improved the vitamin A, riboflavin and selenium nutriture in the supplemented group with a concomittant regression of precancerous lesions present on the palate. Clinically complete remission of white, red and combination lesions was seen in 57% of subjects on supplements whereas 8% on placebo showed a positive response. Further progression of these lesions was seen in 10% of the supplemented group compared with 47% in the placebo group ($P<0.001$). In the non-lesion group, new lesions appeared in 12% on supplements while more than 38% on the placebo developed new lesions ($P<0.02$). The results, coupled with the observation of a better nutritional status of vitamin A, riboflavin and selenium in those who had a clinical response, suggested that a cocktail of nutrients as a prescriptive approach rendered the subjects at risk refractory to carcinogens in the environment. Since the study was a small straight trial and not double-blind, a large study with factorial designs could provide answers to whether single nutrients can produce similar responses. Dietary intervention might perhaps be the long-term strategy for prevention of cancer.

Keywords: nutrient intervention, oral precancers, reverse smokers

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INTRODUCTION

CANCER IS no longer considered to be an inevitable consequence of ageing. A major fraction of human cancer appears to be potentially preventable [1, 2]. Several endogenous and exogenous factors have been identified as causative or promoting factors in cancers at several sites. Alternatives to therapy of late disease are, therefore, being pursued vigorously in all professional circles for the control of cancer. With sophisticated molecular and biochemical tools, risk factors which predispose an individual to cancer are now being identified. Epidemiology as a discipline has contributed significantly to dietary hypothesis of several cancers [3]. Many macro- and micronutrients, as well as non-nutrients have been causally related [4].

Though India lacks nationwide cancer registration and systematic death registration, approximately 3.2×10^5 persons are estimated to die from cancer annually, giving a crude mortality rate of 38/100 000 [5]. Cancer of the oral cavity is an important contributor to cancer morbidity and mortality and to overall international cancer burden. Tobacco chewing and smoking are the risk factors for oral cancers in India. About 48.2% of cancers in men and 20.5% of cancers in women are related to tobacco, of which a major proportion is in the oral cavity, pharynx, larynx, oesophagus (74.7%), while lung cancers account only for 15%. Control of cancers of the head and neck, lung, cervix and breast which account for 50–55% of the cancer load in India will have a maximum measurable effect on the incidence of cancer [6]. Though scientific evidence still is not as strong regarding the quantitative

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relationships of cancer with foods and nutrients as in the case of tobacco and alcohol, dietary modification as a means of prevention appears to be a fascinating alternative for prevention of epithelial cancers [7]. Of the several approaches suggested to strengthen or refute existing evidence in support of causal diet and cancer links, intervention with nutrients is gaining rapid recognition. As micronutrient deficiencies are common in India and have been related to oral and upper aerodigestive tract cancers [8], it was considered necessary to study the impact of nutrients on oral preneoplastic lesions, commonly encountered in a high risk area [9]. In our case–control studies on oral and oesophageal cancers, we have observed that nutrients such as vitamins A, C, E, riboflavin and trace metals zinc and selenium were significantly low in cases as compared with controls. In the present study, we included these nutrients, except vitamin C and E as they were more expensive, and less stable compounds.

This paper encapsulates the results of a placebo controlled nutrient intervention trial undertaken as a prescriptive approach to study the impact of a cocktail of micronutrients on reverse (lit end inside the mouth) chutta smokers with or without lesions. A high risk group of reverse smokers in Andhra Pradesh, India, where carcinoma of the hard palate is endemic, was selected.

The objectives of the study were: (1) to quantitate dietary intake of food groups and nutrients and assess nutritional status with respect to micronutrients such as vitamin A, riboflavin, folate and minerals such as iron, magnesium, zinc, selenium and copper; (2) to evaluate the clinical response of precancerous lesions to micronutrient supplements; (3) to estimate certain internal dosimeters such as micronuclei and DNA adducts in epithelial cells.

SUBJECTS AND METHODS

Area and sampling procedure

The study was conducted in four villages in the northeastern coastal area of Andhra Pradesh where reverse smoking is the prevailing habit.

Subjects were considered eligible if they were reverse smokers, less than 80 years of age and eager to take medication. Care was taken not to include pregnant women and those who were likely to increase their family size. A total of 298 subjects with or without oral lesions participated in the clinical trial (Table 1). The sampling was purposive with subjects willing to give blood and saliva.

All the subjects underwent a complete oral examination and the lesions over the palate were carefully recorded initially, and 12 months after the clinical trial. Before treatment began, a black and white photograph of the individual and baseline colour photographs of the lesions were taken to facilitate identification of individuals and for the evaluation of clinical response, respectively. A simple questionnaire was administered to record their habits of smoking and chewing. Dietary intake was quantitated in a subsample by a 24-h diet and food frequency questionnaires. Random blood samples were collected before and after the study period in a subsample to estimate micronutrients. Oral epithelial cells were collected and buccal cell smears prepared for measuring DNA adducts and micronuclei at baseline level and after 12 months from the trial period. Heights and weights of all individuals were recorded and body mass index calculated.

Table 1. Subjects participating and lesion status in different groups

	Initial number	Final number	% followed
Total no. of subjects	298	203	68
Supplemented			
Lesions	99	63	64
Non-lesions	51	34	66
Placebo			
Lesions	97	64	66
Non-lesions	51	42	82

Clinical

Palatal lesions were mapped according to Gupta *et al.* on nine arbitrary zones [9]. The lesions were recorded as white, red, combinations or ulcers in the areas marked for evaluating clinical response. In addition, clinical photographs were taken. The oral examinations were made by medical doctors who were trained by and calibrated to dentists who had experience in the field of epidemiology [10]. The oral examination was conducted in daylight.

Assessment of response

Every third month, an investigator made a door-to-door survey for assessing toxic reactions to supplements and changes in smoking habits. A short questionnaire was administered to detect toxicities. The outcome of treatment was assessed clinically by two medical officers and were compared for consistency. Colour photographs of the lesions were also examined. The end-points for clinical recovery were categorised as complete regressions when all the lesions disappeared, and as partial regressions when the lesions disappeared in a few areas or disappeared completely with appearance of fresh lesions in other areas. Lesions were considered to deteriorate if there were fresh lesions in addition to the existing lesions. In non-lesion subjects the appearance of new lesions was noted.

Treatment

After the baseline assessments were completed, vitamin A (retinol acetate), riboflavin, zinc (sulphate) and selenium (selenomethionine) containing capsules were administered bi-weekly under supervision over a period of 1 year. The dosage schedules were changed every fourth month as indicated in Table 2. The dosages were adjusted to avoid toxicity and at the same time to give sufficiently permissible amounts of these nutrients. Therefore, doses of vitamin A, selenium and riboflavin were reduced after the initial 4 months while zinc, which was tolerated well, was increased. During the last

Table 2. Nutrients and dosages administered to the supplemented group

	Months		
	1–4	5–8	9–12
Vitamin A (U)	25 000	10 000	25 000
Riboflavin (mg)	50	15	30
Zinc (mg)	12.5	25	25
Selenium (μg)	100	50	50

Administered bi-weekly under supervision.

4 months vitamin A and riboflavin were again increased in order to saturate the tissue levels.

Two villages were randomly allocated to nutrient supplements while the other two received placebo. Patients were followed-up for a period of 1 year (Table 1).

Biochemical

The blood samples were collected in heparinised tubes. A small aliquot of whole blood was removed for folate estimation. The remaining blood was then centrifuged. Aliquots of whole blood, plasma and the saline-washed cells were then stored at −20°C. They were transported within a week to the institute where further analysis was completed. The following procedures were adopted: for vitamin A, high-pressure liquid chromatography [11], glutathione reductase in red cells (EGR) for riboflavin nutriture [12], red cell folate by microbiological procedure [13], ferritin by ELISA technique [14] and trace metals, such as zinc, copper and magnesium, by atomic absorption spectrophotometry and selenium by fluorometric procedure [15]. Albumin and haemoglobin were estimated by routine procedures. For vitamin A and other nutrients, quality control samples were run along with test samples. The coefficient of variation was well within the accepted limits (vitamin A 6.9%, selenium 5.1% and zinc 10.1%).

Statistical analysis

A proportion test was carried out to test statistically significant differences in the clinical response and odds ratios were calculated at 95% confidence intervals (CI). Student's *t*-test was employed for testing differences if any in the nutritional status.

The methodology and results of diet survey, DNA adducts and micronuclei in epithelial cells are being reported separately.

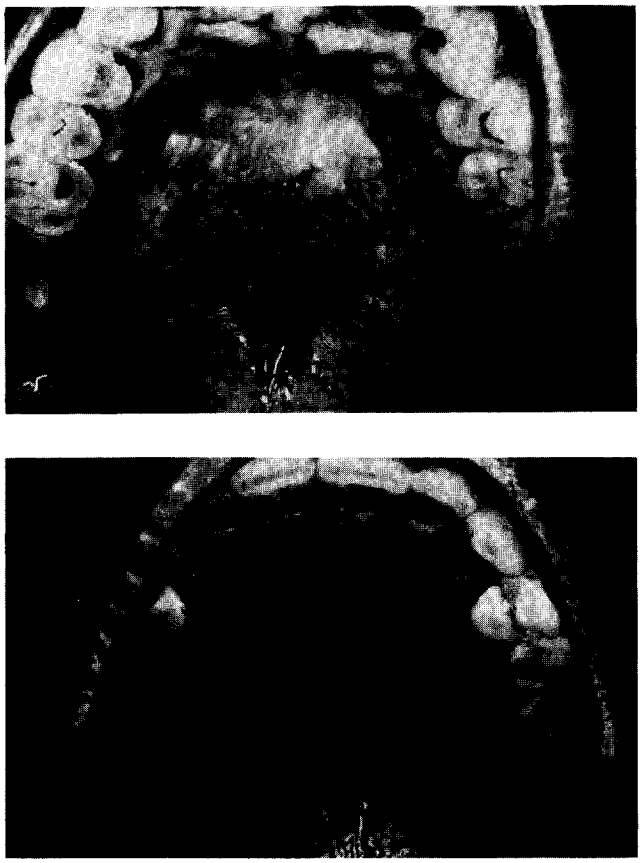

Fig. 1. (a) Before intervention, white patch on the palate. (b) After 1 year of intervention, clear palate.

RESULTS

Clinical

Both cases and controls of the study population were mostly illiterate, engaged in agricultural labour. Out of 298 subjects selected, 79 were males and 219 females aged between 25 and 70 years and they were distributed equally in placebo and nutrient-supplemented groups (Table 1). The relatively high proportion of women in our study population reflects the fact that more women than men in this population smoke in reverse direction and are at higher risk of developing oral precancerous lesions and palatal cancer. In all, 196 (66%) subjects had lesions and 102 (34%) had no lesions. Several types of lesions were encountered over the palate. The lesions which were encountered on the palate were either white (Fig. 1) or red patches or a combination of red and white lesions with a few of them having ulcers. In the lesion group, 50% had red lesions and 40% had white lesions. In Table 3, the distribution of lesions in the supplemented and the placebo group are indicated. The two treatment groups were identical with regard to the presence/absence of lesions and the distribution of various types of lesions. The subjects belong to the low income group. Most of them were agricultural labourers living in huts with low thatched roofs and limited ventilation. The surroundings were unhygienic with smoke emanating from primitive cooking systems. All the subjects were reverse smokers with smoking habits initiated at a very young age. The unprocessed tobacco rolled in the form of cigars, known locally as chuttas, were 10–12 cm in length. They smoke with the reverse end of the chutta in the mouth (Fig. 2). At the end of 1 year, 64–66% of people with lesions and 66–82% without lesions were followed-up, with the total follow-up being 68% (Table 1). The pattern was similar in both groups. The drop in the follow-up rate was mainly due to natural calamities (floods), migration due to family feuds, with only about 5% refusing to take medications regularly.

Table 3. Palatal lesions and their distribution

	Initial		Final	
	Supplemented	Placebo	Supplemented	Placebo
Red	49	48	14	36
White	42	37	8	25
Combined/ulcers	8	12	8	13

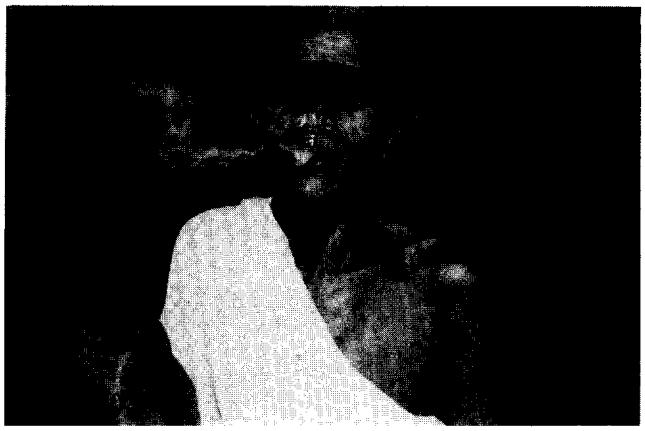

Fig. 2. A woman smoking with the burning end of the chutta in the mouth.

Table 4. Initial nutritional status

Parameter	Supplement group	Placebo group
Body mass index	18.4±0.196 (73)	18.0±1.88 (76)
Haemoglobin (g%)	9.97±1.79 (70)	10.68±1.71† (76)
Albumin (g%)	3.09±0.42 (76)	3.07±0.62 (77)
E.G.R. (A.C.)	1.28±0.17 (40)	1.37±0.23* (38)
Folate (ng/ml of RBC)	79.8±17.2 (67)	75.7±17.41 (65)
Vitamin A (μg/dl)	67.5±24.51 (73)	71.7±26.16 (76)
Ferritin (μg/l)	19.9±16.13 (62)	20.1±16.06 (77)
Selenium (ng/ml)	98.1±16.23 (76)	96.8±15.71 (77)
Zinc (μg/ml)	0.91±0.197 (76)	0.94±0.226 (77)
Magnesium (μg/ml)	18.2±2.72 (76)	18.8±2.72 (77)
Copper (μg/ml)	0.98±0.17 (76)	1.02±0.18 (77)

Mean±S.D. *$P<0.05$. †$P<0.02$. Figures in parentheses indicate number of subjects. A.C., activation coefficient.

Nutritional status

Initial. The general nutritional status, as reflected by body mass index and serum albumin levels, was similar in supplemented and placebo groups, though the haemoglobin levels were significantly higher by about 0.7 g% in the latter group. No differences were observed in red cell folate and serum ferritin levels (Table 4). The activation coefficient of erythrocyte glutathione reductase was higher ($P<0.05$) in the placebo group. The two treatment groups were virtually identical with respect to serum vitamin A levels and other micronutrients, such as selenium, zinc, magnesium and copper, reflecting a homogeneity in nutritional status in both the groups.

When the same parameters were tested between those with and without lesions, plasma vitamin A, the activation coefficient of glutathione reductase, folate and ferritin were found to be significantly elevated in the non-lesion group. The other micronutrients, such as selenium, zinc and copper, were identical, irrespective of lesion status, while magnesium levels were elevated in the lesion group (Table 5).

Final. Owing to seasonal variations and cross-sectional comparisons, the final vitamin/mineral status was compared between supplemented and placebo groups, without any reference to the initial status. Vitamin A levels were significntly higher in the supplement group (Table 6). The activation coefficient of glutathione reductase was also low in the supplemented group ($P<0.02$), with no differences in other nutrients.

However, when within-group comparisons between those with and without lesions were made, the vitamin A levels were significantly elevated in the supplemented non-lesion group, and was higher when compared with the placebo group (Table 7). The activation coefficient of glutathione reductase was elevated in the placebo groups in individuals with and without lesions. These values were significantly different from those supplemented with no lesions. Though no differences were seen within groups between those with and without lesions, plasma selenium levels were higher in individuals with no lesions in the supplemented group. However, in the supplemented group those with lesions had significant low levels of selenium. The placebo lesion and non-lesion groups had significant low levels of selenium when compared to the non-lesion supplemented group. The zinc levels were similar in all the groups.

A group of clinical responders versus non-responders in the supplement group and responders in the supplement group versus non-responders in the placebo group (there were few responders in the placebo group) were compared (Table 8). It indicated that responders had high plasma vitamin A

Table 5. Comparison of initial nutritional status of lesion and non-lesion groups

Parameter	Lesion	Non-lesion
Body mass index	0.18±0.019 (94)	0.19±0.019 (45)
Haemoglobin (g%)	10.2±1.57 (92)	10.5±2.10 (45)
Albumin (g%)	3.01±0.51 (96)	3.2±0.55 (47)
E.G.R. (A.C.)	1.29±0.18 (58)	1.47±0.24† (15)
Folate (ng/ml of RBC)	18.3±15.58 (79)	24.7±17.27* (35)
Vitamin A (μg/dl)	64.5±20.73 (94)	76.3±30.99† (46)
Ferritin (μg/l)	18.3±15.58 (79)	24.7±17.27* (35)
Selenium (ng/ml)	98.2±15.48 (95)	96.2±16.11 (47)
Zinc (μg/ml)	0.93±0.22 (96)	0.90±0.21 (47)
Magnesium (μg/ml)	18.8±2.56 (96)	17.9±2.72* (47)
Copper (μg/ml)	0.99±0.17 (96)	1.00±0.19 (47)

Figures in parentheses indicate number of subjects. All values are mean±S.D. *$P<0.05$. †$P<0.01$. A.C., activation coefficient.

Table 6. Nutritional status at the end of 1 year

Parameter	Supplemented	Placebo
Haemoglobin (g%)	10.2±1.75 (47)	11.2±2.44† (50)
Albumin (g%)	3.02±0.39 (49)	3.21±0.50 (50)
E.G.R. (A.C.)	1.3±0.30 (48)	1.61±0.33‡ (51)
Folate (ng/ml of RBC)	76.9±18.13 (44)	80.5±16.13 (45)
Vitamin A (μg/dl)	51.1±12.71 (49)	44.4±14.44† (55)
Ferritin (μg/l)	23.6±20.01 (44)	28.3±24.01 (39)
Selenium (ng/ml)	87.0±27.33 (50)	88.3±20.0 (51)
Zinc (μg/ml)	0.96±0.227 (47)	1.0±0.211 (38)
Magnesium (μg/ml)	0.72±0.17 (47)	0.76±0.18 (38)
Copper (μg/ml)	17.3±1.79 (47)	17.7±1.66 (38)

Figures in parentheses indicate number of subjects. All values are mean±S.D. *$P<0.05$. †$P<0.02$. ‡$P<0.01$. A.C., activation coefficient.

(52.5 μg/dl), low activation coefficient of glutathione reductase (1.23) and high plasma selenium (97.6 ng/ml). These values, when compared with those from non-responders in supplemented and placebo groups, were found to be significant. However, zinc levels were low in responders (0.85 μg/ml).

Response to therapy

At the conclusion of 1 year of treatment, oral examinations carried out yielded significant differences in response rates in both the lesion and non-lesion groups, even though the subjects had not changed their smoking habits significantly during the study period (Figs 3, 4). Only 3 subjects, 2 in the supplement and 1 in the placebo group reduced smoking. Of these, 2 had lesions, 1 in each group. Of these subjects on supplements, 57% had complete regression of all lesions while only 8% on placebo had such a result ($P<0.001$). Partial regressions were, however, not different. Both red and white lesions responded to the same extent (Table 3). It is most interesting and important to observe that only 10% of subjects in the supplemented group deteriorated as against 47% on placebo. Among those who had no lesions, 38% developed new lesions on placebo whereas only 12% developed on nutrient supplements ($P<0.02$). The odds ratios for clinical improvement for those with and without lesions were greater than 12.

Toxicity

A simple questionnaire based on known toxic reactions did not elicit any significant differences in adverse effects between the supplemented and placebo groups. The signs and symptoms attributable to toxic reactions, particularly to vitamin A and selenium, were not evident in the treated group.

DISCUSSION

As cancer research is progressing, the role of life style factors is becoming more evident and several factors have been causally related. The current study reported is an attempt to suggest a positive role for micronutrients in prevention of oral cancers. Although the study sample was selected and motivated, it is the first report of its kind on nutrient intervention on precancerous lesions in reverse smokers. There are currently several ongoing chemopreventive trials with antioxidant micronutrients in western countries and China [2].

It is now well established that tobacco and alcohol are the two major risk factors for upper aerodigestive tract cancers [19]. Though patients can have similar exposures to such products, the risk of cancer appears to vary between individuals. In the multistep carcinogenic process, nutrients explored and confirmed for their protective effect are known to be risk modifiers [4].

In India, oral cancers dominate the cancer field in both tobacco chewers and smokers. Our study was located in an area where inverted smoking is commonly practised, particularly in women. The incidence rates of palatal cancers are as high as 14/100 000 in this area. The peculiar habit originated among females and the most common reason for the habit appears to be toothache. The precancerous lesions on the palate are variable. Diffuse whitening (palatal keratosis), excrescences of 1–3 mm (elevated areas with central red dot) or well-defined white and red areas (elevated plaques) or ulcers and depigmented patches are seen in varying combinations. For our assessment purposes only white and red areas or combination lesions greater than 5 mm with a few ulcers were considered. Diffuse whitening was not included. Excrescences which may appear and disappear were also excluded [9]. White and red areas though unstable, regress very slowly and hence a careful clinical appraisal of response was arrived at and the response graded as given in Subjects and Methods.

The selection of the cocktail of nutrients tested was based on several previous observations in oral cancers in India and elsewhere [16–19]. The relative risk of oral cancers in chewers and smokers was much higher when subjects were on low intakes of milk, meat, fish, eggs and vegetables [20, 21]. Our recent efforts in a case–control approach on oral (Prasad, National Institute of Nutrition) and oesophageal cancers [22] suggested a high risk when individuals were on low intakes of vegetables, green or otherwise and low intakes of carotenes/vitamin A and C. Literature is replete with evidence that vegetable and fruit intake is directly correlated to several cancers [23, 24]. Our own observations in the current study (Krishnaswamy, National Institute of Nutrition) indicated a higher risk for lesions in individuals whose intake of protective foods was less frequent (carotenes/vitamin A, vitamin C and iron). However, iron was not considered for supplementation as iron tablets are known to irritate the gastric mucosa.

Vitamin A and iron nutriture appeared to be better in the non-lesion group, while riboflavin nutriture was better in the lesion group. However, serum magnesium levels were also higher in the lesion group which is similar to our observations in case–control studies on oral cancer. On treatment, even though the absolute level of vitamin A in blood was lower after the trial (due to seasonal variations), those without lesions in the supplemented group had better nutriture of micronutrients such as vitamin A, riboflavin and selenium, indicating an impact of the supplements. The fall in the values at the end of the study is unlikely to be due to methodological problem, as the coefficient of variation was within accepted limits. It is to be noted that biological markers of diet exist only for very few nutrients [25]. Plasma vitamin A does not always

Table 7. Nutritional status at the end of 1 year for nutrients under study

Nutrients	Supplemented		Placebo	
	Lesions	Non-lesion	Lesions	Non-lesion
Vitamin A (μg/dl)	46.9 ± 13.24^{ab} (15)	52.9 ± 12.21^{ac} (34)	44.1 ± 13.85^{b} (38)	44.9 ± 15.92^{c} (17)
E.G.R. (A.C.)	1.38 ± 0.40^{d} (15)	1.28 ± 0.26^{e} (35)	1.63 ± 0.28^{d} (33)	1.57 ± 0.39^{e} (18)
Selenium (ng/ml)	77.3 ± 12.51^{fg} (15)	91.2 ± 30.86^{fh} (35)	87.6 ± 22.09^{gi} (33)	89.6 ± 16.13^{hi} (18)
Zinc (μg/ml)	1.05 ± 0.30 (12)	0.94 ± 0.19 (34)	1.01 ± 0.21 (24)	0.97 ± 0.22 (14)

Figures in parentheses indicate number of subjects. All values are mean ± S.D. Values bearing same superscripts are significant. a, i—$P < 0.01$; b, c, e, g—$P < 0.02$; d, h—$P < 0.05$; f—$P < 0.001$.

Table 8. Comparison of nutritional status at the end of 1 year between clinical responders and non-responders

Nutrients	Supplemented		Placebo
	Responders (18)	Non-responders (12)	Non-responders (32)
Vitamin A (μg/dl)	52.6 ± 8.64	46.9 ± 13.24	44.1 ± 13.85*
E.G.R. (A.C.)	1.2 ± 0.26	1.4 ± 0.40	1.6 ± 0.30‡
Selenium (ng/ml)	97.6 ± 15.39	77.3 ± 12.51§	87.6 ± 22.09
Zinc (μg/ml)	0.85 ± 0.15	1.1 ± 0.30	1.0 ± 0.21†

All values are mean ± S.D. Figures in parentheses indicate number of subjects. *$P < 0.02$. †$P < 0.05$. ‡$P < 0.001$. §Trend.

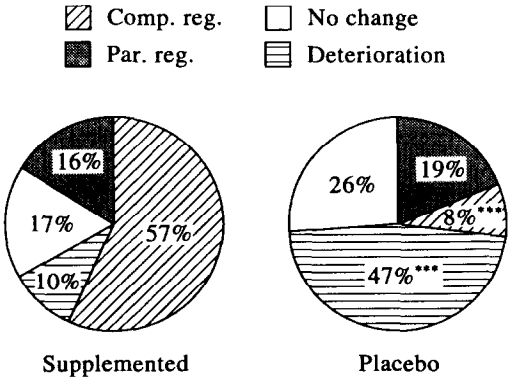

Fig. 3. Clinical response of lesions at the end of 1 year. The complete regression as well as deterioration were significantly different between the groups ($P < 0.001$, Student's proportion *t*-test). Odds ratio = 15.73 (confidence interval 5.55–44.53).

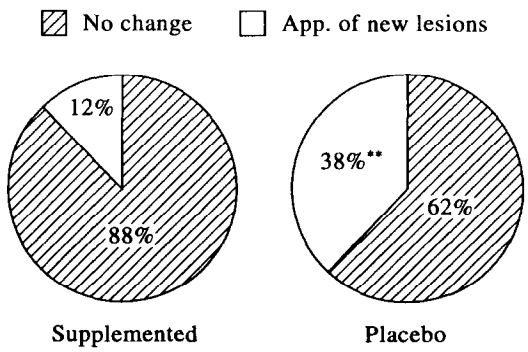

Fig. 4. Clinical response of non-lesion at the end of 1 year. The appearance of new lesions was significantly different between the groups ($P < 0.02$, Student's proportion *t*-test). Odds ratio = 12.19 (confidence interval 3.62–41.08).

correlate with dietary intake. The intervention began after the summer months, which coincided with mango availability in the area, and therefore, vitamin A levels were better before supplements. Blood levels of vitamin A/carotenes often reflect the seasonality of dietary sources of carotenoids. However, the study was completed before the onset of the mango crop and hence we were not able to compare post-treatment values with the basal observations. Unfortunately, analysis of carotenoids which are better markers of intake was not performed. However, if one compared the pre- and post-treatment values, the drop in plasma vitamin A in the supplemented group (12%) was significant as compared to the placebo group (38%; $P < 0.001$).

Vitamin A deficiency and susceptibility to chemically induced neoplasia is well known [26]. Vitamin A is critical for epithelial cell growth and differentiation. Large cohort studies in which blood samples were obtained from healthy individuals suggest a higher risk of lung cancer [27], while others document a relationship between β-carotene and cancer risk [28]. However, synthetic retinoids as preventive/therapeutic agents have gained considerable recognition [29].

Riboflavin appears to have an important role in malignancy particularly in inhibiting formation of carcinogen DNA adducts when given in large doses [30]. Chemopreventive

trials on oesophageal cancer in China suggest a beneficial role in squamous cell carcinoma of the oesophagus [31]. Our findings of a better riboflavin nutriture (low activation coefficient) in the supplemented subjects without lesions, reiterate its role in cancer prevention [32]. Its efficacy can be partially explained by its role in maturation of collagen and wound healing and its therapeutic efficacy in oral mucocutaneous lesions [33, 34].

Even though zinc has attracted considerable attention in the field of cancer, so far no clinical trials have been undertaken. Because of the low doses employed in the present trial, it failed to be reflected in plasma levels. On the other hand, selenium was supplemented in a more easily available form, namely, selenomethionine. However, the doses employed were low in order to avoid toxicity. Hence, though the plasma levels were higher in those without lesions in the supplemented group, it is difficult to comment on the clinical response in relation to plasma selenium levels. Our own case–control study on zinc and selenium levels in oesophageal and oral cancer [35] and other prospective cohort and case–control studies of cancers [36, 37] where nail tissue zinc and selenium were available, suggest an association and causal relationship. Further selenium and zinc might help in building up tissue stores of vitamin A carrier/cellular binding proteins and its metabolism.

Our attempts to measure biological markers of nutrients in general have been of limited value. However, with the remarkable clinical response, one is tempted to conclude that the differences observed in plasma levels in subjects with and without lesions may be coupled with better tissue stores to exert an aggregated and crucial effect at target sites.

The remission of established palatal lesions and the inhibition of development of new lesions in the non-lesion group in the supplemented subjects suggest a plausible curative and protective effect for micronutrients. The doses of micronutrients administered, to begin with, were higher except for zinc and subsequently they were reduced to avoid toxicity. The doses employed were the lowest as compared to all previous studies and raise positive questions regarding dose–response relations and pharmacological effects. The cocktail of nutrients used might have had synergistic effects, even at low doses [38]. The complete response rate of 57% were similar to that observed by Stich and co-workers [39, 40], who administered very high doses of vitamin A in their study (200 000 U/week). In our placebo group, the appearance of new lesions was similar to those observed in the control cohort by Gupta *et al.* [41], in their behaviour intervention studies. Our spontaneous regression rates were, however, higher (8%) as compared to 3% observed by Stich and co-workers.

The odds ratios indicated that on the placebo, the risk of developing new lesions was greater with a very small proportion showing clinical improvement of existing lesions, while on supplementation of nutrients, the opposite was observed.

The results are so striking that even though the study is not randomised and double-blind, they provide strong evidence for micronutrients in cancer prevention. Though we were unable to obtain biopsies and histological evidence, it was obvious that almost all the lesions responded in a similar fashion. It is necessary to point out here that the epithelial dysplasia, as reported by Mehta *et al.* [42], in such lesions is 23%, the highest being in the red areas (52%). The red areas are, therefore, considered to reflect the severe clinical form, the dysplasia being characterised by atrophic epithelium, increased numbers of mitosis and civatte body accumulation.

The pathogenesis of 90% of head and neck cancers is firmly linked to environmental risk factors [43]. The individual susceptibilities appear to be related to not only the intensity and duration of exposure to carcinogen but also to nutrients in the diet [18]. Kellerman *et al.* [44] presented data which suggest that the susceptibility in lung cancer is genetically determined by enzymes such as aryl hydrocarbon hydroxylase. Our own results in the undernourished on activation/deactivation enzymes suggest a greater risk of developing carcinogenic metabolites [45] which form carcinogen–DNA adducts [46]. Further, poor nutritional status may compromise immune competence and increase damage due to peroxidations. The crucial balance between pro- and anti-oxidants might further compound the insults in the poorly nourished individuals. Our results, therefore, emphasize the fact that there is an increased susceptibility to carcinogens in the undernourished segment of the population and a combination of micronutrients has a positive aggregate impact on preneoplastic lesions.

Limitations

Although the results of the study are encouraging, it is necessary to keep in mind the limitations of the study. The study was a straight-trial and not a double-blind study. Although the villages were randomised, the individuals within the group were not randomised. However, the observer bias to a certain extent has been overcome by two clinicians evaluating the clinical response. The number of subjects participating in the trial were small. The data were not analysed with respect to age, as it was difficult to obtain accurate age in this population. Further seasonal variation in the nutrient intake and cross-sectional comparisons have compounded the interpretation of nutritional status.

However, only large-scale studies with factorial designs can provide answers to whether or not single nutrients can have similar effects and provide information on the optimal doses. Dietary intervention may perhaps be the long-term strategy for prevention of cancer. However, such interventions are time consuming, and call for intensive programmes of nutrition education, coupled with agricultural and horticultural interventions. A very recent study by Vijayaraghavan [47] has shown that horticultural intervention can result in increased consumption of antioxidant-rich food groups such as green leafy vegetables and fruits.

Public health action should be directed towards increasing the consumption of dark green and yellow vegetables and fruits which possess a package of protective substances. It is possible to envisage a programme where 'proscription' can be coupled with 'prescription', either dietary or chemoprophylaxis which renders the subject at risk refractory to carcinogens in the environment.

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