Research Article
Altered Trace Element Level and Antioxidant Activity in Whole Blood of Oral Leukoplakia and Cancer patients in Comparison with Healthy Controls
Niharika Swain, Jay Gopal Ray

Abstract
Background: Leukoplakia, the most common oral precancerous lesion is a deleterious consequence of prolonged tobacco intake either in smoke or smokeless form. Apart from the well-known risk factors involved in malignant transformation of such potentially malignant disorder, imbalance in trace element profile and associated oxidative stress has recognizable impact in the process of carcinogenesis. Aim: To evaluate the level of the trace elements (Zinc, Copper, Manganese and selenium) and antioxidant enzyme activity (Superoxide dismutase) in whole blood of leukoplakia and oral cancer patients. Materials and Methods: A case control study comprises of 3 study groups of healthy controls (n=30), oral leukoplakia (n=60) and oral cancer (n=60) patients. Trace element concentration and Superoxide Dismutase enzyme activity in whole blood was measured by Energy Dispersive X-Ray Fluorescence and Photo-spectrometry respectively. The results were analyzed statistically by using One Way-ANOVA and Student’s t tests. Result: The data analysis revealed remarkable alteration in the trace elemental concentration as Zinc and Selenium concentration was lowered in both precancer and cancer patients as compared to that of the controls. Whereas concentration of Copper was progressively and significantly increased from Leukoplakia to cancer group. Manganese showed an inverse pattern in both disease groups. Superoxide Dismutase enzyme activity was significantly (p ≤0.05) lowered in both leukoplakia and cancer patients. Conclusion: The present study highlighted about relationship between alteration in the level of trace elements, oxidative stress and leukoplakia. Concerted efforts would, therefore, help in early detection, management and monitoring the efficacy of available treatment.

Key words: Trace Elements; Whole Blood; Leukoplakia; Squamous Cell Carcinoma; Antioxidant Enzyme; Superoxide Dismutase.

Introduction
Oral cancer is the sixth most common human malignancy and it is a major cause of cancer morbidity and mortality worldwide. The incidence and mortality of oral cancer varies widely across the world. Globally about 500,000 new oral and pharyngeal cancers are diagnosed annually. In south-central Asia, cancer of the oral cavity ranks among the three most common types of cancer. In India, the age standardized incidence rate of oral cancer is reported at 12.6 per 100,000 populations. The development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions. Leukoplakia is the most common precancer representing 85% of such lesions. It has been suggested that a vast majority of oral squamous cell Carcinomas in India arise from pre-existing Leukoplakia. In two studies from India, annual malignant transformation rates of 0.06-0.3% have been reported. In studies from Western countries somewhat higher figures have been mentioned; an annual malignant transformation rate of approximately 1% is probably a reasonable average figure for all types of leukoplakia together. International Agency for Research on Cancer (IARC) conducts focused research on cancer etiology and prevention. Thus providing evidence on global cancer incidence, prevalence, the causes of cancer, mechanisms of carcinogenesis and the most effective strategies for cancer prevention and early detection. In this regard trace elements have been extensively studied in recent years to assess whether they have any modifying effects in the etiology of cancer. Many research works also revealed association of deficiency or imbalance in trace elements in both blood and tissue of cancer patients compared to controls. Trace elements like Zinc (Zn), Copper (Cu), Manganese (Mn) and Selenium (Se) act as...
essential components of antioxidant enzymes. Among one of the best studied antioxidant enzyme, Superoxide Dismutase (SOD) act as first line of defense in living organisms. There are different isoenzymes of SOD. The one present in mitochondria is Mn-dependent and that present in cytoplasm is copper dependent. Diminished amounts of manganese-containing Superoxide Dismutase have been found in all the tumors examined to date. Lowered amounts of the copper-zinc-containing Superoxide Dismutase have been found in many, but not all tumors. Different studies already emphasized that Mn-SOD can act as a tumor suppressing enzyme.

Therefore it is reasonable to assume that variations in blood level of these biochemical markers maybe associated with the pathogenesis of oral cancer. The importance of these elements in cancer was reported by Schwartz which opened the door for new diagnostic and therapeutic endeavors in many areas of medicine and specifically in the areas of oncology. Biochemical alterations of trace element concentration and antioxidant enzyme in the whole blood of such patients can help not only in the early diagnosis, but also in designing various treatment modalities. Hence the present study was carried out to evaluate the level of the trace elements and Superoxide dismutase enzyme activity in oral leukoplakia and oral cancer patients.

Materials and Methods
The present study was carried out in Dr. R. Ahmed Dental College and Hospital, Kolkata, in association with UGC-DAE Consortium for Scientific Research, Kolkata, with the permission of ethical committee. Sixty patients with oral leukoplakia and 60 patients with Oral squamous cell carcinoma (OSCC) with histopathologically proven lesions were included in the study. For comparison thirty normal subjects were also selected. The age group of the patients ranged from 31–50 years. All the subjects were exclusively males and smokers for 10 years (5-8 cigarette/day). Smokeless tobacco associated lesions were not included in the study because of small sample size. The methods used in the study were:

1) Determination of trace elemental status by Energy Dispersive X-Ray Fluorescence (EDXRF) method a highly sensitive, non-destructive and multi-elemental technique.

2) Determination of Superoxide Dismutase enzyme activity by photospectrometry. The principle of this method is based on the oxidation of reduced form Nicotinamide adenine dinucleotide (NADH), mediated by Superoxide radical in a purely chemical system. Coenzyme oxidation occurs in the presence of suitable concentration of Ethylenediaminetetraacetic acid (EDTA), Mn and Mercaptoethanol through a free radical chain of reactions involving thiol oxidation and univalent O₂ reduction. The addition of SOD to the reaction mixture causes a proportionate inhibition of the rate of NADH oxidation, thus confirming the involvement of superoxide ion in the process and providing the basis for SOD activity determination.

Finally the results obtained were carefully recorded, analyzed by using One Way-ANOVA and Student’s-t statistical tests, corroborated and evaluated in reference to aims and objectives of the study.

Results
Trace element variation in precancer and cancer group
Elemental profile of blood of Leukoplakia and oral Carcinoma patients is illustrated in Table 1. In these patients significant (p ≤0.05) depletion in concentrations of Zn (Graph 1), Mn (Graph 2) was observed in comparison to that of normal individuals.

Concentration of Se in blood of patients showed a trend of gradual depletion from control to disease groups but the depletion was statistically non-significant. Mn showed an inverse pattern when the values in blood were compared with each other as shown in Graph 2. Mn reflected a slight increase in malignant patients in contrast to significant decrease in leukoplakia patients. Blood of patients with leukoplakia and malignancy showed significant (P ≤0.05) increase in concentration of Cu when compared to that of controls. The elevation in concentration in Copper showed a linear progression from precancer to Cancer group (Graph 2).

SOD enzyme activity
Data of the present investigation showed a significant (P ≤0.05) decrease in Superoxide Dismutase activity in both leukoplakia and carcinoma patients revealing the underlying oxidative stress as shown in Graph 3.
Table 1: Comparison of concentration of Zn, Cu, Mn & Se in blood samples of controls, leukoplakia & carcinoma patients. [ppm=parts per millennium, *=Significant at 5% level (p < 0.05), "C"= Control, **=Significant at 1% level (p <0.01), "LKP" for Leukoplakia, ***=Significant at 0.1% level (p < 0.001), "CA"=Carcinoma, n.s=Not Significant, M=Mean and SD=Standard Deviation]

<table>
<thead>
<tr>
<th>Element</th>
<th>Control M±SD (ppm)</th>
<th>LKP M±SD (ppm)</th>
<th>CA M±SD (ppm)</th>
<th>t- value C - LKP</th>
<th>t- value LKP-CA</th>
<th>t-value C- CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>67.49±3.894</td>
<td>59.64±6.475</td>
<td>64.95±2.893</td>
<td>3.617*</td>
<td>1.332 n.s</td>
<td>-0.919 n.s</td>
</tr>
<tr>
<td>Cu</td>
<td>6.302±1.247</td>
<td>6.473±0.863</td>
<td>7.805±0.155</td>
<td>4.79*</td>
<td>2.28*</td>
<td>1.604 n.s</td>
</tr>
<tr>
<td>Mn</td>
<td>3.143±0.368</td>
<td>1.282±0.786</td>
<td>3.16±0.32</td>
<td>10.69**</td>
<td>3.11*</td>
<td>0.996 n.s</td>
</tr>
<tr>
<td>Se</td>
<td>2.033±0.725</td>
<td>1.68±0.621</td>
<td>0.71±0.39</td>
<td>2.81 n.s</td>
<td>-0.855 n.s</td>
<td>-1.977 n.s</td>
</tr>
</tbody>
</table>

Discussion

Data of our present study designed to evaluate the correlation between elemental alteration and pathogenesis of precancerous lesion. As described in the previous section a significant depletion in Zinc titre in blood of both disease (leukoplakia and carcinoma) groups was observed, which further establishes essentiality of this element. Few but some studies already cited about low zinc level in serum of potentially premalignant condition like in oral leukoplakia\(^ {13}\) and in oral carcinoma patients also. In our study, zinc value was significantly reduced as compared to control in blood of both leukoplakia and carcinoma affected patients. Zinc is also thought to function as an antioxidant through a number of proposed mechanisms. Zinc is a cofactor for Cu-Zn Superoxide Dismutase, part of the primary antioxidant system of vertebrates.\(^ {14}\)

Selenium, an essential trace element, has immunomodulating and antiproliferative properties and can influence immune response by changing the expression of cytokines and their receptors or making immune cells more resistant to oxidative stress.\(^ {15}\) It exhibits an antiproliferative effect modulating cellular proliferation in G1 phase in both normal and neoplastic cells impairing the expression of c-fos and c-myc oncogenes and can also induce apoptosis in keratinocytes because of their pro-oxidant catalytic activity.\(^ {16}\) Various workers have reported reduced selenium concentration in cancer patients. In several case–control studies, low serum or plasma Se concentrations were found to be associated with increased risk of malignant oral cavity lesions\(^ {16,17}\), esophageal and gastric cancers\(^ {18}\), cervical cancer\(^ {19}\), and ovarian cancer.\(^ {20}\) In our study Se showed a small change (depletion) in blood of younger patients affected by both leukoplakia and
Copper participates in the cell metabolism, being present in various enzymes such as tyrosinase, uricase and cytochrome oxidase, mainly those concerned with oxidation. Margalith et al suggested that role of copper ions in biological damage is caused by superoxide radicals or other reducing agents such as ascorbate, which reduce the copper complex. These complexes react with hydrogen peroxide to form hydroxyl radicals that cause damage to protein, RNA and DNA that are not repairable by cellular mechanisms thus initiating the malignant process. A gradual increase of serum Copper level from precancer to the cancer group as compared to normal was already evident. In our study the Cu was significantly elevated in blood affected by both leukoplakia and carcinoma.

Manganese is both a constituent and an activator of several enzymes and proteins in plants, animals and humans. The three primary manganese metalloenzymes in mammals are manganese superoxide dismutase (Mn SOD), pyruvate carboxylase and arginase. In this study leukoplakia patients had significant lower concentration of manganese level in blood when compared to normal individual and carcinoma patients. The depletion in patients with precancerous lesion probably reflects the system’s need to utilize the specific specialist role of Mn in removing the free radicals (i.e. superoxide ion). Increase in the Mn may be the result of the cancerous condition itself which may be considered as a positive-feedback response to fight the oxidative stress and to maintain the homeostasis.

A significant depletion in SOD enzyme activity was detected in both leukoplakia and carcinoma patients which may be contributed to increasing load of free radicals in body due to chemical carcinogens present in tobacco smoke. As SOD which scavenges O_2, and catalase which detoxifies H_2O_2 are amongst the important enzymes present intracellularly, so this depletion could be corroborated to exhaustion of the enzyme.

Conclusion
The trace elemental estimation done in the present study showed the altered level of these elements in blood of patients with leukoplakia and carcinoma and gave us the idea about necessary elements to be given as supplements to counteract the oxidative stress induced by free radicals which can cause serious damage to cells. The antioxidant enzyme SOD activity was found to be decreased in leukoplakia and carcinoma patients who have higher level of oxidative stress due to declined concentration of the trace elements. Thus, the present study enlightens the possible relationship between trace element levels, oxidative stress and both pathogenesis and malignant transformation potential of leukoplakia and it will act as a guideline for further studies involving larger number of samples.

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