RESEARCH ARTICLE

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# Selenium Influences Trace Elements Homeostasis, Cancer Biomarkers in Squamous Cell Carcinoma Patients Administered with Cancerocidal Radiotherapy

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## Abstract

In the perspective of selenium as an antioxidant and anti-carcinogen, so far no strong intervention trials with selenium over radiation-treated oral squamous cell carcinoma cases have been conducted, to examine the response of the disease and the subsequent biochemical alterations. In the present study, untreated oral cancer cases (Gp II) were compared with radiation-treated groups with and without selenium (Gp IIa, IIb), forward to find the trace elements and cancer biomarkers status, at a follow-up of 6 months. Severe alteration in the trace elements levels of Se, Cu, Fe, Zn, Na, K, Ca, Cl, were noticed in Gp II. Though Gp IIa showed slight improvement, administration of selenium (Gp IIb) improved the level of all these elements to a greater extent ($p<0.001$). GpII and IIa showed increased level of bio markers 5'-nucleotidase, PschE, LAP, γ-GTP, LDH, SGOT, SGPT, ACP, ALP, CPK, TNF, CEA, AFP, Scc-Ag. The greater extent of restitution to near normalcy was observed in patients given selenium (Gp IIb) ($p<0.001$). Owing to the fact that selenium scavengers oxidants and hence decelerate carcinogenesis by eliminating tumors, so the tumor released constituents into the systemic circulation declined significantly. Therefore, the outcome of the study suggests selenium as a valuable therapeutic measure as adjuvant for oral cancer patients undergoing cancerocidal radiotherapy.

**Keywords:** Trace elements- cancer biomarkers- selenium- oral cancer- oxidative stress

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## Introduction

Impaired antioxidant defense system observed in cancer patients of multiple sites reflects the excessive free radical production. Radiation toxicity cause enzyme deficiency in radiation-treated cancer patients who may render the system inefficient to manage the free radical attack (Schreurs et al., 1985; Halliwell and Gutteridge, 1990; Sabitha and Shyamaladevi, 1999). Some nutrients alter cancer incidence and growth, acting both as an antioxidant and anti-carcinogens. Foregoing researchers on one of the nutritional anticarcinogen is selenium. Several forms of selenium enter the body as part of amino acids within proteins (Maschos et al., 1998). Selenium compounds which are effectively absorbed in the gastrointestinal tract had been hypothesized to enter red blood cells via diffusion and carried throughout the body (Ganther 1987, Swati et al., 1992). Dietary selenium as in the form of selenite gets incorporated with GPx (Rotruck et al., 1973; Fleming et al., 2001). Excess oxidative stress is implicated in almost all stages of oral cancer development, particularly stage III and IV. In addition to increased flux of oxy-radicals and loss of cellular redox homeostasis observed in cancer conditions, radiation mediated free radical also ensures oxidative stress. Scavenging ROS may hence decelerate carcinogenesis to a certain extent (Feig, 1994; Rahman, 2007). Based on this criteria the present study set out to evaluate the antioxidant and anticarcinogenic potential of selenium in oral cancer patients undergoing radiotherapy, by analyzing the extent of its influence over the level of cancer biomarkers and trace elements. Proliferation and metabolic rates of the tumor cells being higher than most normal cells, the rate of shedding of plasma membrane constituents into the circulation of a tumor-bearing host would also be expected to be higher. So for no strong intervention trials with selenium (both organic and inorganic forms) in radiation-treated oral SCC patients have been conducted, to explore the state of malignancy by assessing metabolic biomarkers and trace elements alterations, to examine the response of the disease.

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## Material and Methods

*Experimental Design*

Oral squamous cell carcinoma patients (Stage III with corresponding Tumor Nodal Metastasis [TNM] classification), who volunteered for being into the present study were categorized into Group I -Normal Healthy individuals; Group II -Untreated oral cancer patients; Patients under Group II were classified into: Group IIa -Radiation-treated oral cancer patients; Group IIb-Radiation-treated oral cancer patients supplemented with selenium; Group IIa followed at 2nd (Group II a1), 4th (Group II a2) and 6th (Group II a3) month; Group IIb at 2nd (Group II b1), 4th(Group II b2) and 6th (Group II b3) month. Radiotherapy was given with a telecobalt beam using anterior and lateral wedge pair or lateral parallel portals (Theratron – 780 – $^{60}$CO; Phoenix – $^{60}$CO; Gammatron – $^{60}$CO) at a dosage of 6000 cGy (200 cGy/day) in five fractions per week for a period of six weeks. Selenium (sodium selenite) was supplemented orally (capsule) after cessation of radiotherapy, at a dosage of 400 µg/day for 6 months. Treatment schedule and selenium supplementation of the patients were carefully recorded and monitored.

*Source of Chemicals*

Sodium selenite capsules procured from Cassel research laboratories, Chennai, India. All the antibodies and chemicals of analytical grade were purchased from Amershan pharmacia Biotech Europe GmbH, Freiburg, Germany; National Institute of Immunology, New Delhi India; Dr.M.G.R. Medical University, Chennai, India; Sigma Chemical Company, USA ; Life Technologies, USA.

Blood samples were collected after overnight fast. The control group consisted of healthy volunteers who were age, sex matched to the radiotherapy groups. Group II had their blood samples taken immediately before radiotherapy. In both selenium supplemented and non- supplemented group blood samples were collected after the end of radiotherapy with follow-up after 2nd, 4th and 6th month. Cancer tissue samples were obtained at the end of the 6th month.

*Estimation of Trace elements*

Selenium concentration was detected by fluorometric method (Olson et al., 1975). Minerals were estimated by atomic absorption spectrometry (Perkin – Elmer Model 2380). Estimation of Copper, Iron, Zinc, Sodium and potassium, Calcium, Chloride level were done according to the procedure described elsewhere (Jha et al., 1985; Thomas et al., 2013; Rogers et al., 1991; Jackson 1973, Sendroy 1944, King, DS Bain 1951).

*Estimation of BioMarkers*

The activity of 5'-Nucleotidase (Luly et al., 1972) pseudo choline esterase (Sur et al., 1987) Leucine aminopeptidase (Samorojski, 1962) γ-glutamyl transpeptidase (Orlowshi and Meister, 1965) lactate dehydrogenase (King, 1965) Aspartate aminotransferase, Alanine aminotransferase (King,1965). Acid phosphatase, Alkaline phosphatase (King,1965). Creatine phosphokinase (Okinaka et al., 1961) were analysed as per procedures given elsewhere. The isoenzymes pattern of lactate dehydrogenase was separated gel electrophoresis (Dietz and Lubrano 1967). The levels of tissue GST-π was quantified by ELISA with a modified version (Sharma and Singh, 1989). The level of serum TNF-α was measured by using ELISA as described earlier using mouse monoclonal antibody against human TNF-α (1:5,000 dilution) and Goat-anti mouse-IgG – HRP conjugated.Carcino Embryonic Antigen (CEA) by the immuno blotting method (Pappas et al., 1983). Quantitation of CEA by ELISA done using ELISA as described earlier using mouse monoclonal antibody against human CEA (1:1,000 dilution) and Goat antimouse IgG HRP conjugated. Alpha Feto Protein (AFP) by the method of immunobinding assay (Hawkes et al., 1982). The level of AFP was measured using ELISA as described earlier using mouse monoclonal antibody against human AFP (1:1000 dilution) and goat-antimouse-IgG-HRP conjugated (1:5000). The levels of squamous cell carcinoma – Antigen (Scc-Ag) was quantified by the method of Electro Immuno diffusion (Rocket Immuno Electrophoresis) (Denis, 1979).

*Statistical analysis*

Statistical analysis of all the study groups was done by students t-test (The levels of significance were evaluated as $p<0.001$). For each group the corresponding values were expressed as mean ± SD. Group II was compared with Group I. Group IIa patients were compared with Group II. Group IIb was compared with Group IIa.

## Results

*Trace elements*

Figure 1 picture the selenium status in plasma of normal, untreated oral cancer, radiation-treated and selenium supplemented patients. Selenium level was found to be drastically decreased with the severity of the disease in oral cancer cases (Group II) in comparison to normal and in radiation-treated group there were no significant changes until 6 months (Compare Group II with Group II a1, a2, a3). Administration of selenium improved the selenium levels in the systemic and consistent manner at the end of 2nd, 4th month by 43% and 53%. A high significance was observed at the end of the 6th month by 64% (Compare Group II b1,b2,b3 with Group II a1, a2, a3).

Based on the results showing high significance at 6 months, the studies carried out at 6 months follow-up of radiation and selenium supplemented groups (Group II a3 and Group II b3) were only presented in this paper, for the following parameters. Table 1 exhibits the levels of essential elements of normal, untreated oral cancer, radiation-treated and selenium supplemented groups. In comparison to normal, untreated oral cancer patients (Group II) showed much lesser levels of essential elements zinc, sodium, chloride and higher levels of copper, iron, potassium and calcium. Following radiation zinc ($p<0.001$), sodium ($p<0.01$) and chloride ($p<0.001$) showed increase while copper, iron, potassium, calcium

Table 1. Level of Essential Elements Copper, Iron, Zinc, Sodium, Potassium, Chloride, Calcium in Serum of Normal, Untreated Oral Cancer, Radiation Treated and Radiation Treated + Selenium Supplemented Oral Cancer Patients

| Essential Elements | Normal (Group I) | Untreated Oral Cancer (Group II) | Radiotherapy alone 6 months (Group II $a_3$) | Radiotherapy + Selenium supplemented groups 6 months (Group II $b_3$) |
|---|---|---|---|---|
| Copper | 106.2 ± 12.3 | $154.6 \pm 16.4^{x\$}$ | $136 \pm 15.9^{y_3\#}$ | $120.7 \pm 13.4^{z_3\#}$ |
| Iron | 128.6 ± 13.5 | $194.6 \pm 22.1^{x\$}$ | $171 \pm 19^{y_3\#}$ | $159.7 \pm 17.7^{z_3\#}$ |
| Zinc | 98.1 ± 10.7 | $50.4 \pm 5.3^{x\$}$ | $64 \pm 8^{y_3\$}$ | $78.6 \pm 8.2^{z_3\$}$ |
| Sodium | 148.0 ± 15.4 | $93.2 \pm 9.8^{x\$}$ | $104 \pm 11.5^{y_3\#}$ | $120 \pm 12.6^{z_3\#}$ |
| Potassium | 4.5 ± 0.52 | $7.2 \pm 0.73^{x\$}$ | 6.5 ± 0.92 | $5.6 \pm 0.61^{z_3\#}$ |
| Chloride | 97.8 ± 9.8 | $56.4 \pm 5.8^{x\$}$ | $66 \pm 7.3^{y_3\$}$ | $77.3 \pm 8.1^{z_3\$}$ |
| Calcium | 9.4 ± 1.2 | $14.7 \pm 1.7^{x\$}$ | $13 \pm 1.6^{y_3\#}$ | $12 \pm 1.3^{z_3*}$ |

Copper, Zinc - mg/dl; Iron - µg/dl; Sodium, Potassium, Chloride, Calcium - mEq/L, Group I – Normal; Group II – Untreated oral cancer patients; Group II $a_3$ – Radiation treated – 6 months, Group II ,$b_3$ – Radiation treated + selenium supplemented – 6 months , Values are expressed as mean ± SD , x- Group II compared with Group I; $y_3$-Group IIa3 compared with Group II;$z_3$- Group IIb3 compared with Group IIa3. Statistical significance are expressed as $^*$ p<0.05; $^\#$p<0.01; $^\$$p<0.001

showed decreased level (p<0.01), (p<0.01), NS, (p<0.01) respectively, after 6 months compared to untreated and oral cancer cases (Group IIa3 versus Group II) In comparison to radiation group selenium supplementation (Group II b3) increased the levels of zinc (p<0.001), sodium (p<0.01), chloride (p<0.001) decreased the levels of Cu (p<0.01), Fe (p<0.01), K (p<0.01) and Ca (p<0.05) after 6 months of selenium intake.

*Bio Markers*

Table 2 shows the activity of biomarkers in serum of normal, untreated oral cancer, radiation-treated and selenium supplemented groups. Untreated oral cancer cases, when compared with normal, showed increased activity of all markers namely 5' nucleotidase, LAP, PschE, gamma GTP, LDH, SGOT, SGPT, ACP, ALP and CPK (p<0.001). Following 6 months of post-irradiation

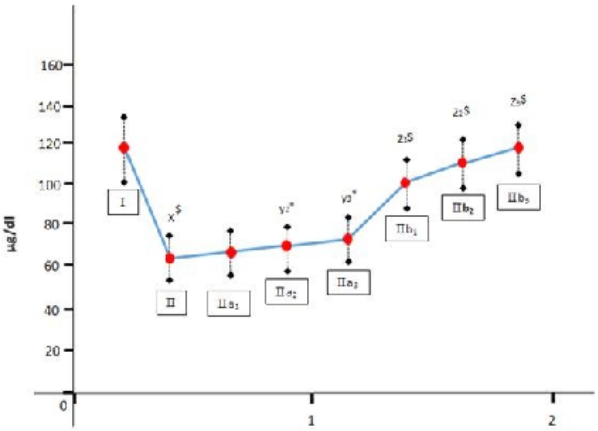



Figure 1. Plasma selenium level in normal and study groups.Group I – Normal, Group II – Untreated oral cancer patients, Group II a1, a2, a3 – Radiation treated – 2,4,6 months respectively, Group II b1,b2,b3 - Radiation treated + selenium supplemented - 2,4,6 months respectively, Values areexpressed as mean ± SD, XGroup II compared with Group I; $y_1$Group IIa1 compared with Group II; $y_2$ Group IIa2 compared with Group II; $y_3$ Group IIa3 compared with Group II; $z_1$Group IIb1 compared with Group IIa1; $z_2$Group IIb2 compared with Group IIa2; $z_3$Group IIb3 compared with Group IIa3, Statistical significance are expressed as $^*$p <0.05; $^\#$p <0.01; $^\$$p< 0.001

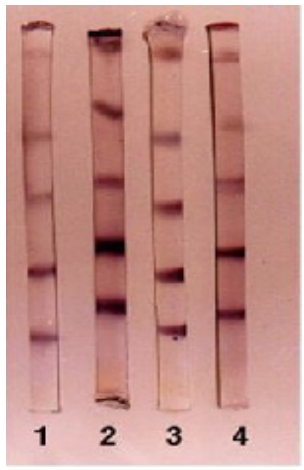



Figure 2. Isoenzyme Pattern of Serum Lactate Dehydrogenase in Normal and Study Groups, Lane 1- Normal, Lane 2 – Untreated Oral Cancer, Lane 3- Radiation Treated, Lane 4 – Radiation + Selenium Treated

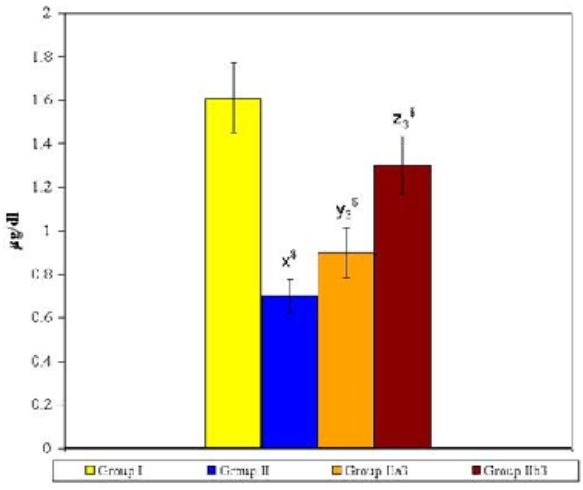



Figure 3. Quantitation of Tissue GST-π Content by ELISA in Normal and Study Groups. Group I – Normal, Group II – Untreated oral cancer patients, Group II $a_3$ – Radiation treated – 6 months, Group II $b_3$ - Radiation treated + selenium supplemented - 6 months, Values areexpressed as mean ± SD, XGroup II compared with Group I; $y_3$ Group IIa3 compared with Group II; $z_3$Group IIb3 compared with Group IIa3, Statistical significance are expressed as $^*$p <0.05;$^\#$p <0.01; $^\$$p< 0.001

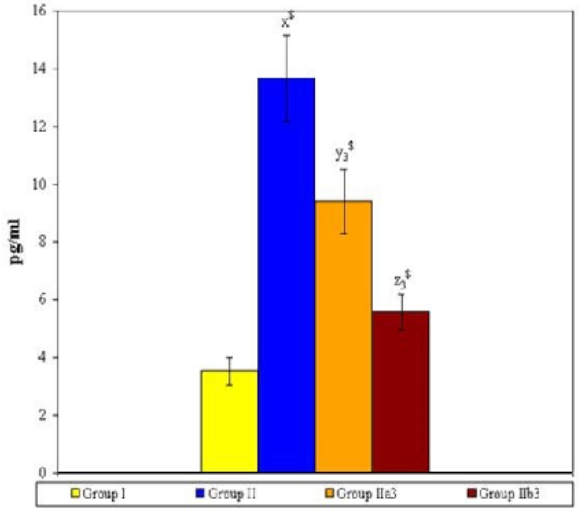



Figure 4. Serum Tumor Necrosis Factor TNF- concentration in Normal and Study Groups. Group I – Normal, Group II – Untreated oral cancer patients, Group II $a_3$ – Radiation treated – 6 months, Group II $b_3$ - Radiation treated + selenium supplemented - 6 months, Values areexpressed as mean ± SD, XGroup II compared with Group I; $y_3$ Group IIa3 compared with Group II; $z_3$Group IIb3 compared with Group IIa3, Statistical significance are expressed as $^{*}p<0.05$;$^{\#}p<0.01$; $^{\$}p< 0.001$

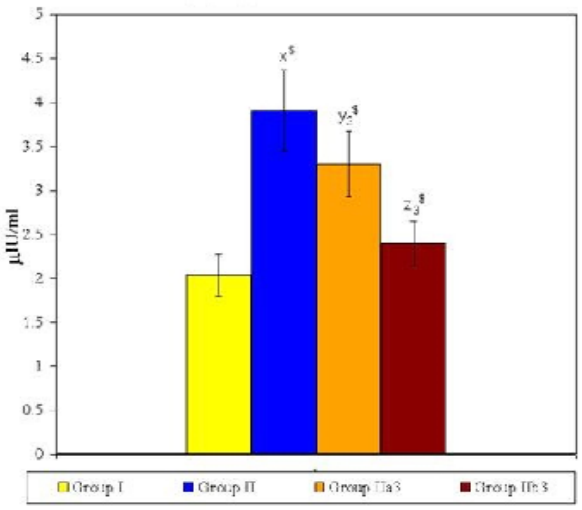



Figure 5. Levels of Carcino Embryonic Antigen (CEA) in Serum of Normal and Study Groups. Group I – Normal, Group II – Untreated oral cancer patients, Group II $a_3$ – Radiation treated – 6 months, Group II $b_3$ - Radiation treated + selenium supplemented - 6 months, Values areexpressed as mean ± SD, XGroup II compared with Group I; $y_3$ Group IIa3 compared with Group II; $z_3$Group IIb3 compared with Group IIa3, Statistical significance are expressed as $^{*}p<0.05$;$^{\#}p<0.01$; $^{\$}p< 0.001$

period the activity of above enzymes was found to be decreased ($p<0.01$) (Compare Group II a3 with Group II). Selenium supplementation brings the activities of these enzymes close to normal levels at 6 months ($p<0.001$).

Figure 2 demonstrates the pattern of LDH isoenzyme of normal, untreated oral cancer, radiation-treated and selenium supplemented groups. The pattern demonstrates a clear trend towards an increase in the levels in untreated group (Group II) when compared to normal (Group I). Comparing isoenzyme pattern of LDH isoenzymes the expression of band 4 (LDH 4) and 5 (LDH 5) was more in the untreated cases suggesting its membrane leakage due to oxidative stress. A slight decrease in the expression of LDH 4 and LDH 5 was observed in radiation-treated group (compare Group II a3 versus Group II) and this became non-discernible in selenium supplemented groups (Group II b3 compared with Group II a3).

Figure 3 denotes the tissue GST - π level in normal, untreated oral cancer, radiation-treated and selenium supplemented groups. Cancer tissue from untreated oral cancer cases (Group II) showed a marked decrease of GST by 155%. In comparison to untreated oral cancer groups (Group II) there was a significant increase by 27% in radiation-treated group while a remarkable increase by 27% was observed following selenium supplementation

Table 2. Activities of Serum Marker Enzymes 5'-Nucleotidase, PschE, LAP, γ-GTP, LDH, SGOT, SGPT, ACP, ALP and CPK of Normal, Untreated Oral Cancer, Radiation Treated and Radiation Treated + Selenium Supplemented Oral Cancer Patients

| Marker Enzymes | Normal (Group I) | Untreated Oral Cancer (Group II) | Radiotherapy alone 6 months (Group II $a_3$) | Raditotherapy + Selenium supplemented group 6 months (Group II $b_3$) |
|---|---|---|---|---|
| 5'Nucleotidase | 7.8 ± 0.92 | $14.1 \pm 1.16^{x\$}$ | $12.8 \pm 1.6^{y_3\#}$ | $9.3 \pm 1.1^{z_3\$}$ |
| Pseudo choline esterase (PschE) | 574 + 61 | $321 \pm 39^{x\$}$ | $368 \pm 57^{y_3\#}$ | $497 \pm 55^{z_3\$}$ |
| Leucine amino peptidase (LAP) | 9.2 ± 1.2 | $12.8 \pm 1.4^{x\$}$ | $11.8 \pm 1.2^{y_3\#}$ | $10.3 \pm 1.3^{z_3\$}$ |
| γ-Glutamyl trans peptidase (γ-GTP) | 10.6 ± 1.3 | $19.3 \pm 2.2^{x\$}$ | $17.7 \pm 1.8^{y_3\#}$ | $15.1 \pm 1.7^{z_3\$}$ |
| Lactate dehydrogenase (LDH) | 44.7 ± 5.3 | $83.1 \pm 9.2^{x\$}$ | $77.2 \pm 8.9^{y_3*}$ | $66.3 \pm 7.5^{z_3\$}$ |
| Asparate transaminase (SGOT) | 12.1 ± 1.4 | $17.3 \pm 2.1^{x\$}$ | $15.8 \pm 1.8^{y_3\#}$ | $13.7 \pm 1.5^{z_3\$}$ |
| Alanine transaminase (SGPT) | 14.6 ± 1.73 | $19.7 \pm 2.3^{x\$}$ | $18.1 \pm 1.8^{y_3\#}$ | $15.9 \pm 1.8^{z_3\$}$ |
| Acid phosphatase (ACP) | 143 ± 16.2 | $197 \pm 20.1^{x\$}$ | $181 \pm 19.5^{y_3\#}$ | $162 \pm 16.3^{z_3\$}$ |
| Alkaline phosphatase (ALP) | 61.7 ± 6.3 | $84.7 \pm 8.6^{x\$}$ | $78.4 \pm 8.3^{y_3\#}$ | $70.2 \pm 7.1^{z_3\$}$ |
| Creatinine phosphokinase (CPK) | 17.5 ± 2.3 | $29.2 \pm 3.2^{x\$}$ | $26.5 \pm 3.5^{y_3\#}$ | $22.3 \pm 2.1^{z_3\$}$ |

5' Nucleotidase, LAP, γ-GTP, LDH, SGOT, SGPT, ACP, ALP, CPK - IU/L, PschE – µmoles/min/mg protein, Group I – Normal; Group II – Untreated oral cancer patients; Group II $a_3$ – Radiation treated – 6 months, Group II $b_3$ – Radiation treated + selenium supplemented – 6 months ,Values are expressed as mean ± SD , x- Group II compared with Group I; y3-Group IIa3 compared with Group II;$z_3$- Group IIb3 compared with Group IIa3. Statistical significance are expressed as $^{*}p<0.05$; $^{\#}p<0.01$; $^{\$}p<0.001$

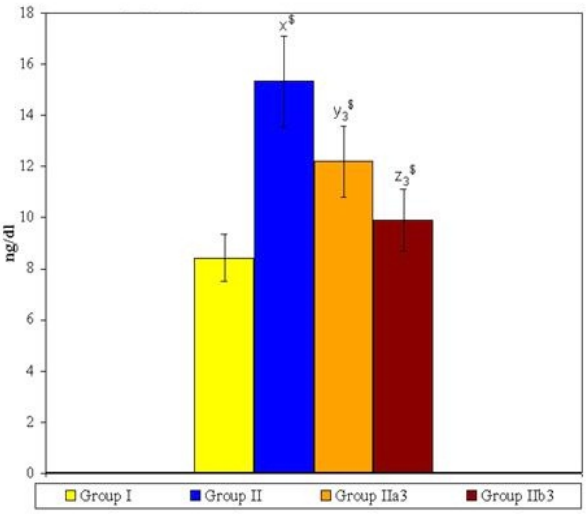



Figure 6. Levels of Alpha Feto Protein (AFP) in Serum of Normal and Study Groups. Group I – Normal, Group II – Untreated oral cancer patients, Group II $a_3$ – Radiation treated – 6 months, Group II $b_3$ - Radiation treated + selenium supplemented - 6 months, Values areexpressed as mean ± SD, XGroup II compared with Group I; $y_3$ Group IIa3 compared with Group II; $z_3$Group IIb3 compared with Group IIa3, Statistical significance are expressed as $^{*}p < 0.05$;$^{\#}p < 0.01$; $^{\$}p < 0.001$

for 6 months (compare Group IIb3 with Group IIa3).

Figure 4 represents the level of tumor necrosis factor in serum of normal untreated oral cancer, radiation-treated and selenium supplemented groups. Untreated oral cancer cases (group II) showed a fourfold increment in the level of serum TNF than normal. The decrease of 31% in TNF level was noticed in radiation-treated group (Group II a3) than the untreated group. Selenium supplemented group showed suppressed the level of the TNF by 41% ($p<0.001$) (Compare Group II b3 with Group II a3).

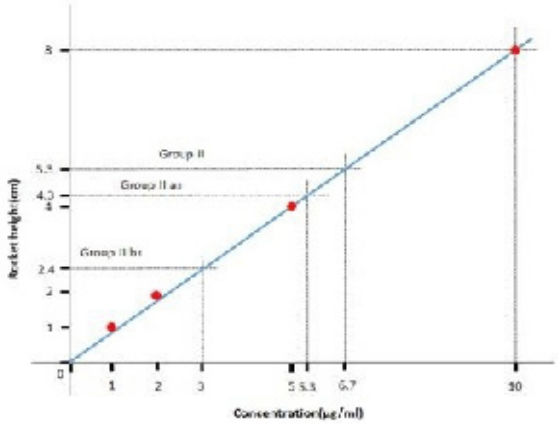
Figure 5 shows the level of CEA of normal, untreated oral cancer, radiation-treated and selenium supplemented groups. Around 100% elevation of CEA level was observed in untreated cases compared to normal. In comparison to untreated cases, the radiation group showed a significant decrease by 16% at 6 months, while selenium supplemented group showed a further decline by 27% in the CEA levels at 6 months when compared to radiation group (Compare II b3 with Group II a3).

Figure 6 denotes the level of AFP of normal, untreated oral cancer, radiation-treated and selenium supplemented groups. Elevation in the levels of AFP by 82% was observed in untreated cases compared to normal (Group II versus Group I). In radiation group the increased level of this marker enzyme in comparison to untreated cases showed a 20% decrease (Group II a3). Selenium supplemented group showed a decline in the AFP levels after 6 months by 19% (Group II b3 compared with Group II a3).

Figure 7a and 7b illustrates the level of squamous cell carcinoma antigen (SCC-Ag) of normal, untreated oral cancer, radiation-treated and selenium supplemented groups. Untreated oral cancer patients showed increased levels of SCC-Ag (5.3 cm) (Group II), when compared with normal (Group I).The radiation group showed


7a

Figure 7a. Rocket Electrophorogram of Squamous Carcinoma Cell Antigen (Scc-Ag) in Normal and Study Group. A – Normal, B – Untreated oral cancer, C – Radiation Treated, D – Radiation + Selenium Treated




7b

Figure 7b. Quantitation of Serum Squamous Cell Carcinoma Antigen (Scc-Ag) in Normal and Study Groups. Group I – Normal, Group II – Untreated oral cancer patients, Group II a3 – Radiation treated – 6 months, Group II b3 - Radiation treated + selenium suppl mented - 6 months, Group II compared with Group I; Group IIa3 compared with Group II; Group IIb3 compared with Group IIa3

a decrease at 6 months (4.3 cm) (Group II a3) in comparison to untreated cases. Much more decrease was observed in selenium supplemented group (2.4 cm), when compared with radiation group (Group II b3 compared with Group II a3).

## Discussion

*Essential elements homeostasis*

Selenium, a trace element present in the active site of the enzyme GPx which metabolizes hydroperoxides is the most essential in the antioxidant defense system. Selenite undergoes thiol dependent reduction (Ganther, 1987) before being incorporated with selenocysteine in the synthesis of the specific seleno proteins (Stadtman, 1996). The reduction in selenium status in oral cancer patients (Figure 1) (Group II) might be due to low levels of circulating selenium. With no improvement in selenium

levels in radiation groups (Figure 1) (Group II a) yet a drastic increase in selenium level was noticed in selenium supplemented groups in a duration-dependent manner of 2,4 and 6 months (Figure 1) (group II b). Increasing the selenium status of the patients, scavenge free radicals directly or that interferes with the generation of free radical mediated events and thus, inhibit the neoplastic process (Cerutti, 1985; Hoppe et al., 1996).

Statistically significant alterations of Copper (Cu), Iron (Fe), Zinc (Zn), Sodium (Na), Potassium (K), Calcium (Ca), Chloride (Cl) levels have been observed in oral cancer patients (Table 1). Cu and Fe components of various oxidation reduction enzymes play an important role in the metabolism of radicals in vivo. Selenium therapy gradually decreased these levels, since the selenium quenching the free radical greatly with redox enzymes. Zn deficiency does impair the structure of oral epithelium and promote carcinogenesis (Rogers et al., 1991). The impact of radiation modifying the Zn content after 6 months of RT may probably due to the decrease in the DNA polymerases which regulates cell proliferation. Selenium supplementation increased Zn levels, regulates the permeability of cell membranes. The significant increase in serum Na level in oral cancer patients following selenium supplementation might be due to increased Na pump activity. Increased level of serum K in cancer condition may be due to the release of K from the intracellular fluid, which seem to have the correlation with erythrocyte deformability and hemolysis which is associated with Zn, selenium, vitamin C and E deficiencies. Selenium supplementation significantly decreased the K levels and this might be probably due to the maintenance of Na and K homeostasis by selenium. The decrease in chloride level in cancer patients attributes to the modulation of chloride shift (Schroeder et al., 1970). Moderate modulation in chloride shift observed in radiation groups was found to reverse to near normal in the selenium group. The increased calcium level in cancer and radiation-treated patients might be due to alterations in cellular ion homeostasis and the invasion of the cancerous cell (metastasis) to the bone. Selenium supplementation reverted back the altered levels to near normalcy and thus maintained homeostasis. Its intercycle relation with other elements was reasoned out for the observed modulations.

*Bio Markers*

Classically, a marker is synthesized by the tumor and released into the circulation, but it may also be produced by normal tissues in response to invasion by cancer cells. The quantity of tumor marker should directly reflect the bulk of malignancy (Kurokawa et al., 1997). 5'-nucleotidase is an established plasma membrane marker in many mammalian cells (Chatterjee et al., 1981; Rosi et al., 1998). The increased levels of 5'-nucleotidase observed in the sera of oral cancer and radiation-treated patients (Table 2) may be due to damage to the cancer cell (oral squamous) causing leakage of 5'-nucleotidase into the extracellular fluid and thence into circulation. Selenium supplementation afforded protection to the plasma membrane which is evident through the lesser activity of 5'-nucleotidase. Pseudo choline esterase (PschE) has a possible role in calcium transport membrane rigidity and lipoprotein metabolism. Increased PschE in post-irradiation period might be due to the subsiding effect of free radical mediated toxicity (Group IIa3) (Table 2). Selenium group (Group IIb3) showed a spontaneous increase in PschE activity indicating the protective role of selenium against membrane rigidity. The elevated proteolytic enzyme leucine amino peptidase (LAP), in cancer and with little decrease in radiation group may result from the passage of enzyme from damaged or altered cells into the plasma. Selenium reduced these levels probably by preserving these cells from damage. Increased γ-GTP activity in carcinogen altered cells as an adaptive part of the response of the cell to unfavorable chemical exposure, by increasing intracellular levels of glutathione. Selenium group (Group IIb) showed less γ-GTP activity level indicating its effect over carcinogen altered cells. LDH enzyme that functions to oxidize pyruvate to lactate in the final step of the glycolytic pathway showed an increased LDH levels in cancer cases (Group II) (Table 2). Significant difference observed with radiation groups (Group IIa) indicates that as long as tumor activity is there, the total LDH activity of the tumor (and its reflection in the serum) does not diminish despite radiotherapy. Selenium supplementation (Group IIb) decreased the levels of LDH in a significant manner indicating the modification in the anaerobic glycolysis, to the destruction of cancer cells. Following radiotherapy (Group IIa), the expression of fourth and fifth isoenzyme fraction of LDH at the end of 6 months diminished slightly indicating a regress in LDH activity (Figure 2). The isoenzyme pattern of LDH clearly shows that the isoenzymes 4 and 5 are higher in untreated oral cancer patients as compared to normal controls. The expression of fourth and the fifth isoenzyme fraction of LDH was more in cancer patients which manifests advanced stage. The increase in serum LDH may be partly due to metastasis (Rao et al., 1978) and the basis for a marked increase in LDH level in serum of cancer patients might also be due to the release of LDH from the necrotic tissue into circulation. Regression of tumors under therapy along with selenium supplementation (Group IIb) is evidenced by decreased expression of LDH 4 and 5. By changing the permeability of membrane or affecting cellular growth selenium affords some protective mechanisms against abnormal cell growth, through the expression of various selenoproteins or through its metabolites. The reduction in LDH activity might control glycolysis rendering protection to membrane integrity.

Aspartate amino transferase (SGOT) and alanine amino transferase (SGPT) functioning in the transamination processes of the cell is of interest in the protein metabolism of tumor tissue. The elevated activities of SGOT, SGPT, ACP, ALP (Table 2) may be connected with the metabolic disturbances of the normal cells and also tumor associated perturbations may release these enzymes in the blood. The activities of serum amino transferases are a measure of the integrity of the cells. Selenium supplementation (Group IIb) decreased the activities of markers to a greater extent by maintaining the integrity of the cells, thus shows the reversal of the metabolic disturbances to near normal. Creatine phosphokinase (CPK) is a sensitive

indicator of muscular damage (Cay and Naziroglu, 1999), maintains the ATP level constant at the expense of creatine phosphate demonstrating the ability of the kinase to buffer the muscle ATP concentration. The elevated level of CPK in cancer and radiation groups (Table 2) might be due to progressive tissue necrosis and cellular degradation. Moreover, radiation damage was found to act directly on the skeletal muscles to enhance the leakage of creatine kinase, especially on the slow twitch oxidative type that repeated the muscle stress which potentiated the radiation effect. With selenium supplementation, the levels of CPK decreased drastically indicating the protection afforded by selenium against muscular damage and cellular degradation.

Glutathione-S-transferases (GST) are a large family of detoxification enzymes that play an important role in protection mechanism against carcinogenesis. Oral cancer tissues (Group II) showed a decreased GST-π activity as a result of increased oxidative stress and depleted glutathione levels (Figure 3). GST is an important preneoplastic and neoplastic marker in correlation to the extent of free radical mediated change caused by various carcinogen induced cancers (Tsuchida and Sato 1992). Selenium gradually raised the expression of GST π in both tissue and lysate, due to its scavenging action against lipid peroxides.

Elevated TNF levels in oral cancer patients (Group II ) and radiation groups (Group IIa) (Figure 4) may be due to ROS mediated activation of nuclear factor NFƘ-B, which regulates TNF-α production at transcriptional and translational levels. The reduction in TNF-α in selenium administered group (Group IIb) may be ascribed to the inactivation and translocation of NFk-B. A relatively high GSSG levels under conditions of selenium excess (Le Boeuf et al., 1985) reduces NFkB function (Combs and Gray 1998). This may be ascribed to the lowered ROS mediated oxidative damage.

Elevated levels of CEA and AFP noted in oral cancer cases (Group II) (Figure 5a,5b; Figure 6a,6b) is associated with malignancy. Elevated CEA levels are found in cigarette smokers and malignancies as well as with inflammatory diseases (Silverman and Stern, 1994). The complete removal of the tumor is associated with a return of CEA levels to normal, whereas incomplete removal results in persistence of elevated levels. The observed decrease in CEA and AFP in correspondence to selenium intake indicated the decrease in tumor burden. SCC-Ag, a fraction of TA-4 is a cytokeratin-type protein and is best expressed in non-keratinizing squamous carcinoma cells. They are reliable indicators of human SCC, tumor burden and tumor growth, and useful markers of epithelial differentiation (Snyderman et al., 1995). Detectable levels of serum SCC-Ag levels clearly indicates the quantitative abnormality of circulating antigen in cancer and radiation groups (Figure. 7a,7b). Selenium supplementation (Group IIb) markedly decreased SCC-Ag levels to near normal proving prognosis of the epithelial tissues.

In conclusion, the improvement in the trace element levels and detection of low levels of serum biomarkers in selenium supplemented groups is attributed to the membrane stabilizing effect of selenium, proving their antioxidative/anticarcinogenic impact on biochemical indices assessed. The outcome of the study suggests selenium as a promising supplement for oral cancer patients undergoing conventional radiotherapy.

*Conflict of Interest*

None Declared.

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