Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

"A COMPARATIVE EVALUATION OF ENZYMATIC ANTIOXIDANT LEVELS IN PRE AND POST THERAPY PATIENTS WITH ORAL CANCER"

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Received: 12 Sep 2014 Revised and Accepted: 10 Oct 2014

ABSTRACT

Objectives: The present study was aimed to evaluate the magnitude of oxidative stress and levels of enzymatic antioxidants in Oral Squamous cell carcinoma (OSCC) patients receiving radiotherapy (RT) and chemo radiotherapy (CRT). Venous blood samples were collected from 20 healthy subjects, 20 disease control patients (without treatment), 20 oral cancer patients who received chemo radiotherapy and 20 oral cancer patients receiving radiotherapy and were analyzed for antioxidant status using various assay techniques.

Methods: The present study measured the levels of three antioxidants enzymes: Superoxide dismutase (SOD), Glutathione Peroxidase (GP_X), Catalase (CAT) in the plasma samples of 20 patients who were proven with biopsy-Squamous cell carcinoma of the oral cancer with clinical stage III/IV and were receiving radio and chemo radiotherapy. Same enzymes were also estimated in 20 healthy individuals and disease control patients (who were admitted in to the cancer clinic).

Results: The plasma levels of antioxidant enzymes (SOD, CAT, and GP_X) were lower in the oral cancer patients as compared to those in the healthy individuals. Superoxide dismutase levels in healthy control patients were found to be 190.4µg/dl, 34.54 µg/dl in disease control patients, 46.16 µg/dl in radiotherapy received group patients and 81.48 µg/dl in chemo and radiotherapy received group patients. Glutathione peroxidase levels were found to be 65.713 µg/dl in healthy control group patients, 13.8 µg/dl in disease control patients, 16.49 µg/dl in radiotherapy received group patients, 34.2 µg/dl in chemo and radiotherapy received group patients. Catalase enzyme levels were found to be 52.37 µg/dl in healthy control group patients, 12.35 µg/dl in disease control group patients, 22.34 µg/dl in radiotherapy received group patients, 27.18 µg/dl inchemo and radiotherapy received group patients. These enzymes also showed significant changes with radiotherapy and chemoradiotherapy. Antioxidant enzymes in the plasma of the oral cancer patients after radiation therapy lowered as compared to the plasma levels of enzymes after chemoradiotherapy.

Conclusion: This present study also showed decreased levels of antioxidant enzymes in the plasma of the oral cavity cancer patients after radiation therapy as compared to the chemoradiotherapy receiving oral cancer patients. An appreciable progress in antioxidant levels were observed in patients after receiving chemoradiotherapy (CRT) and observed to be more effective than after radiotherapy (RT). The reason for this observation was believed that concomitant chemoradiation and radiotherapy caused a reduction in the lipid peroxidation process and an improvement in the antioxidant levels of the oral cancer patients. But the radiation therapy produces high oxidative stress when compared to chemoradiotherapy in Oral Squamous Cell Carcinoma.

Keywords: Oral Cancerr, Oxidative stress, Antioxidant enzymes, Chemo and radiotherapy, Superoxide dismutase, Glutathione Peroxidase, Catalase.

INTRODUCTION

Oral cancer is an important type of cancer among all cancers globally and is one of the six most commonly occurring malignant disorders in the south-east Asian population [1]. Tobacco chewing is the major etiological factor in its development. Other factors include alcohol, genetic factors and diet lacking in micronutrients and vitamins. Tobacco contains large amounts of pro-oxidants that can directly initiate the process of lipid peroxidation which is a chain reaction process producing a continuous production of free radicals which lead to cellular damage [1].

Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, hydrogen peroxide and free radicals play an important role in cancer development. ROS are uncharged molecules containing at least one unpaired electron (or) reactive non radical compounds capable of oxidizing biomolecules. These free radicals and other ROS are called pro-oxidants [2,3]. ROS can cause DNA base alterations, standard DNA breaks damage to tumor suppressor gene and activates the expression of proto-oncogenes [3].

An imbalance between the production of pro-oxidants (ROS) and antioxidants capacity creates oxidative stress [4]. Antioxidants are the most important substances of defense against free radicals damage and are essential for the oxidative damage reduction maintaining health and quality of life. Antioxidant enzymes are mainly involved in the decomposition of ROS. In general, there are two types of antioxidants: Enzymatic and Non-enzymatic antioxidants. These enzymes protect the cell against oxidative injury. Superoxide dismutase (SOD), Glutathione Peroxidase (GP_X), and Catalase (CAT) are the first line enzymatic antioxidant defense systems responsible for scavenging free radicals. Non-enzymatic antioxidants are Vitamin C, E, A, Selenium inhibit both initiation and promotion of carcinogenesis [5].

The typical mode of treatment for early stage oral cancer is chemo radiotherapy (CRT) and radiotherapy (RT) were as advanced oral cancer is resected by surgery followed by external beam radiotherapy. The most common type of treatment is radiotherapy for oral cancer patients. External beam radiation and gamma radiation which is a potential source of field cancerization of the upper aero-digestive tract for radiotherapy are available, but the most commonly used isotopes are Iridium-192 and Iodine-125 [6,7].

Chemotherapy can be administered as adjuvant treatment, as a follow-up treatment after the primary treatment. The patient may develop toxicity of concurrent therapy, as radiation therapy and chemotherapeutic agents may possibly interact with one another. The classic foundation of the chemotherapy/radiation interaction includes spatial interaction, radiation sensitization, and toxicity independence. Spatial interactionrecognizes that radiation therapy will work loc oregionally (i.e., within the irradiated field), while chemotherapy will work systematically. While this definition allows for some chemotherapyantitumor activity within the radiated field,

the major goal forchemotherapy is to eradicate systemic micrometastases [8].

The mechanism of the action of radio and chemotherapeutic agents is to alter cellular homeostasis, thus modifying the signal transduction pathways and predisposing to apoptosis [9]. Some of the studies have demonstrated that increased oxidative stress after radio and chemotherapy while others have reported decreased oxidative stress after chemoradiotherapy concomitantly[10].

The main aim of this study was to evaluate the levels of the enzymatic antioxidants in patients with oral squamous cell carcinoma before and after treatment. Antioxidant levels SOD, GPX and CAT were evaluated in plasma of patients with oral cancer, in healthy individuals and in post therapy individuals to know whether chemoradiotherapy or radiation therapy is responsible for the ROS formation, depletion of antioxidants and development of oxidative stress. The antioxidants detection can be used as a marker in future therapeutic strategy for oral cancer.

This study was taken up with a view of the Indian studies on the effects of radiation therapy and chemo radiotherapy on oxidant-antioxidant status in oral squamous cell carcinoma (OSCC) and to investigate and compare the enzymatic antioxidant alterations in the plasma of oral Squamous cell carcinoma patients receiving chemo radiotherapy (CRT), radiotherapy (RT) receiving patients and healthy individuals.

MATERIALS AND METHODS

Materials

Sample distribution

This comparative study was conducted on 80 patients with biopsy proven Oral Squamous Cell Carcinoma (OSCC) of the oral cavity, registered at the oral cavity cancer department, in various Cancer clinics atWarangal,Telangana,India.

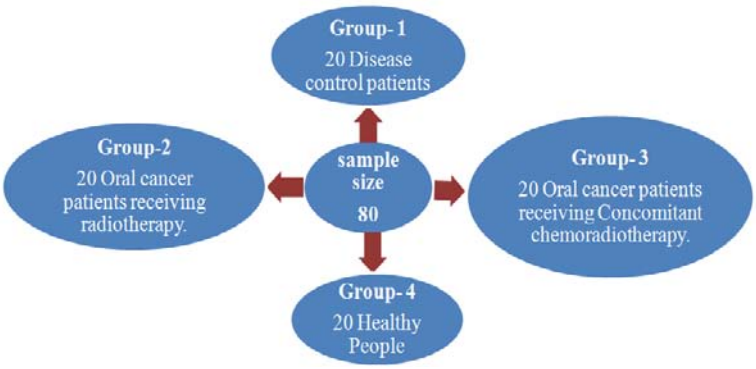
Out of 80 patients, 20 patients of OSCC received radiotherapy (RT), 20 patients of OSCC received chemo radiotherapy (CRT) and 20 were healthy individuals(Control), 20 were disease control patients. The individuals were categorized into 4 groups:

Group-I: Twenty disease control group (without any treatment) who visited the department of oral cancer medicine and radiology. Among these patients 13 were males and 7 were females with the age range from (18-70) having OSCC.

Group-II: Twenty patients who were receiving radiotherapy. Among these patients 13 were males and 7 were females with the age range from (18-70)having OSCC.

Group-III: Twenty patients who were receiving chemo radiotherapy after the completion of radiotherapy. Among these patients 17 were males and 3 were females with the age range from (18-70) having OSCC.

Group-IV: The control group comprises 20 healthy individuals. Informed consent was taken from all subjects before including them in study.



Inclusion criteria

- Patients with an age range from 18-70 years.
- Patients who came to oncology department clinically diagnosed with OSCC.
- Patients who are not on any treatment for OSCC
- Patients who have agreed for the biopsy attended the clinic
- Patients who were receiving Concomitantchemo radiotherapy (CRT).
- Patients who were receiving radiotherapy (RT)
- Normal subjects without any oral lesions and systemic diseases.

Exclusion criteria

- Patients below the age 18 years and above 70 years.
- Patients with any systemic diseases like diabetes mellitus, cardiovascular diseases, renal dysfunction, liver disorders.
- Patients with other type of oral cavity cancers like oral sub mucous fibrosis and Oral leukoplakia.
- Pregnancy

Methods

Source of data

The study was carried out at St. Ann's Cancer Hospital, Warangal, Telangana,India. The study protocol was approved by the Ethical Committee ofKakatiya Medical College,Telangana, India. All subjects were interviewed before clinically examined in the oncologydepartment and relevant demographics data were collected. Informed consent was obtained from the patients. All the study group patients i. e. OSCC group patients were regular tobacco chewers (about 5-8years) and alcoholics.

Collection of samples

Aseptic precautions were taken during collection of blood samples. Approximately 5 ml of over night fasting venous blood samples were collected from the antecubital vein using sterile syringe from each individual in all groups and collected in 5 ml vials. Plasma was separated by using EDTA as an anticoagulant and blood sample was centrifuged at 3000rpm for 15 min. Then separated plasma was collected in a transparent 5 ml vials and stored at -20 to -25^{0}C till further analysis.

Biochemical measurements

Superoxide dismutase (SOD) assay

Superoxide dismutase assay is based on the inhibition of superoxideinduced NADH oxidation. Decrease in the rate of NADH oxidation is dependent on the enzyme concentration, and saturation levels were attainable by recording the corresponding readings, spectrophotometrically at 520 nm [11].

Glutathione Peroxidase (GPx)assay

Estimation of Glutathione Peroxidaseactivitywas measured by using

hydrogen peroxide and the rate of disappearance ofNADPHat37°Candwasrecordedspectrophotometricallyat 340 nm [11].

Catalase (CAT) assay

CAT was assayed by colorimetric method. The colour produced by the reaction of H_2O_2 with dichromate in acetic acid was measured at 620 nm [11].

Statistical analysis

The quantified variables in the study (age, sex, superoxide dismutase (SOD), glutathione Peroxidase (GPx)and CAT levels) were subjected to statistical analysis. All these values were presented as mean ± standard deviation. The data were statistically analyzed using Graph pad prism statistical software. Unpaired Studént s 't' test was performed to compare the levels between control and study groups. *P* value less than 0.05 was considered significant.

RESULTS

Demographics

All afore mentioned groups consisted of (80%) males and a (20%) female, their distribution demographics is shown in figure 1.

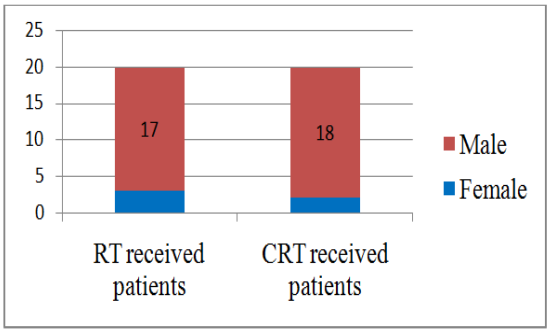


Fig. 1: Distribution of OSCC patients based on treatment pattern

Figure 2 illustrates the comparison of mean GPx levels in between healthy control group, disease control group, radiation (RT) received and CRT received group. In chemo and radiotherapy (CRT) received group the GPx level was increased significantly (p<0.001) compared to disease control and RT group.

Table 1: Distribution of OSCC patients into age groups

Age	OSCC with CT (n=20) (%)	OSCC with RT (n=20) (%)	TOTAL (n=40)	Odds ratio
18-30 yr	4 (20%)	4 (20%)	8 (20%)	1
30-40 yr	6 (30%)	5 (25%)	11 (27%)	1.2
40-50 yr	5 (25%)	4 (20%)	9 (22%)	1.25
50-60 yr	3 (15%)	4 (20%)	7 (17%)	0.75
>=60 yr	2 (10%)	3 (15%)	5 (12%)	0.6

As shown in table 1, the age of the individuals at diagnosis of cancer ranged between 18-70 years with mean age of 44 years. More than half of the OSCC patients were diagnosed between the ages of 25-50 years. 4 were aged younger than 30 years and 16 were aged older than 60 years at presentation.

Table 2: Distribution of risk factors among OSCC patients

Risk factors	Chemotherapy receiving OSCC	Radiotherapy receiving OSCC	Total (%)	Odds ratio
Tobacco chewing, Smoking	7	4	11 (27%)	1.75
Alcohol	0	0	0 (0%)	0
(Paan), Betel quid	4	6	10 (25%)	0.6
Both alcohol and tobacco	9	10	19 (47%)	0.9

As shown in table 2, among 40 patients with OSCC attended the clinic, 19 patients were on both alcohol and tobacco, 11 patients were habituated to tobacco chewing and smoking and 10 patients were on chyni (Paan), betel quid.

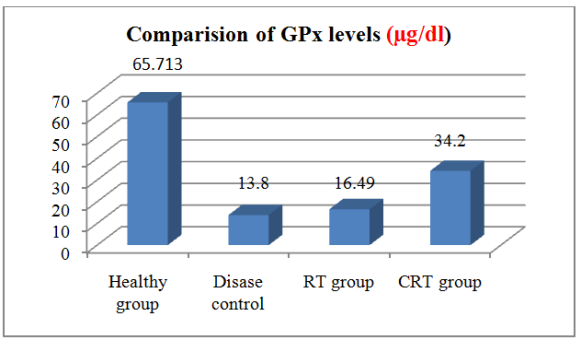
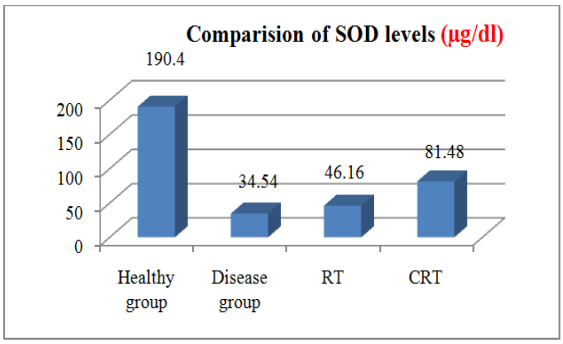


Fig. 2: Comparison of GPx levels in RT and Chemo and radiotherapy (CRT) with oral cancer.



Fig, 3: Comparison of SOD levels in RT and Chemo and radiotherapy (CRT) with oral cancer

Figure 3 shows the comparison of mean SOD levels in between healthy control group,disease control group, radiation (RT) received group and CRT received group. In chemo and radiotherapy (CRT) received group the GPx level was increased significantly (p<0.001) compared to disease control and RT group.

Figure 4 shows the comparison of mean CAT levels in between healthy control group,disease control group, radiation (RT) received group and CRT received group. In chemo and radiotherapy (CRT) received group the GPx level was increased significantly (p<0.001) compared to disease control and RT group.

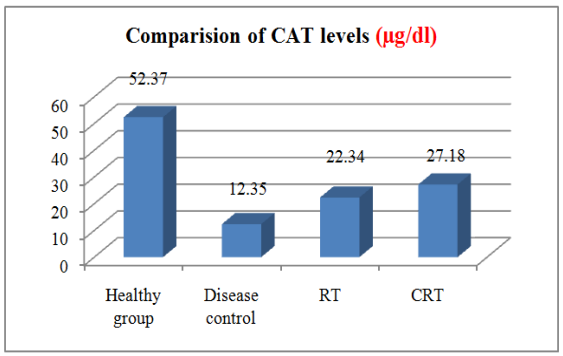


Fig. 4: Comparison of CAT levels in RT and Chemo and radiotherapy (CRT) with oral cancer

A statistically significant difference was observed ($p<0.001$) with higher mean GPx, SOD and CAT values in CRT and RT (post treatment) received the group in comparison with the disease control group (pre treatment). Thus, disease control group showed the lowest mean GPx, SOD, CAT levels when compared with healthy individuals and post treatment (CRT & RT) received group.

DISCUSSION

Oral cancer appears to be one of the major causes of mystery and death. Radiotherapy is one of the clinical means by which oral cancer can be treated [12]. Oxidative stress is a mismatch between the production of damaging ROS and the efficacy of the antioxidant defense. In oxidative stress there is an excessive production of ROS and there is a significant decrease or lack of antioxidant enzymes defense [12].

This study was mainly involved in evaluating and comparing the antioxidant status in between disease control group and oral cancer patients receiving chemo radiotherapy (CRT) and radiotherapy (RT). A majority of oral cancer patients were 80% males and 20% females who had tobacco, betel quid chewing and alcohol consumption. Previous studies have shown that these habits have carcinogenic effects [13].

Antioxidant enzymes such as SOD,GPx and CAT can directly counterbalance the oxidant attack and protect the cells against DNA damage. The plasma levels of enzymatic antioxidants (SOD, GPx and CAT) were lower in the disease control group as compared to those in the healthy subjects [11].

Chemo and radiotherapy can be used for the treatment of oral cancer. But the treatment is used to destruct the ROS and against antioxidant defense system. As it is well known that enzymatic antioxidant (SOD, CAT and GPx) levels increases when patients received chemo radiotherapy or radiotherapy for a long while, but the progress in the antioxidant levels at very initial stages of the therapies were never determined [14]. So, this study showed that the percentage increases of antioxidant levels in receiving both kinds of therapies were determined and the summary of the study is as follows

SOD

The optimum levels of SOD are 164-240µg/dL[11]. Recent studies on SOD revealed that deficiency of SOD levels leads to abnormalities like ocular disturbances, anemia [12]. In disease control patients the concentration of SOD levels were found to be below the optimum levels. A study of these patients case sheets gave an impression that these patients were suffering from anemia grade I-II and visual disturbances[11,13] and also the same individuals showed an appreciable increase in SOD levels after chemo radiotherapy (CRT) [13]. Progress in SOD levels afterchemoradiotherapy and radiotherapy revealed that the chemoradiotherapy is more effective treatment than radiotherapy which was in concordance with the study by Sun Y, Oberley LW.

GPx

The optimum levels of GPx are 27.5-73.6µg/dL [14].Recent studies on GPx revealed that deficiency of GPx levels leads to liver damage [15,16]. In disease control patients the concentration of GPx levels were found to be below the optimum levels. Study of these patients case sheets gave an impression that the bilirubin (direct, indirect and total) levels of these patients were found to be increased [16]. Where the same patients showed that there was an appreciable increase in GPx levels after chemoradiotherapythan after only radiotherapy. The percentage of progress in GPx levels after chemoradiotherapy indicated that the chemoradiotherapy (CRT) is more effective treatment than radiotherapy (RT) which was in concordance with the study by Sun Y, Oberley LW.

CAT

The optimum levels of CAT are 50-60µg/dL[17]. Recent studies on CAT revealed that deficiency of CAT levels leads to abnormalities like respiratory diseases (runny nose, bronchial infections and cough) and gastrointestinal disorders [15,16,17]. In disease control patients the concentration of CAT levels were found to be below the optimum levels. Study on these patients case sheets gave an impression that these patients were suffering from pneumonia, dysphasia (difficulty in swallowing) and digestive problems [17]. Same patients showed that there was an appreciable increase in CAT levels after chemoradiotherapy and radiotherapy. But this study indicated that there was an appreciable increase in CAT levels after chemoradiotherapy than after only radiotherapy which was in concordance with the study by Greenwald R A.

The low activities of these antioxidant enzymes play an important role in progression of lesion and leads to the development of oxidative stress [15]. This suggests that lower antioxidant enzymes activity in oral cancer patients might be due to the depletion of the antioxidant defense system.[18]Some of the studies demonstrated that the adjuvant oral administration of antioxidant supplementation during conventional oral cancer treatment is still a matter of controversy; because the mechanisms of chemo radiotherapy (CRT) and radiotherapy (RT) are based in part on the production of free radicals. [19,20]Previous studies have shown that antioxidants can inhibit neoplastic cell growth by complex mechanisms whereas,[21]. Disease control patients were observed to suffer from respiratory diseases, GIT disturbances, anemia and other co-morbidity conditions before treatment. After the treatment, all these abnormalities frequency were reduced. Thus, this conveys that decreased antioxidants (SOD, GPx and CAT) levels may also result in hematological, respiratory and liver damage. Thus the relationship between them is inversely proportional [21,22].

Some studies have also shown that antioxidants given under controlled circumstances are able to increase the therapeutic efficacy of chemo and radiotherapy by improving tolerance and the sensitivity of the tumor while diminishing the toxic effects on healthy cell system[21]. Therapeutic effect was better in the group of patients received the antioxidant combination in addition to chemo and radiotherapy than in the group those patients were treated with radio and chemotherapy alone [23].

CONCLUSION

A comparative study of enzymatic antioxidant levels like SOD, GPx and CAT in patients after receiving radiation therapy and concurrent chemoradiotherapy regimen represents the best current standard therapy option for many patients with regionally advanced solid tumors, and improves the probability of cure. An appreciable progress in antioxidant levels was observed in patients after receiving chemoradiotherapy observed to be more effective than after radiotherapy. Chemotherapy given with RT leads to improved outcome versus RT alone.

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ABBREVIATIONS

OSCC –Oral Squamous Cell Carcinoma, CRT –Chemoradiotherapy

RT – Radiotherapy, SOD –Superoxide dismutase

GPx –Glutathione Peroxidase, CAT –Catalase

ROS –Reactive Oxygen Species, µg/dL –Microgram per deciliter