# Evaluation Of Biomarkers of Oxidative Stress Levels in Chemoradiotherapy Induced Oral Mucositis in Head and Neck Cancer Patients - A Hospital Based Prospective Study

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**Abstract**
Introduction: Oral mucositis is a painful debilitating inflammation and ulceration in the oral mucosa that occurs as the adverse effect of chemoradiotherapy due to release of free radicals. Aim of the study was to evaluate defensive action of Superoxide dismutase (SOD), Glutathione peroxidase (Gpx) and oxidative stress determinant 8-hydroxy 2-deoxyguanosine(8-OHdG) in chemoradiotherapy-induced oral mucositis in HNC patients. Methods: 40 patients undergoing chemoradiotherapy were evaluated for oral mucositis were included in this study. Oral Mucositis progression according to grades on Day 1,7,30 were analysed using Friedman tests. Salivary SOD, Gpx and 8-OHdG levels were assessed using the enzyme-linked immunoassay (ELISA) method. The data were statistically analysed using the ANOVA test to determine the significance of each salivary marker. Level of significance was set at P<0.05RESULTS- Superoxide Dismutase levels are significantly decreased in HNC prior CRT (Day 1) (0.77±0.11 ng/ml), Day 7 (0.63±0.09ng/ml), Day 30 (0.43±0.08 ng/ml) compared to healthy controls (0.99±0.10 ng/ml (P<0.05). Levels of Glutathione peroxidase were significantly reduced in HNC prior CRT (Day 1) (0.64±0.06 ng/ml), Day 7 (0.53±0.03ng/ml), Day 30 (0.45±0.05 ng/ml) compared to healthy controls (0.77±0.04 ng/ml) (P<0.05). 8-hydroxy 2-deoxyguanosine levels were significantly elevated in HNC prior CRT (Day 1) (6.61±0.57 ng/ml), Day 7 (7.90±0.46ng/ml), Day 30 (08.99±0.70 ng/ml) compared to healthy controls (5.33±0.55 ng/ml) (P<0.05). There was statistically significant difference in grading of oral mucositis between Day 1 and Day 30(p<0.01), Day 7 and Day 30(p<0.01). Conclusion: Levels of superoxide dismutase and glutathione peroxidase were reduced representing lack of antioxidant defense and 8-OHdG levels were significantly elevated denoting the increased oxidative stress in patients with oral mucositis undergoing chemoradiotherapy in Head and Neck cancer. Effective antioxidant therapy as a supportive care can prevent the occurrence and severity of oral mucositis in HNC patients.
Keywords: Oral mucositis, chemoradiotherapy, Saliva, Oxidative stress levels, Head and neck neoplasm

## 1. Introduction

Oral mucositis is an acute debilitating inflammation and ulceration of oral mucosa that occurs as an adverse effect in patients affected with Head and Neck cancer following chemoradiotherapy. Patients with erythema and ulceration show significantly longer hospitalization for the treatment of cancer compared to patients without mucositis[1]. The severity of mucositis increases in patients undergoing a high dose of chemotherapy and repeated radiation cycles [2]. Mucositis has a severe

doi.org/10.31838/hiv22.02.751 Received: 01.07.21, Revised: 14.08.21, Accepted: 19.09.21

impact on a patient's health related quality of life and negatively affects the ability to maintain the intensity of anti-cancer treatment under proper schedule. Moreover, it has taken place as a non-negligible adverse economic effect due to the cost of the cancer treatment.

The initiation phase of mucositis is characterized by radio or chemotherapy-induced direct DNA damage, that causes injury to the basal epithelial, submucosal, and endothelial cells. These cells release endogenous damage-associated molecular patterns that further damage cell membranes, stimulate macrophages, and trigger molecules that release reactive oxygen species[3].

The production of reactive oxygen species (ROS) plays an important role in human metabolism under physiological conditions[4]. When the production of these reactive species exceeds desired levels due to increased production and/or reduced elimination, the consequent imbalance leads to oxidative stress [5]. The free radical induced oxidative stress can induce DNA damage including strand breakage, base modification and DNA-protein cross-linkage [6,7]. Following this, salivary antioxidative enzymes can be predictive factors for oral mucositis.

The enzymatic salivary biomarker metal ion dependent Superoxide Dismutase (SOD) catalyzes the dismutation of superoxide anions to hydrogen peroxide. SOD is secreted in extracellular space regulates the cellular and extracellular redox state in normal and tumorigenic condition [8].

Glutathione peroxidase is a selenium enzymatic salivary biomarker that participates in reduction of hydrogen peroxide due to its ability to scavenge free radicals thereby increasing the production of plasma glutathione levels which is essential in oxidative stress defense [9]. The salivary oxidative stress marker 8-hydroxy 2-deoxy guanosine accumulation causes DNA damage which leads to increased production of reactive oxygen metabolites such as malondialdehyde, methyl peroxidase thereby elevating the level of protein degradation such as kinins and activate arachidonic acids [6].

Previous studies concentrated on estimation of levels of SOD in leukoplakia, lichen planus, bone marrow transplanted patients and in vitro experimental methods such as mice[10–12]. Other studies estimated Gpx levels in Gastrointestinal mucositis and oral submucous fibrosis.[10,13] But the current study assessed the levels of SOD and Gpx particularly among chemoradiotherapy induced oral mucositis patients. Moreover, 8-OHdG levels were estimated only from the urine samples of oral cancer patients. To our authors' knowledge, none of the previous studies analysed the 8-OHdG levels in patients undergoing chemoradiotherapy with oral mucositis in Head and cancer patients.

The debilitating effect of oral mucositis is due to production of accumulating free radicals following which gradual increase in oxidative stress is created that invariably follows in radiotherapy of oral and facial tissues and is observed in more than 75% of patients receiving chemoradiotherapy [8]. Since our team has extensive knowledge and research experience that has translated into high quality publications. Hence, the aim of the present study was to estimate the levels of salivary markers (SOD,Gpx and 8-OHdG) in chemoradiotherapy induced oral mucositis in Head and neck cancer patients.

## 2. Materials and Methods

### Study Design

This was a prospective study for a period of 6 months from December 2020 to June 2021. 40 Head and neck cancer patients were included in this study with 40-65 years of age group undergoing chemoradiotherapy. Healthy controls were 20. They were assessed for oral mucositis and graded based on WHO criteria. Radiotherapy was administered 5 times per week with a total dose of 50-70 Gy. Chemotherapy was based on 21 Days administration of Cisplatin 40 mg and paclitaxel 60 mg. The study was conducted according to ethical guidelines of Dr. Rai CBCC Center, Chennai, India. The present study was approved by the ethical board of the institution –SDC- Institutional Human Ethical Committee and was in accordance with the Helsinki declaration. IHEC Reference number is IHEC/SDC/UG-1769/20/304. Informed consent was obtained from all the patients.

### Inclusion criteria

- Patients undergoing chemotherapy for head and neck cancer
- Patients with previous history of radiotherapy or chemotherapy
- Patients with secondary cancer

### Exclusion criteria

- Patients with systemic illness such as diabetes mellitus, immunocompromised patients, long term antibiotic usage.

### Saliva Collection

Whole unstimulated saliva of 1-1.5 ml for 5 minutes was collected in sterile containers from each of the participants by a spitting method. Low volumes of saliva could only be collected due to radiation induced hyposalivation. Firstly, saliva samples were collected from 20 healthy controls without any HNC or other systemic illnesses. Then saliva samples were collected from 40 HNC patients undergoing chemoradiotherapy, were evaluated for oral mucositis and graded based on WHO criteria. They were collected at three time points :HNC prior undergoing CRT Day 1, Day 7, Day 30 of CRT with oral mucositis present. There are 4 grades of oral mucositis. Grade 0 (None) indicates no signs or symptoms, Grade 1 (Mild) indicates erythema and soreness, Grade 2 (Moderate) indicates ulcers, solid intake, Grade 3 (Severe) indicates ulcers, only liquid intake, Grade 4 (Life Threatening) indicates ulcers-

Alimentation not possible. All samples were immediately stored at -20℃ and were processed for biochemical analysis. The levels of SOD, Gpx and 8-OHdG were estimated.

### Superoxide dismutase Assay

Superoxide dismutase was assayed by the method of Marklund and Marklund . The degree of inhibition of auto- oxidation of pyrogallol, at an alkaline pH, by superoxide dismutase was used as a measure of the enzyme activity.To 0.25 ml of saliva 0.25 ml of ethanol and 1.25 ml of chloroform were added, kept in a mechanical shaker for 15 min and centrifuged at 20000 revolution for 15min. To 0.5 ml of the supernatant, 2.0 ml of 0.1 M Tris-HCl buffer pH 8.2; 1.5 ml of distilled water and 0.5 ml of pyrogallol were added. Change in optical density at 0, 1 and 3 min was read at 420 nm in a spectrophotometer. Control tubes containing 0.5 ml of distilled water were also treated in a similar manner against a buffer blank. The enzyme activity is expressed as Units/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol auto-oxidation.

### Glutathione Peroxidase Assay

Glutathione peroxidase converts reduced glutathione (GSH) into oxidised form using hydrogen peroxide during its reaction. The amount of GSH utilized is estimated by measuring it in the assay mixture before and after the enzyme activity. GSH reacts with DTNB (5,5-dithio-bis- (2-nitrobenzoic acid) to give yellow colour, which is then measured at 420 nm.0.2 ml each of EDTA, sodium azide, GSH, H2O2, buffer and saliva were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of trichloroacetic acid and the tubes were centrifuged. To 0.5 ml of supernatant, 3.0 ml of phosphate solution and 1.0 ml of 5,5-dithio-bis-(2-nitrobenzoic acid) were added and the colour developed was read at 420 nm immediately against blank containing only phosphate solution and DTNB reagent. Graded amounts of standards were also treated similarly. GPx activity is expressed as µmoles of H202 consumed/min/mg protein.

### 8-OHdG

Oxidative DNA damage was quantified using Enzyme-Linked Immunosorbent Assay (ELISA).The plate has been pre-coated with human 8-OHDG antibody. 8-OHDG present in the sample is added and binds to antibodies coated on the wells. Biotinylated human 8-OHDG Antibody is added and binds to 8-OHDG in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated 8-OHDG antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added, and color develops in proportion to the amount of human 8-OHDG. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

## 3. Statistical Analysis

Data was analysed using Statistical Package of Social Sciences (SPSS, version 22, SPSS Inc., Chicago, IL., USA). ANOVA test was used to determine the significance of each salivary marker. Estimation of different levels of SOD, Gpx and 8-OHdG were done using descriptive statistics.

Prevalence of grading of oral mucositis among patients undergoing Chemo-radiotherapy on Day 1, day 7 and day 30 were done using descriptive statistics. Oral Mucositis progression according to grades on Day 1,7,30 were analysed using Friedman tests. Level of significance was set at $p<0.05$

## 4. Results

40 patients were included in this prospective study with 40-65 years of age group undergoing chemoradiotherapy for head and neck cancer. Out of 40 patients, 24 (60%) patients were males and 16(40%) were females. At the end of Day 7 of treatment, (N=18)45.0% (95%CI [30.4, 60.3]) had Grade 0 OM, (N=15) 37.5 % (95%CI [23.8,52.9]) developed Grade 1 OM, (N=7) 17.5 % (95%CI [8.2,31.3]) developed Grade 2 OM. At the end of Day 30, (N=8) 20% (95%CI [9.9,34.2]) developed Grade 1 OM, (N=20) 50% (95%CI [35,65]) Grade 2 OM, (N=12) 30.0% (95%CI [17.6,45.2]) developed Grade 3 OM. (Table 1). There was statistically no significant difference in grading of oral mucositis between Day 1 and Day 7 ($p< 0.09$), there was significant difference between Day 1 and Day 30($p<0.01$), Day 7 and Day 30($p<0.01$) (Table 2,3,4). Boxplot showed the distribution of grades of oral mucositis of same patient at different time points on Day 1(Before Chemoradiotherapy), Day 7, Day 30, n total=40 patients by Friedman's Two-Way Analysis of Variance. (Figure 1).

From the study, the levels of Superoxide dismutase, glutathione peroxidase and 8 hydroxy 2 deoxyguanosine were estimated. Mean and Standard deviation values of SOD, Gpx and 8-OHdG of the healthy controls, HNC prior CRT (Day 1), HNC patients undergoing CRT induced oral mucositis (Day 7), HNC patients undergoing CRT induced oral mucositis (Day 30) were shown in Table 5.

Bar graph depicts the levels of Superoxide dismutase, Gpx and 8-OHdG in healthy controls, HNC prior CRT (Day 1), HNC patients undergoing CRT induced oral mucositis (Day 7), HNC patients undergoing CRT induced oral mucositis (Day 30). From Figure 2, SOD level was highest among healthy controls compared to patients with HNC. Superoxide Dismutase levels are decreased in HNC prior CRT (Day 1) (0.77±0.11 ng/ml), Day 7 (0.63±0.09ng/ml), Day 30 (0.43±0.08 ng/ml) compared to healthy controls (0.99±0.10 ng/ml) the result is found to be statistically significant $P<0.05$. From Figure 3, Gpx level was highest among healthy controls compared to patients with HNC. Levels of Glutathione peroxidase were reduced in HNC prior CRT (Day 1) (0.64±0.06 ng/ml), Day 7 (0.53±0.03ng/ml), Day 30 (0.45±0.05 ng/ml) compared to healthy controls (0.77±0.04 ng/ml) the result is found to be

statistically significant P<0.05. From Figure 4, 8-OHdG level was lowest in healthy controls than patients with HNC. Further, 8-hydroxy 2-deoxyguanosine levels were elevated in in HNC prior CRT (Day 1) (6.61±0.57 ng/ml), Day 7 (7.90±0.46ng/ml), Day 30 (08.99±0.70 ng/ml) compared to healthy controls (5.33±0.55 ng/ml) the result is found to be statistically significant P<0.05.

## 5. Discussion

Oral mucositis involves several stages such as initiation, upregulation, signaling, amplification, ulceration and healing. Exposure to ionizing radiation causes generation of free radicals such as reactive oxygen species and reactive nitrogen species [14].

Free radicals are unstable molecules that have one or more unpaired electrons in outer shells, this instability leads them to obtain the missing electron from DNA, RNA, lipids thus reestablishing stability by damaging molecules vital for cells. This phase plays an important role in initiation of oral mucositis. Free radicals cause DNA breakage and cell death activating transcription factors NF-κ B and proinflammatory cytokines thus causing damage to basal cells of epithelium. In signalling and amplification phase regulation of Tumor necrosis factor α (TNF-α), Interleukin β (IL-1β) and Interleukin 6 (IL-6) further damage the cells of mucosa. Next phase leads to chain of apoptotic events induced by Radiotherapy ,thinning of mucosa results in ulceration with bacterial colonization [15]

Kim et al classified salivary antioxidants in three large groups based on their function. Preventive antioxidants form the first group such as SOD, carotenoids, catalase, glutathione peroxidase, transferrin which inhibit the production of free radicals. Sweeping antioxidants such as Vitamin A and E, albumin, bilirubin that eliminates free radicals inhibit initiation and spreading of free radicals. Lastly protease, transferase, lipases repair the damage caused in tissues [16]

Our study analysed salivary antioxidant levels of SOD, and Glutathione Peroxidase and oxidative stress biomarker such as 8-OhdG in Chemoradiotherapy induced oral mucositis patients in HNC. In our study, Superoxide dismutase levels undergoing chemoradiotherapy in HNC patients are lowered compared to HNC patients' prior CRT and the results are found to be significant. Level of SOD is found to be reduced in non-treated cancer patients and still more reduced in patients undergoing chemoradiotherapy in HNC compared to controls in our study. Similar findings were observed in few previous studies supporting that reduction in levels of SOD in the majority of head and neck malignancies and the level is found to be even more reduced following post-chemoradiotherapy compared to healthy controls[14]

In a study by Evelin et al.,the middle stage 7-10 days after Bone marrow transplantation at the beginning of clinical manifestation of oral mucositis there was an increase in concentration of SOD as a defense mechanism of saliva against oxidative stress [11]. Nagler studied salivary antioxidant profile in healthy adults and determined SOD values are 0.79U/ml for basal saliva,0.80 U/ml for stimulated one [17]. Similarly in our study, SOD in healthy controls were 0.99±0.10 ng/ml, (Day 1) had (0.77±0.11 ng/ml), Day 7 (0.63±0.09ng/ml), Day 30 (0.43±0.08 ng/ml)

In the current study, Glutathione peroxidase is found to be reduced in the HNC patients undergoing chemoradiotherapy compared to the HNC patients' prior CRT. A study conducted by Sabitha et al., reported low levels of Gpx in oral cancer patients treated with radiotherapy proving that radiation causes inactivation of antioxidant enzymes by making the bodily immunity inefficient to manage the free radical attack[18]. The lower levels of Gpx in patients with oral mucositis may be attributed to increased utilization in detoxification of oral mucositis, scavenging of free radicals and to counteract prevailing oxidative conditions caused by an elevation in ROS and pro-oxidants. Another study conducted by Sharma et al., who reported that Gpx levels are reduced in pre-treatment patients with posterior one-third of the tongue carcinoma compared to the healthy population[19]. However, a study estimated the plasma total glutathione level which is found to be lower among post-radiotherapy patients associated with low survival rate[20]. A study conducted by Babiuch et al., reported the activity of Gpx biomarker is considerably lower among healthy controls than in patients affected with oral leukoplakia and the level is even lower in OSCC patients [5]. Gpx is one of the important antioxidant enzymes that detoxifies the hydrogen peroxide to water and thereby increases the level of glutathione which aids in naturally protecting against the damage of inflamed mucosa. However oxidative stress created in the body suppresses the radioprotective potential of Gpx[21].

The level of 8-OHdG is higher among post-chemoradiotherapy patients with HNC in the current study. 8-OHdG is a useful biomarker reflecting oxidative damage among workers occupationally exposed to low-dose radiation.This result is similar to a study conducted by Gao et al., concluded that levels of 8-OHdG are elevated among patients receiving radiotherapy for a prolonged period and a study by Kirhan et al., reported that 8-OHdG level is elevated in patients receiving chemotherapy[22,23]. Studies proved that production of this potential biomarker can lead to accumulation of intracellular reactive oxygen species which can induce mutation in DNA, thus disrupting the function of cells is elevated in cancer patients [24]. As it is elevated in cancer patients, they are more elevated in patients undergoing chemoradiotherapy in HNC. Analyzing the previous studies, consideration of 8-OHdG level in cancer patients can be of good prognostic value[24].

In our study Day 7 after chemoradiotherapy had

more of Grade 0 (37.5 %, n=15), Grade 1(17.5%,n=7) with mild Oral mucositis and Day 30 had more Grade 2 (50.0 %,N =20),Grade 3 (30%,n=12) with moderate to severe oral mucositis. Levels of antioxidant capacity of SOD, Gpx decreased and oxidative stress marker 8-OHdG increased as the days of Oral mucositis progressed from Day 7 to Day 30 indicating the disease progression and severity of Oral mucositis

Future studies involving further meticulous analyses with aged-matched subjects is still warranted. It is also important that the oxidative stress levels in saliva may be affected by other factors such as physical activity, diet and metabolism of each individual, and these were not taken into consideration in this current study. As a future prospect, novel drugs with potential antioxidant properties with less side effect, cost effective drug is warranted to prevent the severity of oral mucositis.

## 6. Conclusion

Reduced level of superoxide dismutase and Glutathione peroxidase and elevated 8-hydroxy 2-deoxyguanosine in HNC patients receiving chemo radiotherapy inferences that high dosage of chemotherapeutic agents and multiple cycles of radiotherapy treated HNC patients with oral mucositis have more oxidative stress compared to HNC patients without any chemo- radiotherapy. As the days of Oral mucositis progressed from Day 7 to Day 30 indicating disease severity, antioxidant levels decreased and oxidative stress marker increased significantly. Hence the future scope of providing effective antioxidant therapy as a supportive care to the patients undergoing chemoradiotherapy at an appropriate time must be analysed.

Table 1: Prevalence of Grading of Oral mucositis according to Day 1,7,30

| | | N | Percentage | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | LL % | UL % |
| Day-1 | Grade-0 | 40 | 100.0% | . | . |
| | Grade-1 | 0 | 0.0% | . | . |
| | Grade-2 | 0 | 0.0% | . | . |
| | Grade-3 | 0 | 0.0% | . | . |
| | Total | 40 | 100.0% | . | . |
| Day -7 | Grade-0 | 18 | 45.0% | 30.4% | 60.3% |
| | Grade-1 | 15 | 37.5% | 23.8% | 52.9% |
| | Grade-2 | 7 | 17.5% | 8.2% | 31.3% |
| | Grade-3 | 0 | 0.0% | . | . |
| | Total | 40 | 100.0% | . | . |
| Day-30 | Grade-0 | 0 | 0.0% | | |
| | Grade-1 | 8 | 20.0% | 9.9% | 34.2% |
| | Grade-2 | 20 | 50.0% | 35.0% | 65.0% |
| | Grade-3 | 12 | 30.0% | 17.6% | 45.2% |
| | Grade-0 | 0 | 0.0% | | |
| | Total | 40 | 100.0% | . | . |

Table 2:Pairwise Comparisons of Gradings of Oral mucositis of same patient at different time points on Day 1(Before Chemoradiotherapy) and Day 7(Friedman's Two-Way Analysis of Variance by Ranks)

| | | Day -7 | | | | | | | | $p^a$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade-0 | | Grade-1 | | Grade-2 | | Total | | |
| | | N | % | N | % | N | % | N | % | |
| Day-1 | Grade-0 | 18 | 45.0% | 15 | 37.5% | 7 | 17.5% | 40 | 100.0% | 0.09 |
| | Total | 18 | 45.0% | 15 | 37.5% | 7 | 17.5% | 40 | 100.0% | |

**Table 3: Pairwise Comparisons of Gradings of Oral mucositis of same patient at different time points on Day 1(Before Chemoradiotherapy) and Day 7(Friedman's Two-Way Analysis of Variance by Ranks)**

| | | Day-30 | | | | | | | | pa |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade-1 | | Grade-2 | | Grade-3 | | Total | | |
| | | N | % | N | % | N | % | N | % | |
| Day-1 | Grade-0 | 8 | 20.0% | 20 | 50.0% | 12 | 30.0% | 40 | 100.0% | <0.001 |
| | Total | 8 | 20.0% | 20 | 50.0% | 12 | 30.0% | 40 | 100.0% | |

Table 4: Pairwise Comparisons of Gradings of Oral mucositis of same patient at different time points on Day 7 and Day 30 (Friedman's Two-Way Analysis of Variance by Ranks)

| | | Day-30 | | | | | | | | pa |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade-1 | | Grade-2 | | Grade-3 | | Total | | |
| | | N | % | N | % | N | % | N | % | |
| Day -7 | Grade-0 | 4 | 22.2% | 9 | 50.0% | 5 | 27.8% | 18 | 100.0% | <0.001 |
| | Grade-1 | 3 | 20.0% | 7 | 46.7% | 5 | 33.3% | 15 | 100.0% | |
| | Grade-2 | 1 | 14.3% | 4 | 57.1% | 2 | 28.6% | 7 | 100.0% | |
| | Total | 8 | 20.0% | 20 | 50.0% | 12 | 30.0% | 40 | 100.0% | |

Figure 1: Boxplot depicts the distribution of grades of oral mucositis of same patient at different time points on Day 1(Before Chemoradiotherapy), Day 7, Day 30, n total=40 patients (Friedman's Two-Way Analysis of Variance)

Table 5 : Table shows the mean and Standard deviation values of SOD, Gpx and 8-OHdG of the healthy controls ,HNC prior CRT(Day 1), HNC patients undergoing CRT induced oral mucositis(Day 7), HNC patients undergoing CRT induced oral mucositis(Day 30).

| Groups | Healthy controls | HNC prior CRT(Day 1) | HNC+CRT+ OM(Day 7) | HNC+CRT+ OM(Day 30) |
|---|---|---|---|---|
| Mean SOD | 0.99 | 0.77 | 0.63 | 0.43 |
| Standard deviation | 0.10 | 0.11 | 0.09 | 0.08 |
| Mean Gpx | 0.77 | 0.64 | 0.53 | 0.45 |
| Standard deviation | 0.04 | 0.06 | 0.03 | 0.05 |
| Mean 8-OHdG | 5.33 | 6.61 | 7.900 | 8.99 |
| Standard deviation | 0.55 | 0.57 | 0.46 | 0.7037 |

**Figure 2** - Bar graph depicts the levels of Superoxide dismutase in healthy controls, HNC prior CRT (Day 1), HNC patients undergoing CRT induced oral mucositis (Day 7), HNC patients undergoing CRT induced oral mucositis (Day 30). Superoxide dismutase level was found to be highest in healthy controls compared to other 3 groups. Levels of superoxide dismutase were reduced in HNC patients receiving chemoradiotherapy with oral mucositis on Day 7 and Day 30 compared to HNC prior CRT (Day 1) and Healthy controls.

Figure 3 - Bar graph depicts the levels of Glutathione peroxidase in healthy controls, HNC prior CRT (Day 1), HNC patients undergoing CRT induced oral mucositis (Day 7), HNC patients undergoing CRT induced oral mucositis (D
ay 30). Glutathione peroxidase level is highest among healthy controls compared to other 3 groups. Levels of Glutathione peroxidase were reduced in HNC patients receiving chemoradiotherapy with oral mucositis on Day 7 and Day 30 compared to HNC prior CRT (Day 1) and Healthy controls.

Figure 4 - Bar graph depicts the levels of 8-hydroxy 2-deoxyguanosine in healthy controls, HNC prior CRT (Day 1), HNC patients undergoing CRT induced oral mucositis (Day 7), HNC patients undergoing CRT induced oral mucositis (Day 30).8-hydroxy 2-deoxyguanosine level was found to be lowest among healthy controls compared to other 3 groups. Levels of 8-hydroxy 2-deoxyguanosine were elevated in HNC patients receiving chemoradiotherapy with oral mucositis on Day 7 and Day 30 compared to HNC prior CRT (Day 1) and Healthy controls.

## 7. Acknowledgement: None

Source of funding: None
Conflict of Interest: None
AuthorContribution:
Revathi.B : Literature search, clinical studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing.
Dr. Sreedevi Dharman: Concept, design, definition of intellectual content, statistical analysis, manuscript preparation, manuscript review
Dr. Selvaraj: Definition of intellectual content, clinical studies, data acquisition, manuscript review.

## Ethical policy and institutional review board statement

Patient declaration of consent: Informed written consent for participation in the study and publication of the data for research and educational purposes was obtained from head and neck cancer patients with oral mucositis undergoing chemoradiotherapy in Dr.Rai CBCC center, Saveetha dental college.

Data availability statement: The data set used in the current study is available on request from Revathi.B (151701030.sdc@saveetha.com)

Total number of pages: 22
Total number of figures: 4

## Reference

1.McGuire DB, Correa ME, Johnson J, Wienandts P. The role of basic oral care and good clinical practice principles in the management of oral mucositis. Supportive care in cancer. 2006 Jun;14(6):541-7.

2.Lalla RV, Sonis ST, Peterson DE. Management of oral mucositis in patients who have cancer. Dental Clinics of North America. 2008 Jan 1;52(1):61-77.

3.Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, Gavish M, Nagler RM. Salivary analysis of oral cancer biomarkers. British journal of cancer. 2009 Oct;101(7):1194-8.

4.Dharman S, Maragathavalli G, Shanmugasundaram K, Shanmugam RK. A Systematic review and meta-analysis on the efficacy of curcumin/turmeric for the prevention and amelioration of radiotherapy/radiochemotherapy induced oral mucositis in head and neck cancer patients. Asian Pacific journal of cancer prevention: APJCP. 2021 Jun;22(6):1671.

5. Babiuch K, Bednarczyk A, Gawlik K, Pawlica-Gosiewska D, Kęsek B, Darczuk D, Stępień P, Chomyszyn-Gajewska M, Kaczmarzyk T. Evaluation of enzymatic and non-enzymatic antioxidant status and biomarkers of oxidative stress in saliva of

patients with oral squamous cell carcinoma and oral leukoplakia: a pilot study. Acta Odontologica Scandinavica. 2019 Aug 18;77(6):408-18.
6.Kumar A, Pant MC, Singh HS, Khandelwal S. Assessment of the redox profile and oxidative DNA damage (8-OHdG) in squamous cell carcinoma of head and neck. Journal of cancer research and therapeutics. 2012 Apr 1;8(2):254.
7.Dharman S, Rajeshkumar K. Synthesis and Characterisation of Novel Turmeric Gold Nanoparticles and Evaluation of Its Anti-oxidant, Anti-Inflammatory, Antibacterial Activity for Application in Oral Mucositis-An In vitro Study. Int. J. Dentistry Oral Sci. 2021 May 20;8(5):2525-32.
8. Pico JL, Avila-Garavito A, Naccache P. Mucositis: its occurrence, consequences, and treatment in the oncology setting. The oncologist. 1998 Dec;3(6):446-51.
9.Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature. 1991 Jan;349(6308):431-4.
10.Gurudath S, Ganapathy KS, Pai A, Ballal S, Asha ML. Estimation of superoxide dismutase and glutathione peroxidase in oral submucous fibrosis, oral leukoplakia and oral cancer-a comparative study. Asian Pacific Journal of Cancer Prevention. 2012;13(9):4409-12.
11. Bachmeier E, Mazzeo MA, López MM, Linares JA, Jarchum G, Wietz FM, Finkelberg AB. Mucositis and salivary antioxidants in patients undergoing bone marrow transplantation (BMT). Medicina Oral, Patología Oral y Cirugía Bucal. 2014 Sep;19(5):e444.
12.Ahmed AA, Selim MA, El-Sayed NM. α-Lipoic acid ameliorates oral mucositis and oxidative stress induced by methotrexate in rats. Histological and immunohistochemical study. Life sciences. 2017 Feb 15; 171: 51-9.
13.Wu CH, Ko JL, Liao JM, Huang SS, Lin MY, Lee LH, Chang LY, Ou CC. D-methionine alleviates cisplatin-induced mucositis by restoring the gut microbiota structure and improving intestinal inflammation. Therapeutic advances in medical oncology. 2019 Feb; 11: 1758835918821021.
14. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. Journal of Oral Pathology & Medicine. 2012 Nov;41(10):736-40.
15.Sonis ST. New thoughts on the initiation of mucositis. Oral diseases. 2010 Oct;16(7):597-600.
16.Kim SC, Kim OS, Kim OJ, Kim YJ, Chung HJ. Antioxidant profile of whole saliva after scaling and root planing in periodontal disease. Journal of periodontal & implant science. 2010 Aug 1;40(4):164-71.
17.Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ. Characterization of the differentiated antioxidant profile of human saliva. Free Radical Biology and Medicine. 2002 Feb 1;32(3):268-77.
18.Sabitha KE, Shyamaladevi CS. Oxidant and antioxidant activity changes in patients with oral cancer and treated with radiotherapy. Oral Oncology. 1999 May 1;35(3):273-7.
19.Sharma M, Rajappa M, Kumar G, Sharma A. Oxidant-antioxidant status in Indian patients with carcinoma of posterior one-third of tongue. Cancer Biomarkers. 2009 Jan 1;5(6):253-60.
20.Bøhn SK, Smeland S, Sakhi AK, Thoresen M, Russnes KM, Tausjø J, Svilaas A, Svilaas T, Blomhoff R. Post-radiotherapy plasma total glutathione is associated to outcome in patients with head and neck squamous cell carcinoma. Cancer letters. 2006 Jul 18;238(2):240-7.
21.de Freitas Cuba L, Salum FG, Cherubini K, de Figueiredo MA. Antioxidant agents: a future alternative approach in the prevention and treatment of radiation-induced oral mucositis. Altern Ther Health Med. 2015 Mar 1;21(2):36-41.
22.Gao Y, Wang P, Wang Z, Han L, Li J, Tian C, Zhao F, Wang J, Zhao F, Zhang Q, Lyu Y. Serum 8-Hydroxy-2′-deoxyguanosine level as a potential biomarker of oxidative DNA damage induced by ionizing radiation in human peripheral blood. Dose-Response. 2019 Jan 7;17(1):1559325818820649.
23. Kirhan I, Kas F, Taskiran H, Buyukhatipoglu H, Gönel A, Koyuncu I. Evaluation of Micro-RNA Levels, Apoptosis and Oxidative Stress Markers in Patients Recieving Chemotherapy. Combinatorial Chemistry & High Throughput Screening. 2020 Jan 1;23(1):17-27.
24.Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M, Bekele BN, Raber-Durlacher J, Donnelly JP, Rubenstein EB. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2004 May 1;100(S9):1995-2025.