***https://doi.org/ 10.33472/AFJBS.6.5.2024. 5082-5098***

**African Journal of Biological**

# Comparative Study of Total Antioxidant Capacity, Ceruloplasmin and Malondialdehyde In Tobacco Chewers/Smokers, Patients with Oral Potentially Malignant And Malignant Lesion

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Keywords: Ceruloplasmin, Malondialdehyde, Oral Malignancy, Oxidative Stress, potentially malignant Lesions, Total Antioxidant Capacity
Running title – Comparative Study of TAC, Ceruloplasmin and MDA in Tobacco cheweres/ Smokers, Patients with OPML and Malignant Lesion.
Type of article – Original article , Type of study – Experimental Study.
Date of submission – 01/02/2024

**Abstract**

Oxidative stress is known to produce free radicles which further damages the cell and it is also known to play an essential role in progression of carcinogenesis. In this study, lipid peroxide, that is Serum Malondialdehyde (MDA), Plasma Total antioxidant Capacity (TAC) and Serum Ceruloplasmin levels were estimated in various study and control group to evaluate its role in early diagnosis and progression of oral malignancy. Method: Total samples studied were 384. 4 groups were studied with 96 samples in each group. Group A consisted of healthy control subjects who do not have any clinical conditions or diseases. Group B involved subjects who had a history of tobacco/areca nut chewing and smoking habit. Group C consisted of potentially malignant lesion patients (PML) such as erythroplakia, leukoplakia, oral submucous fibrosis (OSMF) and lichen planus and Group D included newly diagnosed Oral Squamous cell carcinoma patients (OSCC).

Serum MDA was estimated using Thiobarbituric Acid Reactive Substance (TBARS) method. Plasma TAC was estimated using Ferric Reducing Ability of Plasma (FRAP) method and Serum Ceruloplasmin was estimated by A Ravin's method. Results: Serum MDA levels were increased in the study groups when compared with healthy controls. TAC and Ceruloplasmin levels were reduced in the study groups as compared with healthy controls. Conclusion: increased levels of serum MDA and decreased levels of plasma TAC and Serum Ceruloplasmin in various study groups indicate association of

Article History
Volume 6, Issue 5, 2024
Received: 09 May 2024
Accepted: 17 May 2024
doi: ***10.33472/AFJBS.6.5.2024. 5082-5098***

## Introduction

Oral cancer is a severe type of cancer that is a matter increasing global concern. oral cancer and oropharyngeal cancer are on the surge with 377,713 and 98,412 new cases and 177,757 and 48,143 mortality worldwide per year, respectively (GLOBOCAN 2021, IARC, WHO) [1,12] The main factors correlated with mouth cancer include hazardous exposure to substance abuse, such as higher drinking, smoking, and betel nut chewing behaviors [2]. Additionally, viruses, nutritional deficiencies fungus infections, poor oral hygiene, radiation exposure, and other chronic physical and chemical stimulations may be co-factors. In addition, age, gender, geographical culture, ethnicity, and lifestyle habits also affect the incidence of oral cancer [3].

Oxidative stress is defined as a state in which there is an imbalance between free radicals and antioxidants. Additionally, the oral cavity is also susceptible to reactive oxygen species (ROS) created by inhalation of oxidizing agents in air pollution and tobacco smoke. The recognition that ROS (free radicals) and oxidative stress play a crucial role in the cause and progression of major degenerative diseases has led to enormous and worldwide interest in exogenous and endogenous antioxidants [4].

Tobacco chewing and smoking causes imbalance of oxidants and antioxidants which stimulates oxidative stress. It further causes increased lipid peroxidation which then leads to oxidative DNA damage, cellular damage, and disturbance in antioxidant defense system which can induce malignant process. The heat (generated during smoking) as well as pH. (change during chewing) of body fluids due to tobacco consumption affects formation and stabilization of free radicals [5].

In the last stage of the peroxidation process, peroxides are decomposed to aldehydes like malondialdehyde (MDA), which can be detected by thiobarbituric acid that gives a pink color easily

measurable. It is termed thiobarbituric reactive species (TBARS). This method is one of the most widely used assays to assess peroxidation in the whole organism [6].

Calcium hydroxide content of lime in the presence of areca nut is a major factor responsible for the formation of ROS, which causes oxidative damage in the DNA of buccal mucosa cells of betel quid (BQ) chewers [7]

Continuous local irritation by pan masala, gutkha, or areca nut due to its fine particulate nature induces injury-related chronic inflammation, oxidative stress, and cytokine production leading to cell proliferation, cell senescence, or apoptosis; miscoding DNA adduct; and inhibit DNA repair activity.[8]

In this study, we evaluated antioxidant capacity and lipid peroxide levels which may aid in early diagnosis and detection of oral malignancy. And these parameters can be included in the panel of early detection of malignancy along with the other biomarkers.

**Material and Methods**

This study was a cross sectional study. Total samples studied were 384. Present study was done in 4 groups,

| ***Groups*** | ***Sample size (n)*** |
|---|---|
| *A* | 96 |
| *B* | 96 |
| *C* | 96 |
| *D* | 96 |

**Group A** consisted of healthy control subjects who do not have any clinical conditions or diseases. **Group B** involved subjects who had a history of tobacco/gutka chewing, alcohol abuse and smoking

habit for more than 10 years. The subjects included in this group had no history of any illness and diseases expect for the above-mentioned habits.

The subjects studied in this group were

*i) Tobacco/ Gutka chewers – 57*

*ii) Smoking – 22*

*iii) Alcohol – 16*

**Group C** consisted of potentially malignant lesion patients such as erythroplakia, leukoplakia, submucous fibrosis and lichen planus and

**Group D** included newly diagnosed Oral squamous cell carcinoma patients.

This study was approved by Ethics Clearance Committee (157/2020-2021)

Blood samples were taken in the plain bulb and EDTA bulb, which was then centrifuged at 3000 rpm for 5-7 mins to obtain serum and plasma samples for further study.

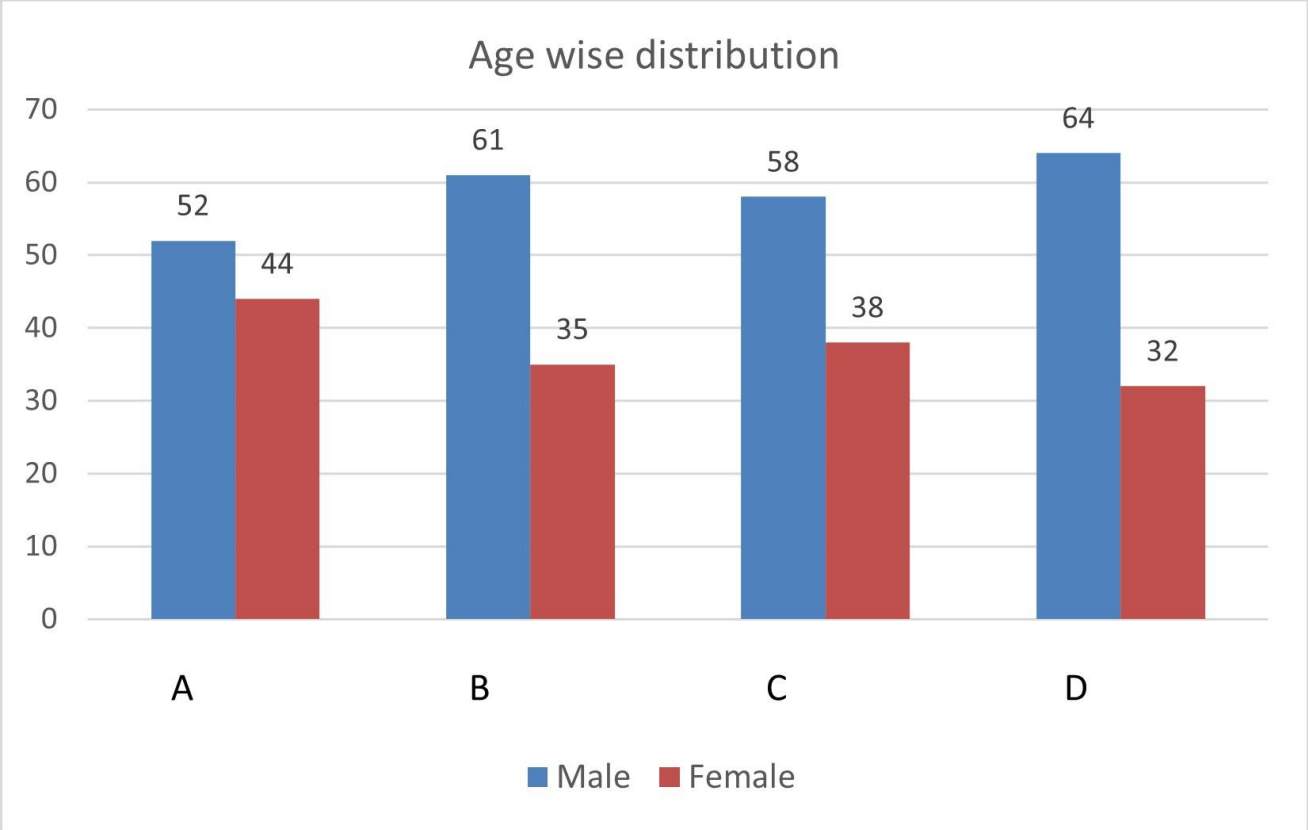
Plasma TAC was measured by FRAP method at 593nm by spectrophotometer. Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values were obtained by comparing the absorbance change at 593 nm [9].

Serum ceruloplasmin was measured by Herbert A. Ravin method at 530nm spectrophotometrically. Ceruloplasmin oxidizes P- phenylenediamine in presence of oxygen to form a purple-colored oxidized product which was measured at 530nm by spectrophotometer [10].

Serum MDA was measured using TBARS method. Malondialdehyde reacts with thiobarbituric acid to form pink colored thiobarbituric acid reactive substances which was then measured at 530nm by spectrophotometer [11]

## Results

**Graph 1-** Age wise distribution of the samples among the various groups.




**Graph 1-** It shows age wise distribution of the samples among the various groups. In Group A there were 52 males (54%) and 44 females (46%). Group B consisted of 61 males (63%) and 35 females (37%). In group C there were 58 males (60%) and 38 females (40%) and in group D there were 64 males (66%) and 32 females (44%)

One-way ANOVA test was done for comparison of the groups along with post Tukey Kramer's test.

| **Groups** | **N** | **Mean** | **SD** | **SEM** | **Min** | **Max** | |
|---|---|---|---|---|---|---|---|
| A | 96 | 1129.3 | 154.5 | 15.7 | 874 | 2160 | |
| B | 96 | 562.18 | 140.6 | 14.36 | 331 | 1201 | **P<0.001** |
| C | 96 | 388.18 | 87.1 | 8.89 | 217 | 593 | |
| D | 96 | 293.54 | 63.7 | 6.50 | 158 | 402 | |
| **Healthy Controls (Mean ±1.96x S.D)**<br>1129.3±154.58 | | | **95% C.I**<br>975 to 1283 | | | | |
| **Healthy Controls (Mean ±1.96 x SEM)**<br>1129.3±30.9 | | | **95% C.I**<br>1098.1 to 1159.9 | | | | |

**Table 1.** Comparison of Serum Total Antioxidant Capacity in Healthy Control and Study Groups.

| **Groups** | **N** | **Mean** | **SD** | **SEM** | **Min** | **Max** | |
|---|---|---|---|---|---|---|---|
| A | 96 | 3.079 | 0.72 | 0.07 | 1.900 | 4.800 | |

**Table 1** shows comparison of plasma TAC was done among the various groups. Group A, B, C and D showed mean values as 112.9.3±154.5, 562.18±140.6, 388.18±87.1 and 293.55±63.72 respectively. The mean differences on comparison of group A with group B was 567.19, group A with group B was 741.19, group A with group C was 835.83, group B with group C was 174.0, group B with group D was 268.64 and group C vs D was 94.64 All of the above-mentioned comparison was statistically significant ($p<0.001$). 95 % confidence interval (C.I) was calculated for healthy control group by calculating mean with standard deviation (Mean ± S.D) and the mean range was from 975 to 1283. Mean was also calculated with SEM and the range obtained was 1098.1 to 1159.9

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| B | 96 | 6.98 | 1.27 | 0.13 | 4.200 | 8.900 | **P<0.001** |
| C | 96 | 8.42 | 1.50 | 0.15 | 5.100 | 11.900 | |
| D | 96 | 11.25 | 1.10 | 0.11 | 9.200 | 13.600 | |
| **Healthy Controls (Mean ±1.96x S.D)** 3.079±1.411 | | | **95% C. I** 1.66 to 4.49 | | | | |
| **Healthy Controls (Mean ±1.96 x SEM)** 3.079±0.137 | | | **95% C. I** 2.942 to 3.216 | | | | |

**Table 2** - Comparison Of Serum Malonyaldehyde In Healthy Control And Study Groups.

**Table 2** shows comparison of serum MDA was done among all the four groups. The mean for all the groups were 3.07 ± 0.72, 6.98 ± 1.27, 8.36 ± 1.67 and 1.73 ± 0.37. The mean difference in comparison of group A with group B was 3.906, group A with group C was 5.348, group A with group D was 8.172, group B with group C was 1.442, group B with group D was 4.266 and group C with group D was 2.824. The comparison among the groups was statistically significant ($p<0.001$).

95% confidence interval was calculated for healthy control group by calculating mean with S.D (Mean ± S.D) and the mean range was from 1.66 to 4.49. Mean was also calculated with SEM and the

| **Groups** | **N** | **Mean** | **SD** | **SEM** | **Min** | **Max** | |
|---|---|---|---|---|---|---|---|
| A | 96 | 27.92 | 7.95 | 0.81 | 14.80 | 45.50 | |

range obtained was 2.942 to 3.216

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| B | 96 | 52.16 | 7.49 | 0.76 | 31.20 | 90.60 | **P<0.001** |
| C | 96 | 78.04 | 8.13 | 0.83 | 61.00 | 141.0 | |
| D | 96 | 87.16 | 15.54 | 1.58 | 39.50 | 124.0 | |
| **Healthy Controls ( Mean ±1.96x S.D)**<br>27.92±15.58 | | | **95% C.I**<br>12.34 to 43.5 | | | | |
| **Healthy Controls (Mean ±1.96 x SEM)**<br>27.92±1.587 | | | **95% Confidence Interval**<br>26.33 to 44.30 | | | | |

**Table 3 -** Comparison Of Serum Ceruloplasmin In Healthy Control And Study Groups.

**Table 3** shows comparison of serum Ceruloplasmin was done among all the four groups. The mean for all the groups were 27.92 ± 7.95, 52.16 ± 7.49, 78.04 ± 0.83 and 87.16 ± 15.54. The mean difference in comparison of group A with group B was 24.248, group A with group C was 50.12, group A with group D was 59.248, group B with group C was 25.87, group B with group D was 35.00 and group C with group D was 9.125. The comparison among the groups was statistically significant ($p<0.001$). 95 % confidence interval was calculated for healthy control group by calculating mean with S.D

(Mean ± S.D) and the mean range was from 12.34 to 43.5. Mean was also calculated with SEM and

the range obtained was 26.33 to 44.30

**Discussion**

Both oral cancer and oropharyngeal cancer are on the rise, with 377,713 and 98,412 new cases and 177,757 and 48,143 deaths worldwide per year, respectively (GLOBOCAN 2021, IARC, WHO) [12].

Additionally, the significant mortality rate of patients with oral cancer has not declined significantly over the past 40 years, with a 5-year mortality rate that is almost 50%. Oral squamous cell carcinomas (OSCC), which accounts for over 90% of oral neoplasms. In addition to the time needed for the process of obtaining at an accurate diagnosis of oral cancer, other data demonstrates that 30% of oral cancer patients delay seeking care for more than 3 months after they first discover signs and symptoms of the disease. [13]

Cancer is a disease of cellular behavior that is characterized by changes in cell surface glycosylation and serum glycoprotein. It is also linked to other kinds of transformation processes. Oral PMLs and oral malignancies that are detected early enough to receive treatment reduces mortality while also giving survivors a better quality of life. Most neoplasms can be prevented and treated if they are found in time, notably oral PMLs such oral leukoplakia and Oral Submucous fibrosis, which usually appear before severe oral malignancies. [14]

The discovery of relevant biomarkers should be done in order to increase the survival rates, to help in early diagnosis, and to improve quality of life due to high rates of morbidity and mortality linked to poor prognosis and diagnosis for oral cancer. Body fluids (such as saliva and blood serum) have received a lot of attention in the field of biomarker development for oral cancer and are thought to be useful tools in this context.

In general, reactive oxygen species (ROS) are products of regular cellular metabolism. Around 1–2% of the respiratory intake of oxygen by humans is converted into ROS which are further divided into two subgroups: (i) free radical species such as superoxide, hydroxyl radical and (ii) non-radical species such as hydrogen peroxide. Free radicals contain unpaired electrons and they are

characterized as "highly reactive chemical species due to such property. Because of their chemical instability, they have the tendency to directly react with various cellular macromolecules such as DNA, lipids and proteins and thus it can participate in the onset of majority of human diseases including various cancers.

ROS production contributes to carcinogenesis by mechanisms such as protein oxidation, lipid peroxidation, and oxidative DNA damage, all of which have the potential to serve as reliable biomarkers in oral cancer. To that end, the use of oxidative stress indicators could be crucial in improving the detection, diagnosis, and prognosis of oral carcinogenesis [15].

Antioxidants (vit C and E), Lipid peroxide (MDA) plays a significant role in oral cancer and precancer patients and therefore can be significant for the diagnosis of oral leukoplakia, lichen planus, OSF and OSCC. [16]

In the present study, serum ceruloplasmin, plasma TAC and serum MDA were estimated and were statistically significant while comparing among the groups. Statistical analysis was done through ANOVA test followed by Tukey Kramer's post test.

In Table 1, comparison of plasma TAC was done among the various groups. Group A, B, C and D respectively. Plasma TAC was seen to be decreased in group B, C and D when compared with the healthy controls. Malignant lesion patients were seen to have the lowest mean when compared with the other groups. These results indicate that antioxidant levels were seen decreasing as the stage progresses leading to oxidative stress. Similar studies were done by Korde et. al. Their study shows that the oxidative stress was increased in the study groups by evaluating TAC and TNO (Total nitric oxide) -MDA levels in PML patients and oral malignant patients. TNO-MDA level was elevated and TAC levels were decreased indicating increased oxidative stress. The positive TNO-MDA association suggest that oxidative DNA damage which is a vital phenomenon for carcinogenesis. [17].

Total antioxidant capacity showed its lowest levels in oral squamous cell carcinoma patients. TAC is a significant diagnostic marker that can be utilized in diagnosis of oral squamous cell carcinoma. [18]. These studies were seen to be correlating with our results.

In Table 2, comparison of serum MDA was done among all the four groups. The serum MDA was seen to be increased in group B, C and D when compared with healthy controls. The mean MDA level was seen to be the highest in malignant lesion patients indicating highest lipid peroxide levels. MDA is used as an indicator for lipid peroxidation levels caused by oxidative stress.

Our results correlate with chole et al's study which shows increased MDA levels in oral cancer and precancer which could be served as a valuable marker in prevention and clinical intervention. Therefore, Increased serum malondialdehyde in oral cancer and oral precancer would serve as a valuable marker for both preventive and clinical intervention, and may deserve further investigation for the early diagnosis, treatment, and prognosis. [19]. According to Nielsen et al, daily smokers had a slightly higher average concentration of plasma MDA than nonsmokers, and plasma MDA correlated with daily exposure to cigarette smoke. Nielsen et al demonstrated a positive correlation between plasma MDA and weekly alcohol consumption. [20]

In Table 3, comparison of serum Ceruloplasmin was done among all the four groups. It was seen increased in the groups B, C and D when compared with the healthy controls. Malignant lesion patients were seen to be having highest mean values of ceruloplasmin when compared with the other groups. Ceruloplasmin. Our results were seen to be correlating with the Patil et al study. They observed that serum ceruloplasmin was raised in OSMF, OSCC and oral leukoplakia group compared to healthy controls. Therefore, they concluded that ceruloplasmin can be used as a potentially reliable marker for assessing and monitoring PMLs and frank OSCC along with clinical diagnostic procedures. [21]

They concluded that the serum ceruloplasmin levels were significantly increased in the PML and Malignant groups as compared to Healthy Control group ($p < .001$). Serum ceruloplasmin can be used as diagnostic marker for oral premalignant and malignant lesions. [22]

## Conclusion

Plasma TAC levels were found to be decreased and Serum ceruloplasmin and serum MDA levels were raised which indicates increased oxidative stress with progression of oral malignancy and pre malignant lesions. Thus, it could be concluded from our observations that these parameters can be helpful in early diagnosis and prognosis of oral malignancy can be further added as an adjunctive diagnostic marker for oral cancer.

## Conflict of Interest

Nil

## Author's Contribution

All the authors contributed equally in this article by executing the idea, verifying the analysis and contributing in results and discussion.

## Acknowledgement

I would like to express my gratitude to the management, Department of Oncology and Radiotherapy and Department of Oral Medicine, Krishna Vishwa Vidyapeeth for their guidance and constant support.

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