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Estimation of serum antioxidative enzyme glutathione peroxidase, serum copper and zinc ratio in patients with oral potentially malignant disorders and oral cancer -A comparative biochemical study

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Abstract

Oral cancer is a life-threatening disease that most commonly arises from OPMDs, necessitating the need for early detection. This cross-sectional study assessed serum levels of antioxidants and trace elements like glutathione peroxidase (GPx), copper (Cu), and zinc (Zn) as potential biomarkers in 75 participants categorized into three groups, with 25 each, Group I (OPMDS), Group II (OSCC), and Group III (healthy controls). Serum levels of GPx, Cu, and Zn were analyzed by using ANOVA followed by Tukey's post hoc tests. Results showed GPx levels were noticeably lower in Groups I and II compared to Group III. Copper levels were highest in Group II, followed by Group I and lowest in Group III, showing statistical significance. Zinc levels varied significantly between Groups II and I but not between Groups I and III. The Cu: Zn ratio was highest in Group I, followed by Group III and lowest in Group II, but this difference was not statistically significant. Findings suggest that reduced GPx levels may heighten oxidative stress. Elevated serum copper and altered Cu: Zn ratio suggest their role in cancer progression.

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So, evaluation of these biomarkers can be one of the alternative diagnostic methods for early detection of oral cancer. Standardization of such methods may improve oral cancer screening and prognosis.

Key words : Oral cancer, potentially malignant lesions, glutathione peroxidase (GPx), copper, zinc, Cu: Zn ratio.

Oral cancer is a critical life-threatening disease with a high global incidence. According to the 2020 GLOBOCAN report, approximately 0.37 million new cases & 0.17 million new deaths were recorded due to lip and oral cavity cancer, with the majority of them in Asia.[34] In India, due to oral cancer, there were annually 77,000 incident cases & 52,000 deaths, approximately representing about 1/4th of global incidences.[39] Several factors, such as genetic and epigenetic influences, microbial interactions, habitual behaviours, and lifestyle choices, play a significant role in the development and progression of oral cancer.[22] The majority of cases arise from oral potentially malignant disorders (OPMDs); these lesions have potential for malignant transformation depending upon the quantity and duration of risk factors like tobacco use.[31] The early identification of these suspicious oral lesions is essential to reduce the risk of malignant transformation and the mortality rate.[26]

Various methods have been implemented for diagnosing these potentially malignant lesions; amongst them, biopsy remains the gold standard.[18] However, finding screening techniques that are simple, affordable, and reliable by minimizing patient discomfort remains a challenge.[1] Investigating and identifying predictive biomarkers that can estimate the probability of malignant transformation is necessary to provide individualized OPMD management and thereby reduce the incidence of oral cancer.[25] One of such alternative approach includes an estimation of antioxidant levels in the body fluids like serum, as they reflect an individual's oxidative stress status.

Anti-oxidants can be broadly categorized into three forms: they are enzymatic forms, which include both endogenous and exogenous antioxidative enzymes, mineral forms and vitamin forms. Enzymatic forms include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase & glutathione reductase (GSH). Mineral forms include copper, zinc, iron, selenium & magnesium, etc. Vitamin forms include vitamin A, C & E.[5] In this present study, we considered one of the antioxidative enzyme glutathione peroxidase (GPx), mineral forms Copper and zinc levels, as they were interrelated and were necessary for DNA, RNA synthesis, which was required for the maintenance of normal cellular activity.[4,27] Due to complex interactions between these two trace elements within the body, any imbalance in their levels can be predicted by the Cu: Zn ratio. Higher levels of Cu: Zn ratio often signify the potential risk of malignancy.[19,20,28] With this background, this cross-sectional study was performed with an aim to analyse GPx, copper & zinc levels and also Cu: Zn ratio.

A total of 75 patients were included, who were further divided into 3 groups, with

25 in each group. Group I included patients with clinically and histopathologically diagnosed potentially malignant disorders; Group II consists of patients with confirmed oral squamous cell carcinoma (OSCC); and Group III included controls as healthy individuals. Patients under 18 & above 65 years or with any systemic illness or with any previous history of treatment for the potentially malignant lesions and oral squamous cell carcinoma were excluded. After clinical and histopathological confirmation, the study objectives were explained & and informed consent was obtained from the patient. Later, Phlebotomy was performed using a 3ml syringe with a 24-gauge 1 1/2 needle, and 2ml blood was drawn from the antecubital vein. After clotting, the serum was separated by centrifugation at 3000 rpm and stored at 4ºC until the analysis was done by adding Ellman's reagent (DTNB), a color reagent (Figure-1). And the values were tabulated and subjected to statistical analysis by using ANOVA followed by Tukey's post hoc tests.

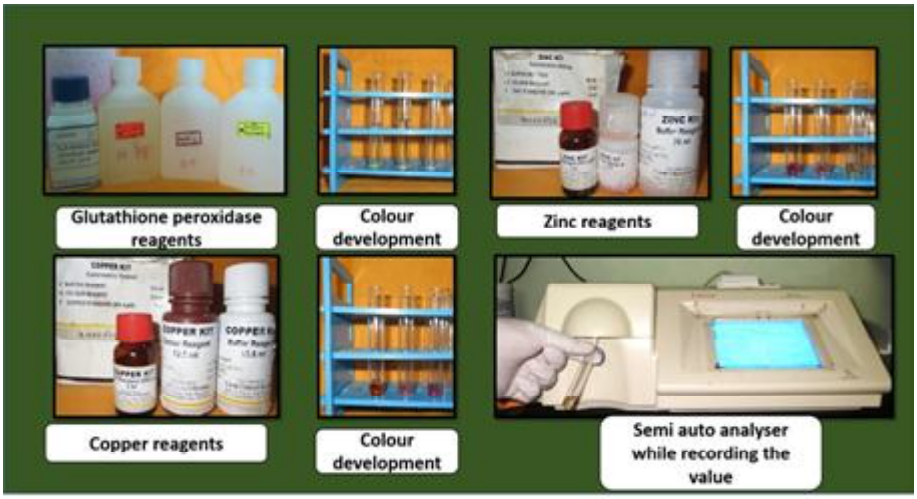

Figure 1. Procedure and Reagents used

Out of 75 subjects, most of the subjects in Group I were Oral Leukoplakia, Oral Lichen Planus, OSMF patients and Group II were oral squamous cell carcinoma patients within the age group of 40 to 60 years, and a few subjects belonged to the age range of 20 to 40 years. When the mean values of copper, zinc, glutathione peroxidase, and copper: zinc ratio were compared across the groups, the copper values were higher in the three groups, with more prevalence of females in group I and group III, males in group II(Table-1,2,3). (Graph-1,2,3,4) depicts intergroup comparative analysis of Gpx levels, copper levels, zinc levels, and cu: Zn ratios, which showed a significant difference between the three groups with higher mean values in group III (3.08±0.58) for Gpx, group II (191.40±26.46) for copper and zinc levels, group 1 (1.46±0.48) for cu: Zn levels. Values of post hoc analysis depicted a significant difference between all three study groups for Gpx levels, copper levels, but in the zinc and cu: Zn ratio group II showed a significant (p=0.000) difference with group I

& group III, whereas no significant difference between group I & III.

Oral cancer is a prevalent malignancy globally, with particularly high rates in India, accounting for 50-70% of all cancer cases.[2,7,16,18] It has one of the highest mortality rates among cancers, predominantly presenting as oral squamous cell carcinoma. Major risk factors include tobacco use along with alcohol

Graph -1. Inter-group comparison of Gpx Levels

Graph -2. Inter-group comparison of copper Levels

Graph -3. Inter-group comparison of Zinc Level

Graph -4. Inter-group comparison of Cu: Zn Levels

consumption, genetic, viral, environmental, social & behavioural factors and also increased age.[16,17,18,23] The present study showed 53% males and 47% females, with the highest female predilection in group II. Oral carcinomas often arise from potentially malignant lesions, which are more common than oral cancer itself and need early detection to prevent progression to malignancy.

Clinical and histopathological examinations are standard for identifying these lesions; sometimes, conventional oral exams may misdiagnose the early cases, as many oral

Table-1. Comparison of mean Copper, Zinc, Glutathione peroxidase levels, and Copper: Zinc ratio among females and males in Group I

	N	Sex	Mean ± *S.D.*	Unpaired t value	P-value
Copper	6	Females	130.16±44.53	.050	.0961
	19	Males	129.15±42.99		
Zinc	6	Females	92.16±53.09	-.415	.682
	19	Males	104.68±67.27		
Gpx	6	Females	2.05±0.63	.895	.380
	19	Male	1.70±0.87		
Cu:Zn ratio	6	Females	1.60±0.64	0.840	0.410
	19	Males	1.41±0.42		

(Statistical analysis: unpaired t test, SD- standard values, *P value < 0.05- Considered Statistically Significant*)

Table-2. Comparison of mean Copper, Zinc, Glutathione peroxidase levels, and Copper: Zinc ratio among females and males in Group II

	N	Sex	Mean ± *S.D.*	Unpaired t value	P-value
Copper	15	Females	164.93±46.11	-1.647	113
	10	Males	191.60±26.65		
Zinc	15	Females	142.53±62.70	-1.780	.088
	10	Males	180.00±26.05		
Gpx	15	Females	1.56±0.69	2.797	.010
	10	Male	0.90±0.31		
Cu:Zn ratio	15	Females	1.30±0.48	1.494	0.149
	10	Males	1.06±0.11		

(Statistical analysis: unpaired t test, SD- standard values, *P value < 0.05- Considered Statistically Significant*)

cancers start as small, asymptomatic lesions.[1]

Oxidative stress, involving free radicals such as hydrogen peroxide & hydroxyl radicals, plays a role in cancer development. Cells counteract this stress using antioxidants like glutathione and enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR).[5,37] Dysfunction in these antioxidants can increase cancer risk.[3]

Trace elements like copper and zinc are crucial in various physiological processes. Copper, involved in oxidation-reduction

Table-3. Comparison of mean Copper, Zinc, Glutathione peroxidase levels, and Copper: Zinc ratio among females and males in Group III

	N	Sex	Mean ± *S.D.*	Unpaired t value	P-value
Copper	14	Females	109.85±33.17	420	678
	11	Males	104.45±30.18		
Zinc	14	Females	79.00±35.45	-284	779
	11	Males	83.45±43.07		
Gpx	14	Females	2.68±0.62	-462	649
	11	Male	2.81±0.62		
Cu:Zn ratio	14	Females	1.39±0.20	1.568	0.131
	11	Males	1.27±0.19		

(Statistical analysis: unpaired t test, SD- standard values, *P value < 0.05- Considered Statistically Significant*)

reactions and tumour angiogenesis, is often elevated in cancer patients.[24,35] Zinc, essential for enzyme function and DNA repair, can influence cancer progression when levels are abnormal due to inflammation and oxidative stress.[12,13] The Cu: Zn ratio may be a better indicator of cancer progression than individual trace element levels.

Studies have measured GPx, copper, and zinc levels in various biological samples, including tumor tissues, blood, and saliva, to understand oxidative stress in cancer and improve diagnostic tools.[4,10,15,19,22,31,36] Monitoring these factors could provide insights into cancer risk, progression, and treatment strategies.

Serum Gpx levels were compared among three groups; they showed decreased levels in group II & group I than group III, which showed statistical significance (Graph -1). These findings are similar to studies conducted by Uikey *et al.*[36], Fiaschi *et al.*[13], Khanna *et al.*[18], Richie *et al.*[26], Gurudath *et al.*[15], Basu *et al.*[9], Shetty *et al.*,[29] and contrary to study conducted by Bagul *et al.*[6] & Deshpande *et.al.*[11] and One possible explanation is that high oxidative stress and lipid peroxidation lead to an increase in free radicals. In response, the body may try to compensate by increasing the levels of glutathione peroxidase (Gpx).[9] When serum Cu levels were compared among the three Groups, it revealed increased Cu levels in group II, followed by group I, and least in group III, which were statistically significant (Graph - 2). These are in accordance with studies done by Khanna *et al.*[18], Balpande *et al.*[8], Shettar *et al.*[28], Tadakamadla *et al.*[34], Ayinampudi *et al.*[4], Kode *et al.*[19], Srilekha *et al.*[32], Bose *et al.*[10] & contrary to Kumar *et al.*[20] one of possible explanation of increased cu levels can be due to chronic inflammation, angiogenesis, oxidative stress, and metabolic alterations associated with tumor development and progression.

Serum Zn levels, when compared among three groups, revealed increased levels

in group II, followed by group I and least in group III, which showed statistical significance (p=0.000), but group I, when compared with group III, did not reveal a significant difference (p=0.139) (Graph - 3). These findings were in accordance with studies done by Ayinampudi *et al.*[4], Baharvand *et al.*[7], Fisher *et al.*[14] & were in contrary with Balpande *et al.*[8], Shettar *et al.*[28], Bagchi *et al.*[5], Shetty *et al.*[29], Hosthor *et al.*[16], Kumar *et al.*[20] & Srilekha *et al.*[32].

When the serum Cu: Zn ratio was compared among Groups, there was an increased ratio in group I, followed by group III & group II, which were statistically insignificant (p=0.387). (Graph - 4). These findings were similar to studies done by Balpande *et al.*[8], Shettar *et al.*[28], Kode *et al.*[19], Shetty *et al.*[29], Fisher *et al.*[14] & were in contrary to Ayinampudi *et al.*[4] and Kumar *et al.*[20].

The study highlights significant differences in antioxidant and trace element levels among individuals with potentially malignant oral lesions, oral cancer, and healthy controls. Key findings include reduced levels of glutathione peroxidase (GPx) in cancerous lesions compared to healthy controls, and increased copper levels and altered copper-to-zinc (Cu) ratios in individuals with oral cancer. These results underscore the potential of these biomarkers in differentiating between cancerous and non-cancerous conditions. Elevated serum copper and altered Cu ratios suggest their roles in cancer progression, while reduced GPx levels may indicate heightened oxidative stress. So, the study supports the potential utility of measuring antioxidant and trace element levels as complementary diagnostic tools for early detection and management of oral cancer. For validating these findings and exploring their clinical applications, further research is needed.

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