

# Interdependence of Antioxidants and Micronutrients in Oral Cancer and Potentially Malignant Oral Disorders: A Serum and Saliva Study

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## Abstract

**Objective:** In our previous studies we have evaluated the role of antioxidants and trace elements in potentially malignant disorders and cancers of the oral cavity, taking into consideration the importance of antioxidants as biomarkers in cancer detection. We felt that other than evaluation, the correlation and interdependence that existed among antioxidants and trace elements require further evaluation in order to develop a better understanding.

**Materials and Methods:** Serum and salivary zinc, glutathione, and superoxide dismutase levels were evaluated in 65 healthy controls, 115 subjects with potentially malignant oral disorders, and 50 subjects with oral squamous cell carcinoma, using the atom absorption photometry, [5, 5-Dithiobis (2 nitrobenzoic acid)], and nitroblue tetrazolium methods, respectively.

**Results:** Serum zinc and serum glutathione showed significant positive correlation ( $r=0.76$ ,  $P=0.01$ ). Similarly, salivary glutathione and salivary zinc levels had a positive correlation ( $r=0.68$ ,  $P=0.01$ ). Serum superoxide dismutase showed a strong positive correlation with serum zinc ( $r=0.64$ ,  $P=0.01$ ). Similarly, there was a moderate positive correlation between salivary superoxide dismutase and salivary zinc ( $r=0.67$ ,  $P=0.01$ ).

**Conclusion:** Our findings showed that trace elements and antioxidants exhibited interdependence in serum, as well as in saliva, in both physiologic and pathologic states such as oral cancer.

**Keywords:** Antioxidants; Serum, Saliva; Micronutrients; Zinc; Copper; Superoxide dismutase; Glutathione

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## INTRODUCTION

Trace elements like zinc, copper, manganese, and selenium act as essential components of

antioxidant enzymes [1]. Several antioxidants and trace elements in the human body are interdependent.

Copper and zinc act as cofactors for the proper functioning of superoxide dismutase (SOD) [2]. Zinc is required to maintain adequate levels of glutathione (GSH) in the blood [3]. Ascorbic acid (AA) is required for the absorption of iron [4].

The individual roles of these micronutrients and antioxidants have been studied previously [2]. However, besides evaluation, the correlation and interdependence that existed among these parameters require further evaluation in order to develop a better understanding. In the past, serum was the most common medium for studying these parameters. However, in recent years saliva has emerged as an equivalent, not a superior medium. Saliva collection is non invasive and easier compared to that of serum [5].

Most of the molecules found in the serum are also detected in the saliva but in lower concentrations [6]. Another important factor is the anatomical proximity of salivary glands to oral cancer and precancerous lesions of the oral cavity [7]. Because of these distinct advantages of saliva, this study was conducted to evaluate the correlation between the levels of SOD, zinc, and glutathione in serum and saliva.

## MATERIALS AND METHODS

A cross sectional study was conducted involving 230 subjects between the ages of 20 and 60 years old, reporting to the Departments of Oral Medicine and Radiology. This study was approved by our institution's Ethics Committee. All subjects signed an informed consent form. Of the 230 study subjects, the control group consisted of 65 healthy controls (Group HC) with no history of systemic diseases or prescription medication intake. The second group consisted of 115 patients with potentially malignant oral disorders (referred to as group PMD), which were clinically diagnosed and histopathologically evaluated (this comprised 65 cases of oral submucous fibrosis, and 50 patients with oral leukoplakia).

The third group, referred to as OSCC, consisted of 50 patients with oral squamous cell carcinoma. Patients with a previous history of malignancy or history of antioxidant/ micronutrient medications were excluded from this study. Serum was collected from antecubital vein using standard aseptic technique. Unstimulated saliva was collected from subjects between 9:00 AM and 12:00 PM to avoid diurnal variation. The subjects were requested not to eat, drink, perform oral hygiene activities or chew gum 60 minutes prior to saliva collection procedure. The subjects were then seated on a dental chair and asked to spit in a graduated container every one minute until 5 ml of saliva was obtained. During saliva collection, subjects were instructed not to speak or swallow. The serum and salivary zinc, GSH, and SOD were analysed by atom absorption photometry, 5, 5-Dithiobis (2 nitrobenzoic acid) (DTNB) method, and nitroblue tetrazolium method (NBT) respectively. Statistical analysis was performed using SPSS version 17 software (Microsoft, IL, USA). Scheffe's test was used in conjunction with ANOVA for comparison between multiple unequal groups, and Spearman's test was used to detect correlation.

## RESULTS

Among the confounding factors, the mean age of the subjects with OSMF and OL in group PMD was significantly different, which could be due to prevalence of Gutkha (tobacco) chewing habits among younger individuals. There was a significant decrease in serum and salivary GSH in groups PMD and OSCC when compared to group HC (Table 1). There was a significant decrease in serum and salivary SOD in groups PMD and OSCC when compared to group HC (Table 2). Similarly, there was a significant decrease in serum and salivary zinc levels in groups PMD and OSCC when compared to group HC (Table 3). Serum zinc and GSH showed a significant positive correlation ( $r=0.76$ ,  $P=0.01$ ) (Figure 1).

**Table 3.** Comparison of serum and salivary GSH levels in the study groups

Parameter	N	Mean (µg/dL)	Standard Deviation	F(ANOVA)	P
<b>Serum GSH</b>					
HC	65	188.04	36.56		
PMD	115	102.87	23.69	14.252	<0.001 hs
OSCC	50	94.79	27.06		
<b>Salivary GSH</b>					
HC	35	94.67	36.59		
PMD	115	51.08	19.11	53.115	<0.001 hs
OSCC	50	47.87	23.17		

ANOVA test hs= Highly significant

**Table 2.** Comparison of serum and salivary SOD levels in the study groups

Parameter	N	Mean	Standard Deviation	F(ANOVA)	P
<b>Serum SOD (U/mgHb)</b>					
HC	65	4.34	0.06		
PMD	115	2.89	0.26	34.213	<0.001 hs
OSCC	50	2.09	0.16		
<b>Salivary SOD (U/mgPr)</b>					
HC1	35	0.17	0.03		
PMD	115	0.11	0.03	23.215	<0.001 hs
OSCC	50	0.07	0.01		

ANOVA test hs= Highly significant

**Table 1.** Comparison of serum and salivary Zn in the study groups

Parameter	N	Mean (µg/dL)	Standard Deviation	F(ANOVA)	P
<b>Serum Zn</b>					
HC	65	161.05	17.81		
PMD	115	147.66	12.86	24.733	<0.001 hs
OSCC	50	103.23	9.11		
<b>Salivary Zn</b>					
HC	65	36.59	4.19		
PMD	115	22.25	5.32	28.219	<0.001 hs
OSCC	50	16.97	5.36		

ANOVA test hs=Highly significant

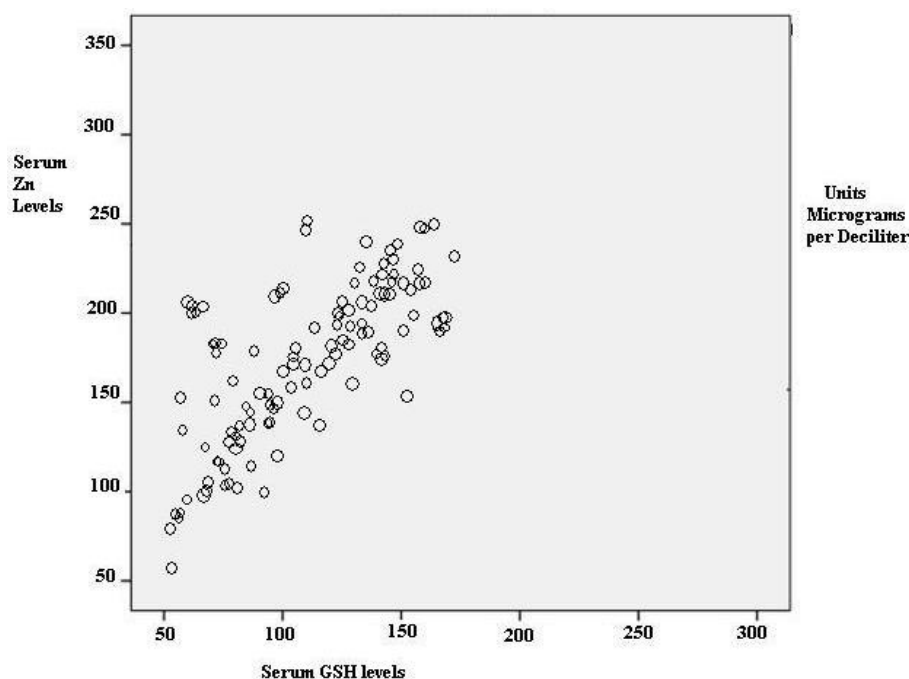


Fig 1. Correlation of serum zinc and serum glutathione levels in the study groups.

Similarly, salivary GSH and zinc showed a significant positive correlation ( $r=0.68$ ,  $P=0.01$ ) (Figure 2). We also found a positive correlation between serum and salivary SOD levels ( $r=0.57$ ,  $P=0.01$ ). There was also a positive correlation between serum SOD and zinc ( $r=0.64$ ,  $P=0.01$ ) (Figure 3). Finally, there was a positive correlation between salivary SOD and salivary zinc ( $r=0.67$ ,  $P=0.01$ ) (Figure 4).

## DISCUSSION

There have been no studies that show a correlation between serum and salivary GSH to date. In the current study, we report a moderate correlation between serum and salivary GSH levels. In a recent study researchers found significant decreases in both serum GSH and zinc levels in patients with OL, but no correlation between them [8]. We have found a significant correlation between the levels of serum and salivary GSH and serum and salivary zinc ( $r=0.76$ ). We also found a significant correlation between salivary zinc and GSH levels.

Glutathione is a major tripeptide non-enzymatic antioxidant present in millimolar concentrations [9].

Superoxide dismutase inactivates superoxide ion by transforming it to hydrogen peroxide [10]. Zinc acts as an antioxidant primarily by the protection of sulfhydryl groups against oxidation and inhibition of production of reactive oxygen free radicals by transition metals. In addition, supra-physiological concentrations of zinc have antioxidant-like effects in organelle-based systems and isolated cell-based systems in vitro [11].

Researchers have stated two key reasons for relating GSH levels with zinc levels. The decrease in GSH may be related to the observation that in zinc-deficient individuals, there is a 7-fold increase in the urinary excretion of glutamic acid. Thus, there may be a decreased availability of this precursor (glutamic acid), which is essential for production and maintenance of GSH. Additionally, zinc is supposed to play an important role in the maintenance of reduced GSH [3].

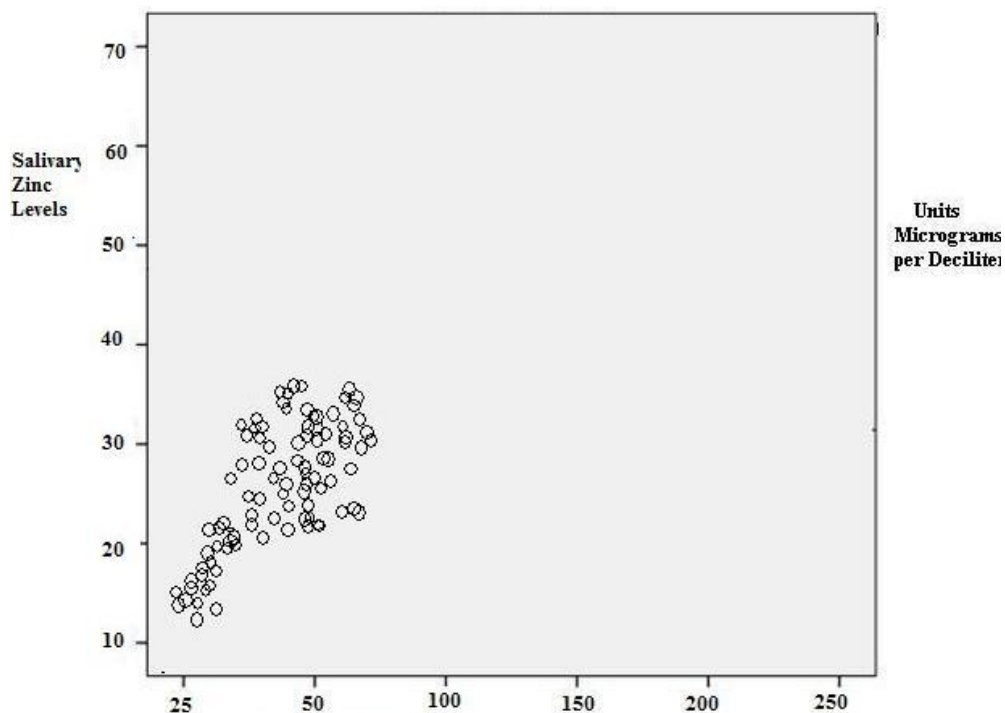


Fig 2. Correlation between salivary zinc and glutathione levels in groups.

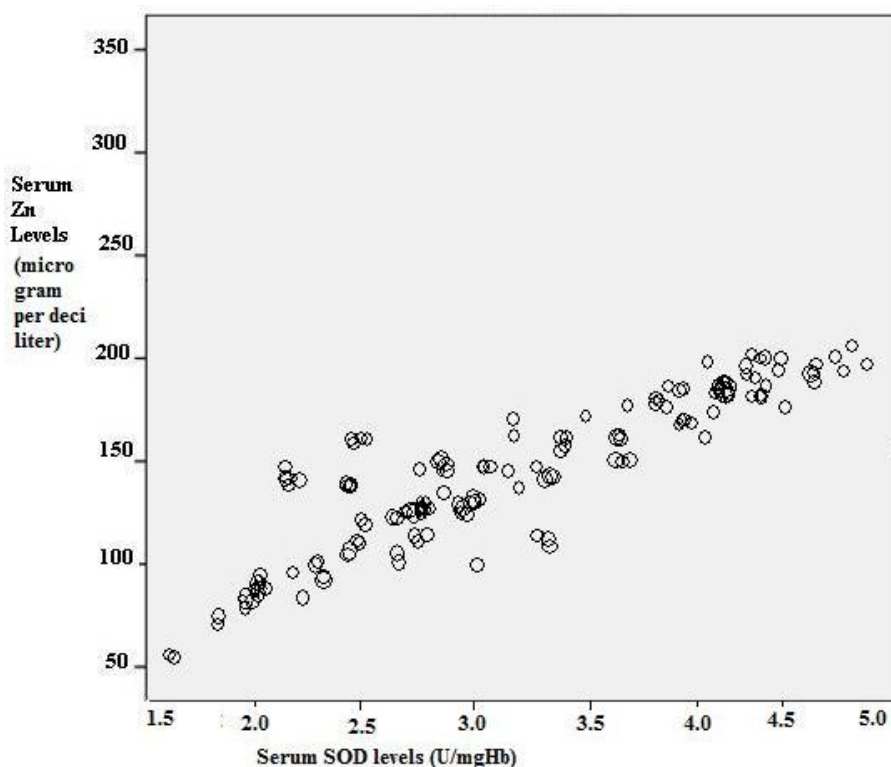


Fig 3. Correlation of serum zinc and SOD levels in the study groups.

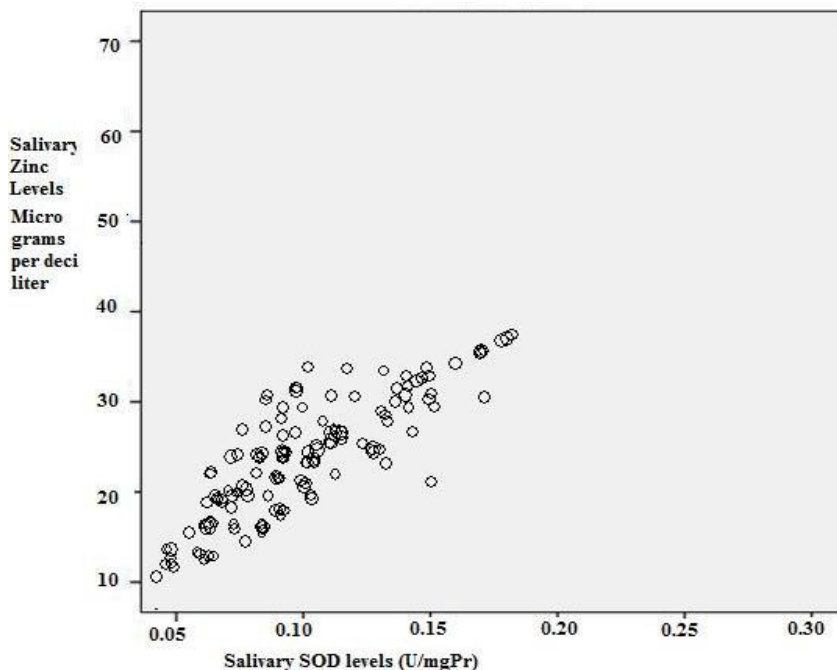


Fig 4. Correlation of salivary Zinc and SOD levels in the study groups.

In the current study, a positive correlation was observed between SOD and zinc levels in serum as well as in saliva, whereas in 2011 Swain et al. reported decreased levels of serum SOD activity and serum zinc levels in patients with OL and OSCC [9].

However, there was no correlation between the two. There are also no prior studies reporting a correlation between the levels of zinc and SOD activity in the saliva, which makes our study unique.

The reason for the correlation between zinc and SOD is that zinc is a cofactor for copper-zinc SOD, which forms a part of the primary antioxidant system of vertebrates [12,13]. Animal experiments have shown that young, growing rats, fed zinc-deficient diets were characterized by low plasma zinc concentrations and decreased plasma SOD activity [14]. Recent studies have also shown a strong positive correlation between serum zinc levels and SOD activity in gastro-intestinal tract tumors, thus reinforcing the fact that their correlation needs to be evaluated and studied in oral malignancies [15,16].

## CONCLUSION

In our earlier studies we highlighted the altered levels of serum and salivary trace elements in patients with oral carcinoma and potentially malignant oral disorders [17-20]. The results from the current study show that the changes in the levels of trace elements and antioxidants are correlated. This is exhibited in serum and saliva of healthy controls, patients with oral squamous cell carcinoma, and subjects with potentially malignant oral disorders.

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