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Title: Determination of role of ceruloplasmin in oral potentially malignant disorders and oral malignancy – A cross sectional study

Running title: Role of ceruloplasmin in OPMDs and oral cancer

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Abstract

Objectives: In the process of carcinogenesis, lipid peroxidation and increased oxidative stress leads to changes in certain antioxidants. The present study was aimed to assess and co-relate serum levels of ceruloplasmin in oral premalignancies and oral cancer so as to gauge its possible association with the process of carcinogenesis and to determine its role as tumor marker.

Material and Methods: The total of 300 participants, were equally divided in six study groups i.e. Oral Submucous Fibrosis (OSMF), Oral Leukoplakia (OL), Nicotina Stomatitis (NS), Oral Malignancy (OM), Controls (C) and Healthy Controls (HC). 5 ml of blood was collected from ante cubetal vein from each participant. The serum was analyzed for ceruloplasmin levels using ERBA CHEM 5 PLUS semi automated chemistry analyzer and diagnostic kit by turbidimetric immunoassay.

Results: There were total 242 males and 58 females, who were between 18 to 82 years of age, with a mean of 45.31 ± 13.97 years. The serum ceruloplasmin levels were significantly increased in OM, OSMF, OL & NS groups as compared to C and HC groups ($p<0.001$). No statistically significant difference was found in intragroup analysis of the disease groups ($p>0.05$).

Conclusion: Serum ceruloplasmin can be used as diagnostic marker for oral premalignant and malignant lesions.

Introduction

Cancer is the most formidable health problems faced by the human being in the today's world. There are enormous variations in global incidence of head and neck cancer. The cancer of the oral cavity is attributed to cause significant morbidity and mortality. The 5-year survival rate for oral cancer is estimated to be 50 precent (Warnakulsuriya, 2009). In the Indian subcontinent, because of cultural, ethnic, geographic factors and due to popularity of the addictive habits, the frequency of oral cancer is noted to be highest as compared to other countries in the world. In India, the occurrence of oral cancer is up to 40% of all cancer thereby representing a major health problem (Sankarnarayanan, 1990).

Amongst all the cancerous lesions, oral cancer is the only one which shows signs and symptoms before developing, which are commonly known as Oral Potentially Malignant Disorders (PMD's). Where premalignant lesions are said to be localized and premalignant conditions are generalized states, both having increased potential for malignant transformation as compared to their normal counterparts. Tobacco and areca nut related habits remain as an epicenter for etiological risk factors for the high incidence of oral PMD's in India. Oral Leukoplakia, oral erythroplakia and palatal changes associated with the smoking habits are considered to be the common oral premalignant lesions, while oral Submucous fibrosis is a common oral premalignant condition (Greenberg et al, 2005).

PMD's like Oral Leukoplakia, Oral Submucuous Fibrosis, Nicotina Stomatitis and Oral Malignancy usually alter body's biochemical mechanism (Sies et al, 1992). Sequential pathological multistage carcinogenesis has been observed in the form of alteration from hyperplasia to dysplasia to neoplasms. Chemical carcinogenesis is a progressive process. Biochemical studies involving the relationship of enzymes, proteins and glycoprotein with malignancy have reported that various substances change quantitatively in the serum during tumor development and are collectively termed as tumor markers or biochemical serum markers (Kadam et al, 2011).

Ceruloplasmin is a metal binding glycoprotein that acts as an endogenous antioxidant (Mark et al, 1998).The plasma concentration of ceruloplasmin alters in acute infections, rheumatoid arthritis, cirrhosis, Wilson's disease and also in oral potentially malignant disorders and oral malignancy (Rao and Rao, 2013).

The purpose of present study is to assess and co-relate serum levels of ceruloplasmin in Oral Leukoplakia, Oral Submucuous Fibrosis, Nicotina Stomatitis and Oral Malignancy and to compare it with that of the healthy individuals so as to gauge its possible role as a biomarker for oral PMD's and oral malignancy and to determine its utility as a diagnostic as well as a prognostic tool in these cases.

Materials and Methods

The present study was conducted in the department of Oral Medicine and Radiology, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth University, Vadodara, Gujarat. The permission to conduct the study was obtained from Institutional Ethics Committee (IEC) with the approval number SVIEC/ON/DENT/BMPG13/D14223 dtd. 13/02/2014.

Subject Selection

The study population comprised of 300 participants, equally divided into six study groups – Oral Leukoplakia (OL), Oral Submucous Fibrosis (OSMF), Nicotina Stomatitis (NS), Oral Malignancy (OM), Controls (C) and Healthy Controls (HC). The control group comprised of 50 participants with tobacco and/or areca nut habit for > 2 years but free from oral lesion and the healthy control group comprised of age and sex matched healthy controls. The lesions were diagnosed clinically according to predetermined diagnostic and staging criteria by two specialists. When required, the diagnosis was confirmed histopathologically. Participants who had undergone treatment for the lesions and having systemic disease were excluded from the study.

Sample collection and analysis

After obtaining the written consent from each and every participant, 5 ml of blood was aspirated from right / left antecubetal vein, under aseptic conditions while maintaining similar diurnal conditions. The blood was collected in a plain vaccute which was then centrifuged at 2000 rpm for 10 minutes to separate the serum. The serum was analyzed for ceruloplasmin levels by using a diagnostic kit - SensIT ceruloplasmin with calibrator, Agappe Diagnostic Ltd. and ERBA CHEM. 5 PLUS semi automated chemistry analyzer. The leftover serum was discarded with necessary biomedical waste guidelines.

Statistical Analysis

The collected data was subjected to statistical analysis by using statistical software, IBM SPSS v. 19.0. The tests applied were descriptive statistics, one way ANOVA test and post HOC Tukey's. The level of significance was set at $p<0.05$.

Results and Observations

The age of the participants was ranging from 18 to 82 years with a mean age of 45.31+ 13.97 years. There were total 242 males and 58 females with the M:F ratio of 4.17:1. (Table 1)

The serum ceruloplasmin levels were estimated in all the groups and the statistical difference was analyzed by using one way ANOVA test. The mean serum ceruloplasmin level was minimum in HC group (73.42 mg/dl) and was maximum in OSMF group (94.89 mg/dl). The difference between the groups was found to be statistically highly significant. ($p<0.001$). (Table 2)

The co-relation of serum ceruloplasmin levels between each study group was performed by Tukey's Post HOC analysis. The difference was statistically significant ($p<0.05$) between OSMF & NS group and OSMF & C group; whereas statistically highly significant ($p<0.001$) between OSMF & HC group and OM & C group. (Table 3)

Intergroup co-relation of serum ceruloplasmin levels was performed using one way ANOVA test for OL, OSMF and NS group which was found to be statistically not significant. ($p>0.05$)

Discussion

The term free radical is generally used to describe a molecular fragment containing one or more unpaired electron in its valance shell and is capable of existing independently (Halliwell and Chirico, 1993). In experimental and clinical medicine they are more commonly described as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Halliwell and Gutteridge, 1999). Oxidative stress is defined as a condition in which the increased concentration of ROS and RNS overwhelms antioxidant defense capacity leading to oxidative damage to biomolecules (Halliwell and Gutteridge, 1999). The important mechanism for carcinogenesis is

oxidative damage to biomolecules and lipid peroxidation. This results in cell signaling leading to generation of cancer (Valko et al, 2006). These harmful effects of free radicals are balanced by enzymatic and non-enzymatic antioxidants (Halliwell et al, 1996).

Ceruloplasmin, a copper containing acute phase reactant plasma protein is known to have antioxidant properties. Several mechanisms have been proposed for antioxidant mechanism of ceruloplasmin. Most of the evidences co-relate its ferroxidase activity with its antioxidant activity (Gonzalez et al, 2008). By its ferroxidase activity, it prevents Fenton reaction, thereby inhibiting production of free radicals. Also, ceruloplasmin is a potential scavenger of superoxide anion radicals (Goldstein et al, 1979).

The concentration of ceruloplasmin increases in several malignancies. Raised concentrations of ceruloplasmin are demonstrated in colon and prostate cancer (Nayak et al, 2003), nasopharyngeal cancer (Doustjalali et al, 2006), uterine cervix cancer (Jeong et al, 2008), laryngeal cancer (Taysi et al, 2003), breast cancer (Ozyilkan et al, 1991) as well as in oral cavity cancer (Akinmoladun et al, 2013). Also, various studies have demonstrated alteration of ceruloplasmin levels in Oral Potentially Malignant Disorders (PMD's) (Rao and Rao, 2013; Rao and Kumari, 2013 & Bathi et al, 2009). Considering this, the present study was designed with the aim of determining and co-relating the serum levels of ceruloplasmin in oral PMD's and oral malignancy.

In the present study, the age of the participants was ranging from 18 to 82 years and males were more (80.67%). This finding was similar to Patil et al (2013), Saraswathi et al (2006) and Khandekar et al (2006). Warnakulasuriya (2009) noted the increased prevalence of oral cancer after the age of 45 years. According to Petti et al (2003), oral pre-malignant diseases affect males at least three times as often as females. Our study has also noted male predilection with 80.67% male participants and 19.33% female participants. The main reason for this is the

consumption of various forms of tobacco and areca nut is more common in Indian males as compared to females (Gupta et al, 1989 & Keluskar and Kale, 2010). In a male dominating Indian society, males being the sole wage earner are privileged to spend their earnings as per his will and wish. The other factor is influence by friends and role models. As males are more exposed to society as compared to females, they are more influenced by these factors.

In our study, the serum levels of ceruloplasmin were determined and compared between all the six groups. The lowest serum levels were noted in healthy control group. It was interestingly noted that, as the severity of the disease increased, the serum levels of ceruloplasmin was also increased accordingly. When arranged in ascending order i.e. from lowest to highest, it was noticed that the mean value of serum ceruloplasmin was gradually increasing from healthy controls (73.42 mg/dl), to control group (78.06 mg/dl) followed by NS group (79.19 mg/dl) and OL group (80.05 mg/dl) and highest values were found in most severe forms of the disease i.e. in OSMF (94.89 mg/dl) and in oral malignancy (92.59 mg/dl). This finding supports our research hypothesis and establishes the serum ceruloplasmin levels as a potential tool to understand the disease progression from normal mucosa to dysplastic changes in mucosa.

The results of our study were supported by various studies on ceruloplasmin levels for oral PMD's and oral cancer. These studies are the study conducted by Rao et al (2013), Akinmoladun et al (2013), Premkumar et al (2011), Bathi et al (2009), Rasheed et al (2007), Jaydeep et al (1997) and Krishnamurthy et al (1986). Various studies on systemic malignancies have also demonstrated increased levels of ceruloplasmin in cases of cancer as compared to that of the healthy controls. These studies are of Pasha et al (2014) for brain cancer, Tysi et al (2003) for laryngeal cancer, Ozyilkan et al (1991) for breast cancer, Nayak et al (2003) for colon and prostate cancer, Doustjalali et al (2006) for nasopharyngeal cancer and Jeong at al (2008) for

uterine cervix cancer. The increase in ceruloplasmin levels in premalignancies and in various malignancies is an indicator of body's antioxidant defense mechanism. The findings of our study and also of the other studies are indicative of role of ceruloplasmin as a biochemical marker of the process of carcinogenesis.

The intergroup analysis yielded statistically not significant differences in between the different clinical stages/types of the diseases. The possible explanation for this finding is lack of balancing of various stages of disease within a specific group.

The limitation of our study is, in this, histopathological examination was not performed for all the participants. A complete histopathological examination would have facilitated the co-relation of serum ceruloplasmin levels with the degree of cellular dysplasia. This could have helped in establishment of possible association between the histological and biochemical changes in process of carcinogenesis, if any. Another limitation is uneven distribution of participants in various clinical staging of the diseases. Equal distribution of the participants could possibly have helped in co-relating serum ceruloplasmin levels with progression of the disease more accurately and more significantly. Future researches should be aimed to overcome these limitations in order to be acquainted with the role of ceruloplasmin in development and progression of malignant process completely in each and every phase of the disease.

Conclusion

The findings of present study demonstrate that the serum levels of ceruloplasmin are strongly associated with progression of the disease process in carcinogenesis. Thus, ceruloplasmin is a reliable biomarker for predication of malignant transformation of oral precancer and a versatile diagnostic tool. In addition, this research has provided a baseline data for future researches

aiming to co-relate the levels of ceruloplasmin with various clinical stages of the disease or with the histopathological grading of dysplasia. Further researches are also required to know the role of ceruloplasmin as a potential prognostic marker in body's oxidative status following medicinal or surgical treatment of the disease.

References

1. Akinmoladun VI, Arinola OG, Kupoluyi TE, Eriba LO (2013). Evaluation of humoral activity in oral cancer patients from a Nigerian referral centre. *J Maxillofac Oral Surg* **12(4)**: 410-413.
2. Bathi RJ, Rao R, Mutalik S (2009). GST null genotype and antioxidants: Risk indicators for oral precancer and cancer. *Indian J Dent Res* **20(3)**: 298-303.
3. Doustjalali SR, Yusof R, Govindasamy GK, Bustam AZ, Pillay B, Hashim OH (2006). Patients with nasopharyngeal carcinoma demonstrate enhanced serum and tissue ceruloplasmin expression. *J Med Invest* **53**: 20-28.
4. Goldstein IM, Kaplan HB, Edelson HS, Weissmann G (1979). Ceruloplasmin: A scavenger of superoxide anion radicals. *J Biol Chem* **254**: 4040-4045.
5. Gonzalez-Cuyar LF, Perry G, Miyajima H, et al (2008). Redox active iron accumulation in aceruloplasminemia. *Neuropathology* **28**: 466–471.
6. Greenberg MS, Glick M and Ship JA (2005). Burket's Oral Medicine. B. C. Decker: New Delhi.
7. Gupta PC, Bhonsle RB, Murti PR, Daftary DK, Mehta FS, Pindborg JJ (1989). An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. *Cancer* **63(11)**: 2247-2252.

8. Halliwell B, Chirico S (1993). Lipid peroxidation: its mechanism, measurement, and significance. *Amer J Clin Nutr* **57**: 715S-724S.
9. Halliwell B, Gutteridge JMC (1999). Oxidative stress. In: Halliwell B, Gutteridge JMC eds., Free radicals in biology and medicine. 3rd Edition. Oxford University Press: New York, pp. 246–350.
10. Halliwell B (1996). Antioxidants in human health and disease. *Ann Rev Nutr* **16**: 33–50.
11. Jaydeep A, Pillai RK, Kannan S, Nalinakumari KR, Mathew B, Nair KM (1997). Serum levels of copper, zinc, iron and ceruloplasmin in oral leukoplakia and squamous cell carcinoma. *J Exp Clin Cancer Res* **16(3)**: 295-300.
12. Jeong DH, Kim HK, Prince AB, Lee DS, Kim YN, Han J (2008). Plasma proteomic analysis of patients with squamous cell carcinoma of uterine cervix. *J Gynecol Oncol* **19(3)**: 173-180.
13. Kadam CY, Katkam RV, Suryakar AN (2011). Biochemical markers in oral cancer. *Biomedical research* **22(1)**: 76-80.
14. Keluskar V, Kale A (2010). An epidemiological study for evaluation of oral precancerous lesions, conditions and oral cancer among Belgaum population with tobacco habits. *Biosci Biotech Res Comm* **3(1)**: 50-54.
15. Khandekar SP, Bagdey PS, Tiwari RR (2006). Oral cancer and some epidemiological factors: A hospital based study. *Indian Journal of Community Medicine* **31(3)**: 157-159.
16. Krishnamurthy S, Jaya S. Serum alphatocopherol lipoperoxides and ceruloplasmin and red cell glutathione and antioxidant enzymes in patients of oral cancer (1986). *Indian Journ Canc* **23**: 36-42.
17. Mark P (1998). Antioxidants. *Clinical nutrition insights*. **24**: 1-4.

18. Nayak SB, Bhat VR, Upadhyay D, Udupa SL (2003). Copper and ceruloplasmin status in serum of prostate and colon cancer patients. *Indian J Physiol Pharmacol* **47(1)**: 1-3.
19. Ozyilkan O, Unlu E, Baltali E, Tekuzman G, Nilufer G, Yener K (1991). The value of ceruloplasmin levels in monitoring the patients with breast cancer. *Journ Islam Acad Science* **4**: 179-180.
20. Pasha MK, Ayesha Q, Narasu ML, Rao KPR, Prabhakar B, Suseela K (2014). Study of magnesium, copper, zinc, iron and ceruloplasmin in liver cirrhosis and ovarian cancer ascitic patients. *British journal of Medicine and Medical Research* **4(21)**: 3902-3911.
21. Patil PB, Bathi R, Chaudhari S (2013). Prevalance of oral mucosal lesions in dental patients with tobacco smoking, chewing, and mixed habits: A cross-sectional study in South India. *J Fam Community Med* **20**: 130-135.
22. Petti S (2003). Pooled estimate of world leukoplakia prevalence: a systematic review. *Oral Oncol* **39**: 770-780.
23. Premkumar P, Bharathan S, Nagini S (2011). Oxidant antioxidant status in patients with oral squamous cell carcinoma in tissue and plasma – A biochemical analysis on 30 samples. *Asian J Exp Biol Sci* **2(2)**: 316-327.
24. Rao AHT, Kumari S (2013). Altered levels of ceruloplasmin, glutathione, beta-carotene in serum of chronic cigarette smokers in comparison with non-smokers. *Res J Pharm Biol Chem Sci* **4(3)**: 15-21.
25. Rao AHT, Sripathi RBH (2013). Estimation of blood levels of serum ceruloplasmin and serum copper as tumor markers in oral leukoplakia and oral malignancies. *Res J Pharm Biol Chem Sci* **4(1)**: 1-7.

26. Rasheed MH, Beevi SS, Rajaraman R, Bose SJC (2007). Alleviation of oxidative and nitrosative stress following curative resection in patient with oral cavity cancer. *J Surg Oncol* **96**: 194-199.

27. Sankaranarayanan R (1990). Oral cancer in India: an epidemiologic and clinical review. *Oral Surg Oral Med Oral Pathol* **69(3)**:325- 330.

28. Saraswathi TR, Ranganathan K, Shanmugam S, Ramesh S, Narasimhan PD, Gunaseelan R (2006). Prevalence of oral lesions in relation to habits: Cross-sectional study in South India. *Indian J Dent Res* **17(3)**: 121-125.

29. Sies H, Stahl W, Sundquist AR (1992). Antioxidant Function of Vitamins. *Ann NY Acad Sci* **669**: 7-20.

30. Taysi S, Akcay F, Uslu C, Dogru Y, Gulcin I (2003). Trace elements and some extracellular antioxidant protein levels in serum of patients with laryngeal cancer. *Biological Trace Element Research* **91**: 11-18.

31. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress induced cancer. *Chem Biol Interact* **160**: 1-40.

32. Warnakulasuriya S (2009). Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* **45**: 309–316.

Tables

Table 1: Distribution of participants according to age and sex

Group	Age			Sex	
	Minimum Age (Yrs.)	Maximum Age (Yrs.)	Mean Age (Yrs.)	Male	Female
OL	24	75	46.32 ± 12.86	47	03
OSMF	18	79	43.66 ± 15.41	45	05
NS	20	80	51.74 ± 13.29	50	00
OM	30	82	50.20 ± 12.17	29	21
C	19	70	40.26 ± 13.39	49	01
HC	21	65	39.68 ± 12.45	22	28
Overall	18	82	45.31 ± 13.92	242	58

(OL – Oral Leukoplakia, OSMF – Oral Submucous Fibrosis, NS – Nicotina Stomatitis, OM – Oral Malignancy, C – Control, HC – Healthy Control, Yrs. – Years)

Table 2: Serum levels of Cerulopalsmin (One way ANOVA)

Particulars	n	Serum Ceruloplasmin Levels				p-value	95% Confidence Interval	
		Min (mg/dl)	Max (mg/dl)	Mean (mg/dl)	Standard Deviation		Lower Bound	Upper Bound
OL	50	22	172	80.05	27.523	<0.001 HIGHLY SIGNIFICANT	72.23	87.88
OSMF	50	37	191	94.89	31.932		85.82	103.97
NS	50	23	132	79.19	25.473		71.95	86.43
OM	50	52	199	92.59	26.513		85.05	100.12
C	50	36	134	78.06	20.409		72.26	83.86
HC	50	19	143	73.42	24.370		66.49	80.34
Total	300	19	199	83.03	27.216		79.94	86.13

(OL – Oral Leukoplakia, OSMF – Oral Submucous Fibrosis, NS – Nicotina Stomatitis, OM – Oral Malignancy, C – Control, HC – Healthy Control, n – number of participants, Min – minimum, Max – Maximum, md/dl – milligram/deciliter)

Table 3: Intergroup Comparison of serum ceruloplasmin levels (Tuckey's Post Hoc Analysis)

		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
OSMF	OL	14.839	5.253	0.056	-0.23	29.91
	NS	15.704	5.253	0.036	0.63	30.77
	OM	2.307	5.253	0.998	-12.76	17.38
	HC	21.476	5.253	0.001	6.41	36.55
	C	16.837	5.253	0.019	1.77	31.91
OL	OSMF	-14.839	5.253	0.056	-29.91	0.23
	NS	.864	5.253	1.000	-14.20	15.93
	OM	-12.533	5.253	0.165	-27.60	2.54
	HC	6.637	5.253	0.805	-8.43	21.71
	C	1.997	5.253	0.999	-13.07	17.07
NS	OSMF	-15.704	5.253	0.036	-30.77	-0.63
	OL	-.864	5.253	1.000	-15.93	14.20
	OM	-13.397	5.253	0.113	-28.47	1.67
	HC	5.773	5.253	0.882	-9.30	20.84
	C	1.133	5.253	1.000	-13.94	16.20
OM	OSMF	-2.307	5.253	0.998	-17.38	12.76
	OL	12.533	5.253	0.165	-2.54	27.60
	NS	13.397	5.253	0.113	-1.67	28.47
	HC	19.170	5.253	0.004	4.10	34.24
	C	14.530	5.253	0.066	-0.54	29.60
HC	OSMF	-21.476	5.253	0.001	-36.55	-6.41
	OL	-6.637	5.253	0.805	-21.71	8.43
	NS	-5.773	5.253	0.882	-20.84	9.30
	OM	-19.170	5.253	0.004	-34.24	-4.10
	C	-4.640	5.253	0.950	-19.71	10.43
C	OSMF	-16.837	5.253	0.019	-31.91	-1.77
	OL	-1.997	5.253	0.999	-17.07	13.07
	NS	-1.133	5.253	1.000	-16.20	13.94
	OM	-14.530	5.253	0.066	-29.60	0.54
	HC	4.640	5.253	0.950	-10.43	19.71

(OL – Oral Leukoplakia, OSMF – Oral Submucous Fibrosis, NS – Nicotina Stomatitis, OM – Oral Malignancy, C – Control, HC – Healthy Control)