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Estimation of Blood Levels of Serum Ceruloplasmin and Serum Copper as Tumour Markers in Oral Leukoplakia and Oral Malignancies

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ABSTRACT

Trace elements and proteins have been studiedextensively to assess whether they have modifying effects in the etiology of cancer. The objective of this study is to evaluate diagnostic utility of plasmatic levels of ceruloplasmin and copper as a tumour marker in oral leukoplakia and oral malignancies. Serumlevels of ceruloplasmin and copper were estimatedin30 age-matched subjects in 3rd and 5th decade of life. These subjects were grouped into three groups of, one control group comprising of normal, healthy individuals and the other two study groups comprising of clinically and histologically diagnosed leukoplakia and squamous cell carcinoma(SCC) respectively. The levelofceruloplasmin was found to be significantly elevated in patients with leukoplakia; as well as the level of copper was found significantly elevated in patients with SCC.Ceruloplasminwas significantly elevated in leukoplakia indicating detoxification process of body against the free radicals generated during carcinogenesis. Copper was significantly elevated in SCC indicating angiogenesis that occurs in tumour progression. Further studies with follow up may be essential to correlate the significance of variations of these tumour markersso as to consider these as a tool in early diagnosis and prognostic indicator.

Keywords: Ceruloplasmin; Copper; Antioxidants; Angiogenesis; Oral leukoplakia; Oral squamous cell carcinoma.

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INTRODUCTION

Leukoplakia, a pre-malignant lesion and squamous cell carcinoma, a malignant neoplasm are quite commonly seen occurring in the oral cavity. Sequential pathological multi-stage carcinogenesis is observed in the form of alterations from hyperplasia to neoplasms.

A very intimate relationship between oxidative challenge and malignancy has been well documented. Oxidative challenge results due to the generation of free radicals (highly reactive molecules with unpaired electrons) under the effect of ionizing radiations or by chemical carcinogenesis. These reactive free radicals are implicated in triggering or transforming non-malignant cells to malignant ones by either DNA damage in the form of strand breakage or by modulating gene expression [1].

One significant characteristic of free radical reactivity is lipid peroxidation which results in deleterious effects on cell leading to their damage. Many chemical carcinogens are shown to be metabolically converted to free radicals which is facilitated by lipid peroxidation [2]. To combat these oxidative challenges our body has evolved quite a lot of anti-oxidant systems. These are detoxifying agents and act as scavengers of free radicals. They commonly constitute ceruloplasmin, tocopherols, beta-carotene, glutathione, superoxide dismutase, etc.

Recently there is high evidence that tumour evoked angiogenesis has a high requirement of copper. Tumour growth is dependent on angiogenesis, which is dependent upon release of growth factors, which are dependent on copper status.

In the present study, the concentration of ceruloplasmin in blood samples from patients with oral leukoplakia and oral squamous cell carcinoma were observed to gauge the possible use of the anti-oxidant ceruloplasmin and the angiogenetic agent copper as tumour markers, to aid in the diagnosis and assessment of prognosis of these lesions.

MATERIALS AND METHODS

The material for the present study comprised a total of 30 subjects reported to Department of Oral and Maxillofacial Surgery; Yenepoya Dental College and Hospital; Mangalore. These entire individual were age-matched in range of 3rd and 5th decade of life. These subjects were grouped into three groups:

Group 1 → 10 healthy subjects in control group without any habit and oral lesions.
Group 2 → 10 patients who were clinically and histologically diagnosed as oral leukoplakia.
Group 3 → 10 patients who were clinically and histologically diagnosed as squamous cell carcinoma of the oral cavity.

From all these patients of clinically diagnosed leukoplakia and squamous cell carcinoma, biopsies were taken and were subjected to histopathological evaluation and graded according

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to existing grading systems. 2ml of venous blood was drawn and was centrifuged, serum separated and transferred into vaccutainers for transport to laboratory for analysis.

Estimation of serum ceruloplasmin was done using Method of Ravin [3], which is a colorimetric assay with precision and reliability. Estimation of serum copper was done using Bathocuproinedisulfonic acid method which is a colorimetric assay of precision and reliability.

RESULTS AND OBSERVATIONS

In the present study, descriptive statistics of ceruloplasmin and copper was done for all the groups and this includes mean, median and standard deviation. In addition, the results were analyzed using Kruskal-Wallis test, Mann-Whitney 'U' test and assessed for significance.

Table No 1: Descriptive Statistics of Serum Ceruloplasmin in Group I and II

Groups	Mean	Standard Deviation	Median
Normals (Group I)	31.2000	3.35989	31
Leukoplakia (Group II)	42.9000	7.23341	43

Table No 2: Descriptive Statistics of Serum Copper in Group I and Group III

Groups	Mean	Standard Deviation	Medican
Normals (Group I)	98.8000	11.17338	99.5
Squamous Cell Carcinoma (Group III)	117.8000	24.31643	123.5

In the present study, when inter-group comparison was done for serum ceruloplasmin levels between normal (Group 1) and leukoplakia (Group 2) using Mann-Whitney 'U' test for significance, it was found to be very highly significant with increased serum ceruloplasmin level in Group 2 (p=0.001). Likewise when inter-group comparison was done for serum copper levels between normal (Group 1) and oral squamous cell carcinoma (Group 3) using Mann-Whitney 'U' test for significance, it was found to be highly significant with increased serum copper level in Group 3 (p=0.049).

In addition, an increase was seen in the mean serum ceruloplasmin value in Group 2 (mean=42.9000) on comparison with Group 1 (mean=31.2000). Likewise an increase was also seen in the mean serum copper value in Group 3 (mean=117.80000) on comparison with Group 1 (mean=98.8000).

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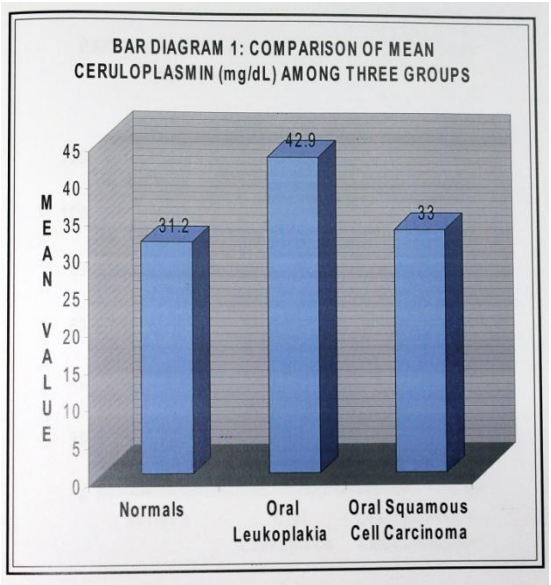
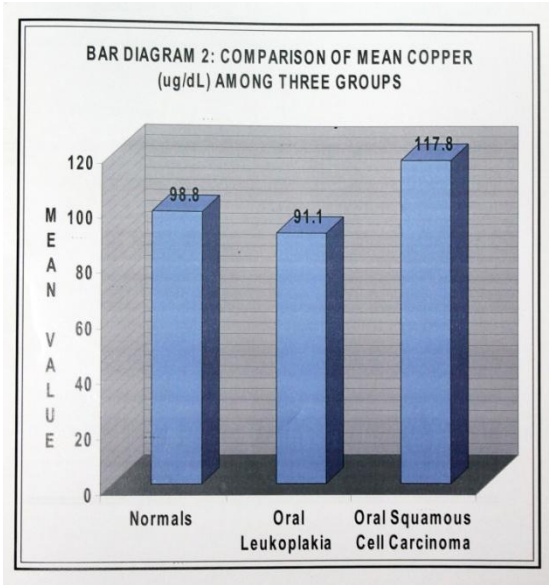
Table No 3: Inter-group Comparison between Group I and II

Groups		Mean	Standard Deviation	'Z' Denoting Mann-Whitney 'U' Test of Significance	'p' Value	Remarks
Serum Copper	Normals (Group I)	98.8000	11.17338	1.25000	P=.221	
	Leukoplakia (Group II)	91.1000	12.62669			
Serum Ceruloplasmin	Normals (Group I)	31.2000	3.25989	3.33500	P=.001	Very Highly Significant
	Leukoplakia (Group II)	42.9000	7.23341			

Table No 4: Inter-group Comparison between Group I and III

Groups		Mean	Standard Deviation	'Z' Denoting Mann-Whitney 'U' Test of Significance	'p' Value	Remarks
Serum Copper	Normals (Group I)	98.8000	11.17338	2.01200	P=.049	Very Highly Significant
	Squamous Cell Carcinoma (Group III))	117.8000	12.62669			
Serum Ceruloplasmin	Normals (Group I)	31.2000	3.25989	0.26500	P=.791	
	Squamous Cell Carcinoma (Group III)	33.02522	7.23341			





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DISCUSSIONS

Ceruloplasmin is a glycoprotein, which belongs to the group of acute phase reactants and is a principal copper containing protein of plasma. It is one of the most potent free radical inhibitor both in tissue homogenates and in simple lipid emulsions. The protein is one of the acute phase reactants whose concentration in plasma rises after tissue injury for protecting the organism as a whole from the possible ill-effects of local damage since one of the ill-effects is the release of free radical oxidation products [4].

Copper is an essential trace element. It effects activity of many enzymes [copper/zinc-superoxide dismutase, ceruloplasmin, cytochrome oxidase, tyrosinase, dopamine hydroxylase and lysine oxidase] both as a cofactor and as an allosteric component [5,6]. These enzymes are essential for cellular respiration, defense against free radicals, melanin synthesis, and formation of connective tissue and for iron metabolism. In addition, copper dependent transcription factors play an important role in gene expression [7]. Judah Folkman introduced a sweeping hypothesis in 1971 stating that if tumours are to grow and thrive, they must develop a blood supply. Every increment in tumour growth requires an increment in capillary growth [8,9].

Copper is believed to be the switch that turns on the angiogenesis process in tumour cells. Growth factors in angiogenesis require binding to copper in order to function properly. Copper-binding molecules are non-angiogenic when free of copper, but become angiogenic when bound to copper. Several angiogenic factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), tumour necrosis factor alpha (TNF-a) and interleukin-1 (IL-1) are found to be copper activated.

Activation factors bind to endothelial, “switch” them from G0 into G1 phase and force proliferation. Brem and his co-workers have shown that copper reduction by copper deficient diet or/and penicillamine (copper-chelating molecule) blocks angiogenesis by “switching” endothelial cells back to G0 phase or apoptosis and by down regulating the angiogenic activity of VEGF, bFGF, TNF-a, IL-1 and probably IL-6 and IL-8 as well [10].

Copper metabolism is profoundly altered in neoplastic disease. It has been found that serum copper concentration correlates with tumour incidence, malignant progression and recurrence in a variety of human cancers.The cellular deposition of copper is also altered in tumour tissues from cytoplasm in normal tissue to intranuclear and perinuclear zones in tumour [11].

It is very clear that free radicals play an essential part in carcinogenesis by damaging the cellular material and in transforming the normal cells to malignant cells. To destroy these free radicals and to reduce the reactive oxygen intermediates, the body synthesizes antioxidants in the form of ceruloplasmin, tocopherol, etc. and their levels have shown a marked increase in blood during leukoplakia.

Also the angiogenetic factors play a very important role in the development of carcinomas. Formation of new blood vessels by a tumour enables tumour growth, invasion and metastasis. Among these, especially copper is believed to be the switch that turns on the angiogenesis process in tumour cells. It has been observed that abnormally high serum copper levels are found in patients with many types of progressive tumours.

In the current study, the levels of ceruloplasmin was found to be significantly elevated in oral leukoplakia when compared to oral squamous cell carcinoma and normal. Also the levels of copper was found to be significantly elevated in oral squamous cell carcinoma when compared to oral leukoplakia and normal.

Serum ceruloplasminhas been shown to be a powerful antioxidant, a scavenger of free radicals and superoxide ions, the results of the present study strongly suggest this effect of ceruloplasmin. The explanation for this appears to be an increase in serumceruloplasmin consequent to decreased catabolism of this enzyme [12] with an increased compensatory antioxidant defenses in serum, to combat the transformation of non-malignant cells to malignant cells.

Serum copper has been shown to be a powerful angiogeneticagent, the results of the present study strongly suggest this effect of copper. The explanation for this appears to be the way in which copper effect activity of many enzymes both as a cofactor and as an allosteric component. These enzymes are essential for formation of connective tissue and angiogenesis. The copper levels are more significantly elevated in progressive malignancy or rapidly growing tumours which require angiogenesis to supplement its nutritional needs and not in slow growing or static tumours whose angiogenetic needs are limited.

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