Original Article

Role of Serum Ceruloplasmin as Tumor Marker in Early Diagnosis of Oral Squamous Cell Carcinoma

Early Diagnosis of Oral Squamous Cell Carcinoma

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ABSTRACT

Objective: To compare serum Ceruloplasmin (CP) levels in histopathologically diagnosed oral squamous cell carcinoma (OSCC) patients with healthy controls and to determine its role as tumor marker of early diagnosis and malignant transformation of OSCC.

Study Design: Comparative cross-sectional study

Place and Duration of Study: This study was conducted at Department of Oral Pathology and Oral & Maxillofacial Surgery, King Edward Medical University Lahore, in duration of 6 months from February 2019 to August 2019.

Materials and Methods: Sample size of 90, compromising: control group A, 45 healthy subjects; group B, 45 histopathologically diagnosed OSCC patients were included. After obtaining informed consent from all patients, serum CP levels were estimated by using immunoturbidimetric analysis for quantitative determination of CP level on the serum samples. All the data was collected on predefined pro-forma and analyzed using SPSS version 26. The mean values of CP were compared using ANNOVA and One-way ANNOVA test. Independent T-Test was applied to subgroups of OSCC to analyze the OSCC disease progression.

Results: CP levels were significantly increased in histopathological grades of OSSC in comparison to control group (p-value < 0.001). However, there was no significant difference between CP values of well differentiated and moderately differentiated histopathological grades of OSCC.

Conclusion: Serum CP levels can help to diagnose early stages of OSCC, thus establishing it as noninvasive tumor marker of malignant transformation. However, role of serum CP levels to establish progression of disease is still ambiguous.

Key Words: Oral squamous cell carcinoma**,** Ceruloplasmin**,** Malignant transformation**,** Well-differentiated squamous cell carcinoma**,** Moderately-differentiated squamous cell carcinoma, Immunoturbidimetric analysis.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC), a fatal debilitating disease with rising incidence has irreparably low survival rate for the past many decades[1]. OSCC is one of the most common cancer that dominates globally, constituting about 90% of all oral malignancies and is ranked as the 8th most common cancer world-wide [2].

However, in Pakistan it is 2nd most common malignancy according to cancer registry of Shaukat Khanum memorial Hospital Pakistan and accounts for 15% of all newly diagnosed cancer cases in comparison to 3% detected globally, which makes it a threat to public health in Pakistan [1]. European Oral Cancer Foundation reports 80-90% survival rate if cancer is diagnosed at an early stage [3]. The late stage diagnosis of cancer leads to high death rate of about 43% at five years from diagnosis, even after treatment, it is associated with severe incapacitating morbidities in survivors [3]. There is extensive data on concept of delay in diagnosis of oral cancer [4]. Delay can either be 'patient delay' or 'professional delay', patient delay defined as "the interval between detection of awareness of a bodily change to the first consultation with a healthcare professional", and professional delay defined as "the interval between first professional consultation and definitive histological diagnosis of malignancy" [4, 5]. Other factors of delay are cognitive and psychosocial variables that involves fear, anxiety and lack of symptom recognition [6]. The gold standard for diagnosis of OSCC is scalpel biopsy, which significantly induces anxiety and fear among the patients to prevent them to seek medical consultation [6]. Therefore, less invasive and least expensive diagnostic measures are highly needed for early detection of OSCC, one of such methods is detection of tumor markers in serum [7].

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These markers are the specific substances released into serum either by the cancer cells or the host while combating the cancer[8]. Tumor marker such as Ceruloplasmin (CP) is a glycoprotein and a principle Copper (Cu) carrying enzyme normally produced by the liver, it is an antioxidant and an acute phase reactant[9]. CP synthesis rises in response to free radical tissue injury, therefore, serum CP levels can be used as diagnostic marker of oxidative damage which is one of the vital steps in the pathogenesis of OSCC [9]. Upsurge of pro-oxidants and deficiency of antioxidant in the body also appear to play an important part in the progression of severity of malignancy [10]. Based on above narrated factors, aim of this study was to compare serum levels of CP of various histopathological grades of OSCC with control group in order to determine its significance as a tumor marker of early diagnosis and establish its role in malignant transformation.

MATERIALS AND METHODS

It was a comparative cross-sectional study based on nonprobability convenient sampling, conducted in the department of Oral and Maxillofacial Surgery and department of Pathology, King Edward Medical University Lahore. After ethical clearance from the university (ref no.406/RC/KEMU), a total of 90 subjects were enrolled in the study and were divided into two groups: Group A (45 healthy controls with no oral lesions); Group B (45 OSSC). Group B was further divided into subgroups based on histopathological grading that is, well-differentiated (WD), moderately-differentiated (MD), however as the sample of OSCC was collected randomly poorly-differentiated (PD)-OSCC was not histologically diagnosed in the sample of 45 cases of group B (OSCC). Both genders ranging from age 10-65 years who had not undergone any treatment for OSCC were included in the study. Patients with chronic systemic disease (diabetes, hypertension, liver and renal failure), pregnant females and those taking oral contraceptives were excluded from the study. Control group comprised of healthy patient with no oral lesion. After obtaining informed consent from all the subjects, 5ml blood was drawn from a major vein and centrifuged at 1500g for 10 minutes to obtain serum. The CP levels were determined through Immunoturbidimetric test (for quantitative determination of CP in human serum) by using CP OSR6164 Assay on the AU640 Analyzer. Data was analyzed using SPSS version 26. Mean ± S.D was used to present quantitative data such as age and serum CP. ANNOVA was applied for comparison of control and OSSC group, while comparison of control with subgroups (WD- OSCC and MD-OSSC) was done by using One-way ANNOVA. p-value $\leq$ 0.05 was considered as significant for both the tests. Independent T-Test was applied to subgroups to analyze the OSCC disease progression.

RESULTS

The study comprised of 90 participants who were categorized into 2 groups: Group A, 45 healthy controls; Group B, 45 patients of OSCC. In group A, 75.60% (n=34) were male and 24.40% (n=11) were female, whereas, in group B 66.70% (n=30) were male and 33.30% (n=15) were female, showing gender distribution of 2:1 male to female ratio in OSSC. Mean age of the group A was 23.38±3.28 years, and mean age of group B was 45.50±18.55 years. In group B, 73.30% (n=33) of the participants were in the age range of 50-65 years, 24.40% (n=11) were of 30-49 years, while 2.20% (n=1) were below 30 years of age, showing prevalence of OSCC in fifth and sixth decade of life.

The mean values of serum CP in both groups was compared using ANNOVA test. The mean CP value of group A was 238.53±51.56, and group B was 361.27±62.89. The mean difference of serum CP between two groups was statistically significant (p-value <0.001) as depicted in table 1.

Table No.1: Comparison of serum CP levels (mg/l) of control with OSCC group

Serum CP Levels (mg/l)			
Groups	Mean± Std. Deviation	95% Confidence Interval for Mean	p-value
Group A(control)	238.53±51.56	223.04-254.02	<0.001
Group B (OSCC)	361.27±62.89	342.37-380.16	

Table No.2: One-way ANOVA for comparison of mean serum CP levels (mg/l) of control (group A) with the sub groups of OSCC (Grade I, II)

Group	sub groups	No. of samples	Mean± Std. Deviation	95% Confidence Interval for Mean	p-value
Control (Group A)	-/-	45(100%)	238.53±51.56	223.04 -254.02	<0.001
OSCC (Group B)	WD-OSCC	31(68.89%)	361.35±56.83	340.51 -382.20	<0.001
	MD-OSCC	14(31.11%)	361.07±77.04	316.59 -405.55	<0.001

The mean difference of serum CP between control (Group A) and subgroups of OSCC (Group B) was statistically significant (p-value <0.001).

Serum CP was 238.53±51.56 mg/l in group A (Control) and 361.27±62.89 in group B (OSCC). The mean difference was statistically significant (p-value <0.001). One-way ANNOVA, test was applied to compare the mean CP value of group A with subgroups of group B; WD-OSCC and MD-OSCC. The results of control (group A) with the subgroups of OSCC (group B) were found to be statistically significant, as shown in table 2.

In order to determine the role of CP as a marker of disease progression in OSCC, serum CP levels of subgroups WD-OSSC and MD-OSSC were compared. Independent T-Test was applied to these subgroups, where mean difference of serum CP level between subgroups of WD-OSCC cases and MD-OSCC patients was statistically insignificant (p-value 0.989) as presented in table 3.

Table No.3: Comparison of serum CP levels (mg/l) between sub groups of OSCC WD-OSCC cases (Grade I) and MD-OSCC cases (Grade II)

OSCC Group B			
Sub Group OSCC	No. of sample	Mean± Std. Deviation	p-value
WD-OSCC cases (Grade I)	31(68.89%)	361.35±56.83	0.989
MD-OSCC cases (Grade II)	14(31.11%)	361.07±77.04	
Total	45(100%)		

Independent Samples Test-0.014 Difference was statistically insignificant between two sub groups of OSCC (Group B).

DISCUSSION

OSCC, one of fatal debilitating cancers is still currently diagnosed at later stages impacting the prognosis of the disease [1]. The delay factors can be multiple owing to availability of health care facilities and patients' anxiety and fear resulting in reluctance to seek consultation [6]. Presently used diagnostic techniques are invasive and expensive for many patients leading to further delay in treatment. Scalpel biopsy, the gold standard for diagnosis of OSCC is painful and apprehensive for the patients; also biopsy can result in dissemination of malignant cells[11]. Therefore, it is need of the hour to research and develop diagnostic methods which are noninvasive and inexpensive. Thus, estimation of serum CP could be one of the noninvasive methods that can be used in an effort to establish early diagnosis of OSCC. CP is among the primary enzymatic antioxidant which acts through several mechanisms: i) ferroxidase activity which inhibits iron-dependent lipid peroxidation and $HO^{\bullet}$ formation from H_2O_2 ; ii) Superoxide Dismutase (SOD) activity by reacting with H_2O_2 and scavenging of superoxide anion radicals; iii) Cu regulation and hemostasis by inhibiting copper-induced lipid peroxidation through Cu ions binding with CP; iv) Iron regulation and hemostasis, CP helps to incorporates iron into transferrin preventing the formation of toxic Fe products [12]. These all mechanisms play a pivotal role in pathogenesis of OSCC resulting in biochemical alteration in levels of serum CP.

In this research, the serum CP levels were significantly increased (p-value <0.001) in OSCC (group B) being 361.27±62.89 mg/l as compared to controls (group A) being 238.53±51.56 mg/l, these findings are in accordance with study conducted by Singh et al., (2015) [13]. Similar findings were also reported by Shah et al., (2017) and Nayyar et al., (2020) in relation to the mean serum CP levels, which was significantly increased in OSCC group [7, 14]. Carcinogenesis occurs due to oxidation of DNA, resulting from imbalance between pro-oxidants and anti-oxidants leading to oxidative stress, this equilibrium is disturbed either by increased levels of Reactive Oxygen Species (ROS) and Reactive nitrative species (RNS) or by antioxidants depletion [15]. Therefore, CP with major oxidase activities tries to combat oxidative stress that occurs along with increased lipid peroxidation [13, 16]. Such changes in plasma of OSCC patients cannot be recouped by the antioxidant defense system resulting in insignificant increase in serum CP levels in OSSC patients [13, 16, 17]. Furthermore, first step of malignant transformation of a lesion is tumor angiogenesis initiated by rise in serum CP levels [18]. CP bounded by Cu binds with the angiogenic growth factors and boosts its affinity for endothelial cells and secretion of angiogenic molecules, such as Fibroblast growth factors (FGF) and interleukin-1 α (IL-1α) [19]. CP plays an vital role in the stabilization and nuclear transport of hypoxia induced factor-1α, thereby regulating vascular endothelial growth factor (VEGF) expression (an important angiogenic growth factor) [20]. Numerous researches carried out on inhibition of CP have successfully demonstrated suppression of tumor growth and angiogenesis [20].

The role of CP as a marker of disease progression in OSCC was not established, as the difference in serum CP levels between WD-OSCC (grade I) cases and MD-OSCC (grade II) was statistically insignificant (p-value 0.989). This finding is consistent with the study conducted by Singh et al.,(2015), where although, values of serum CP were increasing gradually as the OSCC progressed from WD-OSCC to MD-OSCC to PD-OSCC but the mean difference remained statistically insignificant (p-value 0.3556) [13]. No other correlation with the findings of the present research was found in literature to study the relation of levels of CP in serum with the dysplastic changes involved in advancement of OSCC disease severity, therefore comprehensive studies are required to unveil further role of serum CP levels as a marker of disease progression of OSCC.

In this study, the mean age of OSCC was found to be 45.50 ± 18.55 years, whereas the peak incidence of occurrence for OSCC was the 5th decade of life. These findings coincide with a study done by Naseer et al.,

(2016) on frequency of delayed diagnosis of OSCC in Pakistan [21].

In this research, the higher incidence of OSCC was noted in males, with male to female gender predilection in ratio of 2:1. These findings were in accordance with Chaturvedi et al., (2012) and Gadbail et al., (2017) who also reported male to female predilection of 2:1 [22].

CONCLUSION

Estimation of serum CP levels is a useful indicator to diagnose early stages of OSCC, thus establishing it as noninvasive tumor marker of malignant transformation. However, role of serum CP levels to establish progression of disease was not ascertained through this study.

Recommendation: Extensive studies including equal distribution of all histopathological grades with larger sample size are required to unveil further the role of serum CP levels as a marker of progression of disease severity.

Author's Contribution:

Concept & Design of Study:	Shahrayne Rashid, Saadia Manzar, Farhat Kazmi
Drafting:	Shahrayne Rashid, Saadia Manzar
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