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Evaluation of Serum Bilirubin Levels in Oral Cancer

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KEYWORDS

Hepatobiliary, haematological, clinicopathologic, squamous, prognosis.

ABSTRACT:

Background: Aberrant level of serum bilirubin, marker of hepatobiliary and haematological disorders, was associated with patient prognosis in several human malignancies. In this study, we aim to evaluate the predictive value of serum bilirubin for clinicopathologic characteristics and survival of patients with oral squamous cell carcinoma (OSCC).

Methods: This study reviewed 50 patients with OSCC and 50 normal controls matched for age and gender. The association between levels of preoperative direct bilirubin (DBIL), indirect bilirubin (IBIL), total bilirubin (TBIL), and clinical variables were analysed.

Results: Lower Total Bilirubin and Indirect bilirubin were found in OSCC patients compared with normal controls. Significantly lower direct bilirubin was found in OSCC patients and it was an independent prognostic factor. Patients with higher DBIL had longer overall survival than those with lower DBIL.

Conclusion: Lower DBIL was associated with a poorer prognosis and may be regarded as an independent prognostic marker for patients with OSCC.

BACKGROUND:

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer that forms in tissues of the oral cavity (the mouth) or the oropharynx (the part of the throat at the back of the mouth) is a subtype of head and neck cancer [1]. It accounts for approximately 4-5% of all cancers. Amongst the reported cases of HNCs, oral squamous cell carcinoma (OSCC) is the most ubiquitous malignancy developing as a mounting issue across the globe. It is commonly referred to as 'oral cancer' and is extensively widespread in developing countries as compared to other developed countries. Oral cancer has poor prognosis, with overall 5-year survival rates as low as 40%, although, if diagnosed in the early stages (I and II), survival rates can exceed 80% [2]. Up to 50% of oral cancers are diagnosed at an advanced stage (stage III and IV) [3]. Over 5 people in India die every hour every day because of oral cancer and the same numbers of people die from cancer in oropharynx and hypo pharynx. Most oral SCC's are preceded by precancerous lesions [4].

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Epidemiologically, oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide accounting for new cases of oral cavity and pharynx cancer about 11.3 per 100,000 men and women per year. India accounts for about 30% of all new cases annually [5]. India tops in the prevalence of oral cancer in the world and remains the commonest cancer amongst the male population. In women it is the third most common cancer in India after cervical and breast cancer. In India, around 77,000 new cases and 52,000 deaths are reported annually, which is approximately one-fourth of global incidences [5]. As compared to the west, the concern of oral cancer is significantly higher in India as about 70% of the cases are reported in the advanced stages (American Joint Committee on Cancer, Stage III-IV). Lip and oral cavity cancer accounted for 145,000 deaths worldwide in 2012 (2% of total cancer cases). In India, 20 per 100000 populations are affected by oral cancer which accounts for about 30% of all types of cancer. The relationship between serum bilirubin and cancer has been revealed in recent years. Molecular studies on human cancer cells have shown that anticancer effects of bilirubin are due to its ability to drastically increase free radicals inside the tumour cells, thereby alleviating the oxidative stress. Bilirubin has been regarded as a marker of hepatobiliary and haematological disorders for a longer time now. However, recent studies have demonstrated that bilirubin also plays a role as anti-oxidant, anti-inflammatory, and anti-cancer agent. An inverse association between bilirubin and cancer risk has been seen in non-small-cell lung cancer, breast cancer, and colorectal cancer [6]. Specifically, higher serum bilirubin levels predict longer survival in non-metastatic breast cancer [7]. Also, in vitro studies have indicated that bilirubin can induce apoptosis and inhibit proliferation in various neoplastic diseases, such as colon cancer and human adenocarcinoma cells [8]. Therefore, it seems that serum bilirubin has a protective or antitumor effect on malignancies.

The present study was undertaken in the Department of Oral Medicine, Diagnosis and Radiology, Kamineni Institute of Dental Sciences to evaluate the serum bilirubin levels in patients with Oral Squamous Cell Carcinoma and compared them with healthy individuals.

MATERIALS AND METHODS:

A total number of 50 cases of oral squamous cell carcinoma who visited the Department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, Narketpally, were recruited. The inclusion criteria were as follows: (i) The study group includes patients of age above 18 years old; (ii) Patients who are clinically and histopathologically diagnosed with and Oral Squamous Cell Carcinoma; and (iii) Control group includes patients without any oral lesions and without any systemic disorders. The exclusion criteria were as follows: (i) Patients with any evidence of hepatobiliary disorders; (ii) Patients with any evidence of hematologic disorders; (iii) Patients with insufficient survival data or pre-treatment hematology test data; (iv) Patient having distant metastasis; (v) Patients on chemotherapy or radiotherapy treatment; (vi) Patients who are pregnant; and (vii) Patients with any previous malignancy. . The control group consisted of 50 subjects with a similar body mass index (BMI), and matched for age and gender. No family history of oral carcinomas or other malignancies was found in the control samples. These subjects were selected randomly among the individuals that were in the same hospital during the same period. The study procedure was explained to all patients, and they all provided their written informed consent. This study was approved by the Clinical Research Ethics Committee of the institute. (KIDS/IEC/OMR/2019/03).

CLINICAL PARAMETERS:

The clinical parameters that were included in this study are as follows: age, gender, clinical staging, serum levels of direct bilirubin, indirect bilirubin and total bilirubin. The following clinical and pathological parameters were included in our analyses: age, gender, tumor site, TNM stage, N classification, and histological differentiation, serum levels of DBIL, IBIL, and TBIL. The morphological gradation of OSCC samples was measured according to characteristics of its atypical squamous cells by senior pathologists. The clinical gradation of OSCC lesions was measured by TNM classifications of the 7th edition of the American Joint Committee (AJCC) cancer staging manual. Serum levels of DBIL and TBIL were measured in the department of microbiology using the modified method of Jendrassik and Gróf which is the assay for direct

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bilirubin with diazotized colorimetric method. It is to measure the concentration of total bilirubin in serum or plasma.

This test is based on Jendrassik and Grof diazotization method whereby direct bilirubin in the sample reacts with diazotized sulfanilic acid. The intensity of the purple-red colour is directly proportional to the concentration of direct bilirubin and can be photometrically measured. Indirect bilirubin is calculated from the difference between the total and direct bilirubin.

The test has been developed to determine bilirubin concentrations within a measuring range from 0.03 to 10 mg/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

STATISTICAL METHODS:

Participants were interviewed using a standardized questionnaire to collect information on age, sex, pernicious habits like smoked or smokeless form of tobacco consumption, alcohol consumption and any other systemic diseases were recorded from this questionnaire. All the data were tabulated and statistical analysis was performed to assess the association of Bilirubin with Oral Squamous Cell Carcinoma and Control patients. The statistical analysis was performed by using SPSS (statistical package for social sciences) 19.0 version software. Comparison of groups is done by ANOVA test. P-value was determined and expressed as

1. ** $P < 0.05$ represents statistically significant.

2. $P > 0.05$ represents statistically non-significant.

RESULTS:

A total of 100 cases were included in this study for the analysis of serum TBIL, IBIL, and DBIL. The OSCC group consisted of 50 patients (38 males and 12 females) see table 1, with age range from 20-90 years and a mean age of 51 years, see table 2. The control group included 50 age and gender matched individuals. The baseline data is shown in the table below:

Table 1: Gender Distribution in Study Group:

	GENDER	CASES	PERCENTAGE
OSCC (n=50)	**MALES**	**38**	**76%**
	FEMALES	**12**	**24%**

Graph I: Carcinoma at various regions of Oral Cavity:

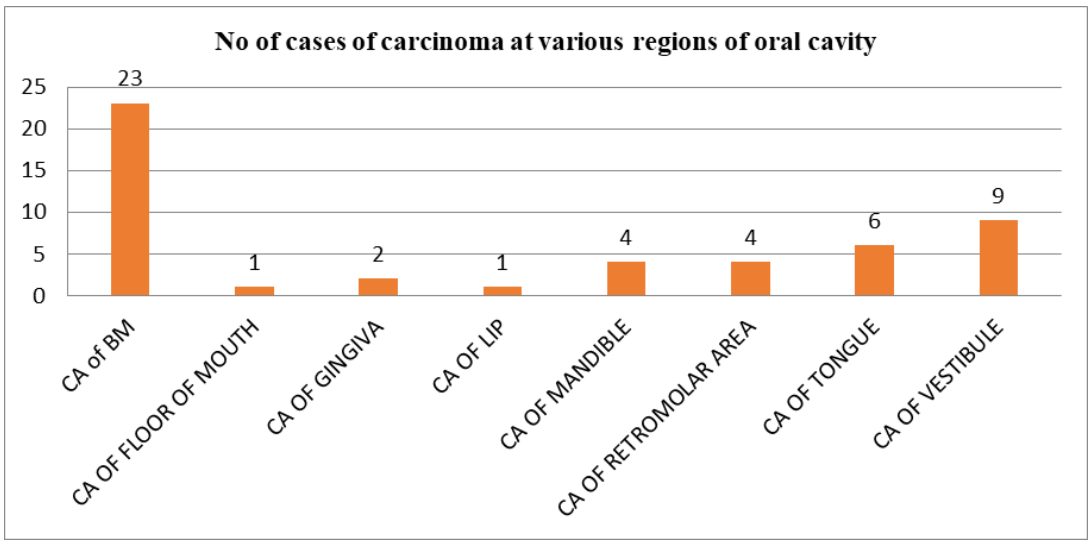

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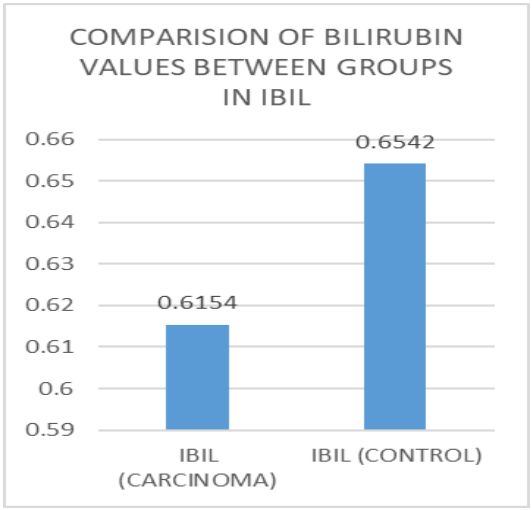
Table 2: Age Distribution in Oral Squamous Cell Carcinoma Group:

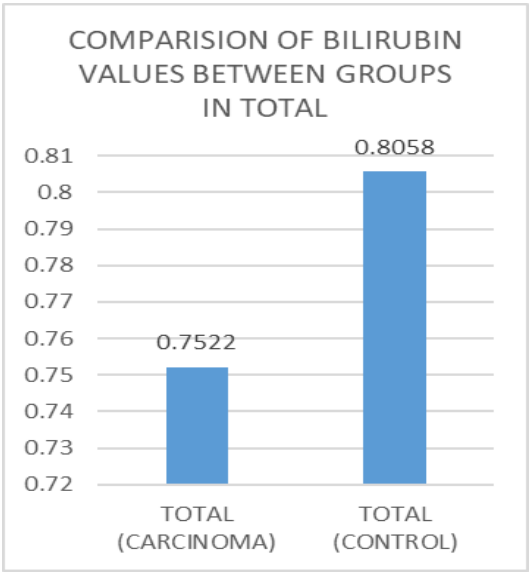
AGE RANGE	CASES	PERCENTAGE
<40	09	18%
41-50	18	36%
51-60	14	28%
>60	09	18%
TOTAL	50	100%

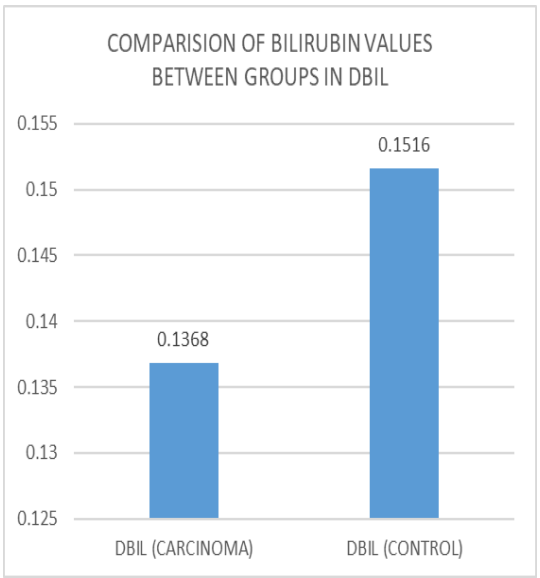
Comparison of the pre-therapy serum levels of TBIL, DBIL, and IBIL between patients and control individuals

TBIL, DBIL, and IBIL serum levels of each subject in this study were evaluated. The mean TBIL, DBIL, and IBIL serum values of patients with OSCC and the matched control group are shown in bar diagram see graph II,III,IV. Lower levels of mean serum TBIL and IBIL were detected in patients compared with control individuals. But correspondingly, a significantly lower level of DBIL was found in patients with Stage IV OSCC than other stages.



Graph II,III,IV : mean TBIL, DBIL, and IBIL serum values of patients with OSCC and the matched control group





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Association between serum TBIL, DBIL, IBIL, and clinicopathologic parameters

We further analysed the relationship between TBIL, DBIL, and IBIL serum levels and clinicopathologic parameters in this study. No significant difference was found in terms of TBIL, DBIL, and IBIL levels between male and female patients. There was no evidence of differences between TNM stages, regional lymph-node metastasis, or between pathological grades.

Next, we investigated whether any type of Bilirubin could be a predictor of survival in patients with OSCC. As we expected, regional lymph node metastasis strongly predicted the overall survival of patients with OSCC. The results of the univariate analysis indicated that DBIL are associated with overall. Patients with higher DBIL had significantly longer overall survival than those with lower DBIL.

To ascertain whether DBIL was an independent parameter of patients' survival, a multivariate analysis was conducted to include lymph-node metastasis as a cofactor. DBIL as well as lymph-node metastasis was independently associated with overall survival. The worst overall survival was observed in patients who had stage IV and lower DBIL, while those with lower stages and higher DBIL showed the best overall survival.

DISCUSSION

Oral cancer has poor prognosis, with overall 5-year survival rates as low as 40%, although, if diagnosed in the early stages (I and II), survival rates can exceed 80%. Up to 50% of oral cancers are diagnosed at an advanced stage (stage III and IV), as most patients are not symptomatic in the early stages and do not seek medical help until they show clear symptoms such as pain, bleeding, or a mass in the mouth or neck if lymphatic spread is already present [9]. When the diagnostic delay exceeds one month, the risk of having an advanced stage oral cancer stage is significantly higher. In most cases, the patient is responsible for a large part of the diagnostic delay; however, delay can also be the result of an incorrect medical approach by not suspecting an oral malignancy and not diagnosing and treating it promptly and adequately. As a general rule, prognosis worsens as the disease becomes more advanced and as the site of the tumour becomes less accessible. Clinical and pathological stage at diagnosis remains the most important factor influencing prognosis. Given the high mortality rate, early detection of oral malignancy and anticipation of diagnosis will result in better prognosis and survival rates and less morbidity from treatment. An early diagnosis is crucial to control a possible malignant transformation of oral premalignant diseases and for increasing the overall survival rate of the patients. Screening involves the detection of the disease at a preclinical state in subjects without even signs or symptoms of the disease. Identification of appropriate biomarkers can lead to early detection of oral cancer. The relationship between serum bilirubin and cancer has been revealed in recent years. Molecular studies on human cancer cells have shown that anticancer effects of bilirubin are due to its ability to drastically increase free radical levels inside the tumour cells, thereby alleviating the oxidative stress [9]. Increased reactive oxygen species damage DNA structure and alters gene expression reducing cell proliferation [10].

We first compared TBIL, IBIL, and DBIL between patients with OSCC and healthy controls and found that both TBIL and IBIL were significantly decreased in OSCC patients compared with their matched healthy controls, but DBIL was increased significantly. The downregulation of bilirubin in patients with OSCC might reflect its protective effects, including potent antioxidant, anti-inflammatory, and anticancer activities. Inflammation can facilitate tremendous cancer progression, including for OSCC, and has been regarded as the seventh hallmark of cancer [11]. Bilirubin is able to inhibit this inflammatory process by preventing the migration of leukocytes into target tissues through the interruption of vascular cell adhesion molecule1 (VCAM-1)-dependent cell signalling [12]. The normal value of bilirubin is approximately stable and does not change with age. Additionally, diet and exercise merely affect the blood level of bilirubin. But liver dysfunction can cause elevated bilirubin levels [12].

In 50 OSCC patients, 22 were well differentiated CA (mean = 0.17mg/dl), 17 were moderately differentiated (mean=0.08 mg/dl) and 11 were poorly differentiated cases (mean = 0.14 mg/dl), in which the serum direct bilirubin is significantly low in moderately differentiated carcinoma followed by poorly differentiated and then in well differentiated carcinoma.

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This difference may be due to the variation in the sample size in each stage. No studies were done on histopathological comparison with bilirubin.

In a study done by Liu X et al, 2425 female patients having stage I tumour (42.47%), 1011 (41.69%) and 384 patients (15.84%) were having stage II and III tumour, respectively [7]. For bilirubin, patients with total bilirubin level >0.2mg/dl had better 5-year OS and their risks of death reduced nearly 40% compared with that of patients with total bilirubin level ≤0.2mg/dl. Among them Stage III patients were with lower serum bilirubin levels and had poor OS. This is similar to present study in which stage IV patients are having lower bilirubin levels with a mean value of 0.1 mg/dl suggesting that bilirubin levels are in inverse association with the progressive stage of the disease, predicting the prognostic role of bilirubin in cancer.

Li N et al in the study showed high-DBIL group had significantly longer OS than the low-DBIL group [13]. The 5-year OS rates were 58.7% and 48.2%, respectively, among the patients with high TBIL and those with low TBIL which is consistent with the present study in which lower DBIL levels have poor survival. This may be explained by the fact that the downregulation of bilirubin in patients with OSCC might reflect its protective effects, including potent antioxidant, anti-inflammatory, and anticancer activities.

Among different types of Bilirubin, it is noticed that though TBIL and IBIL values are varied but DBIL serves as an independent prognostic factor in OSCC patients in the present study. This is similar to the study done by Yang L et al who proved that DBIL was an independent prognostic factor for OS [10].

Certainly, this study has limitations. First, the sample size was less in the study group. Second, gender variation was not assessed due to male predominance in the study group. Then the role of habits was not assessed which may play a role in bilirubin levels as smoking has been regarded as risk factor for OSCC, and several studies have shown an inverse association between smoking and bilirubin levels. Active smokers, even non-smokers with second-hand smoke exposure would have a lower serum bilirubin compared with non-smokers. The alcohol is partly responsible for liver function injury, which may also affect serum levels of bilirubin.

CONCLUSION

The present study based on total 100 samples provided a primary association between serum bilirubin and OSCC incidence. Lower levels of DBIL may be a useful indicator or pointer of OSCC risk. Higher DBIL levels were associated with better overall survival in patients with cancer. To sum up, decreased bilirubin levels – according to clinical/histological staging can be considered as a prognostic indicator for patients with OSCC.

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