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Original Research Article

Assessment of Serum bilirubin as a biomarker of disease status and predictor of survival rate in oral squamous cell carcinoma

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ABSTRACT

Introduction: Carcinogenesis is a complex and multi-step process, which results from various deleterious habits, multiple environmental factors and genetic susceptibility. Inflammation can facilitate tremendous cancer progression. Identifying novel prognostic factors for OSCC is important for early diagnosis, prognosis valuation and choosing more appropriate treatment.

The serum bilirubin plays a chief role in anti-inflammation, anti-oxidation, and anti-tumorigenesis. In different tumor models, thereby alleviating the oxidative stress. Decreased reactive oxygen species damages DNA structure and alters gene expression ultimately reducing cell proliferation. Abnormal level of serum bilirubin, marker of hepatobiliary was associated with patient prognosis in several human malignancies. Therefore, the current study will be carried out to evaluate the predictive value of serum bilirubin for clinicopathologic characteristics and survival of patients with oral squamous cell carcinoma (OSCC).

Materials and Methods: Study was performed retrospectively and it comprised 246 cases of OSCC were selected randomly among the individuals who admitted to the hospital. The pre-operative direct bilirubin (DBIL), indirect bilirubin (IBIL), total bilirubin (TBIL) was compared and evaluate with clinical and pathological parameters. A proportional hazards regression model was used to find out the independent predictors of overall survival (OS).

Result: Significantly lower TBIL (p=0.012) & IBIL (p<0.0035) were found in OSCC patients compared with normal controls. DBIL (p = 0.008) and lymph-node metastasis (p = 0.031) were institute to be self-determining prognostic factors. Cases having lesser DBIL with lymph-node metastasis exhibited the poor OS (p = 0.001).

Conclusion: DBIL and lymph node metastasis was regarded as a self-determining prognostic marker for individuals with OSCC.

1. Introduction

In the Current scenario, the world is heading towards several types of non-communicable diseases, which are also known as modern day epidemics. Amongst all these cancers oral squamous cell carcinoma (OSCC) is the 2^{nd} most common basis of mortality in worldwide. OSCC is the 6^{th} most common cancer reported globally with an annual incidence rate as a 3,00,000 cases.[1]

In the past 20yrs contempt enhancements in the several therapeutic intercessions, such as surgery, chemotherapy and radiotherapy, but survival rate of the individual has not been improved significantly. The diagnosis of this tumor in most cases is carried out at advanced stages of the disease. If the cancer can be detected at the incipient stage and the survival rate could be greatly improved.

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Factors influencing the prognosis of carcinoma include tumor intrinsic factors and host-immune response.[2] Compared with traditional prognostic indicators such as tumor size, clinical stage and degree of differentiation, hematological biochemical indicators are increasingly popular because they are easily obtained, non-invasive and show high predictive efficacy.[3] Uncovering of molecular alteration in such cases might be useful in screening for early finding of malignancy. It has to provides an important indicator for patient's prognosis and could contribute to the future development of treatment modalities based on the presence of specific biomarkers.

Bilirubin, comprising two subtypes i.e. direct bilirubin (20%) and indirect bilirubin (80%), is the end product of hemoglobin metabolism.[4] Serum bilirubin, as an endogenous antioxidant and improving the ability to scavenge oxidative free radicals in cancer patients.[5] Interestingly, many clinical research in last few years have demonstrated that serum bilirubin play as a chief role in anti-inflammation, anti-oxidation and anti-tumorigenesis. In different tumor models, such as colon cancer and adenocarcinoma, bilirubin can promote apoptosis and inhibit production of cancer cell.[6] Therefore, bilirubin act as a diagnostic and prognostic marker in various malignant tumors has been explored, such as small cell lung cancer,[7] pancreatic cancer,[8] ovarian cancer,[9] and colorectal cancer.However, the relationship between serum bilirubin levels and prognosis of OSCC individual has been reported. Therefore, we intend to fill this gap by retrospectively analysing the clinical data of 246 OSCC cases.

2. Material and Method

2.1. Subjects

Total 246 individuals of OSCC were selected at simple random method from the years 2016 and 2020 from the Govt Cancer hospital, Aurangabad. The inclusion criteria were as followes: (i) clinically and histo-pathologically diagnosed cases of OSCC; (ii) age >18 years; (iii) individual had no systemic illness.

The exclusion criteria were as: (i) diagnosed case of hepatobiliary & hemolytic diseases; (iii) Insufficient survival data; (iv) Patient had distant metastasis; (v) patient received chemotherapy or radiotherapy treatment; The control group consisted of a similar body mass index (BMI), which equals to socioeconomic background to the study group. This study was approved with the institutional ethical committee.

2.2. Assessment of Clinicopathological parameter

The various clinical and histopathological parameters were included in our studies like age, gender, tumor site, TNM stage, histological differentiation, pre-therapeutic serum levels of DBIL, IBIL and TBIL. The histological grades of OSCC samples were measured according to characteristics of its atypical squamous cells by pathologists. The clinical staging of OSCC were measure by TNM classifications of 7th edition of the American Joint Committee (AJCC) cancer staging manual.[9] Serum levels of DBIL and TBIL were calculated by using the D.Bili kit and T.Bili kit by the Semiautomatic Biochemistry Analyzer. The serum IBIL was calculated by subtracting level of DBIL from TBIL. (IBIL = TBIL – DBIL)

2.3. Statistical procedures

Statistical analyses were performed with SPSS version 17.0. All data were shown in mean ± standard deviation (SD) formulation. A paired t-test was used to compare clinical parameter between the study group and control groups. The analysis of variance (ANOVA) was used to comparing clinical TNM staging of OSCC. The Kaplan–Meier method was used to analyse overall survival (OS) of the OSCC individual. Chi-square were used to examine the correlation between serum bilirubin with various clinicopathological parameters. Independent predictors of survival were analysed using the Cox proportional hazards model. All tests were P values < 0.05 were considered significant.

3. Results

3.1. Baseline features

The study group comprised total 246 cases (166 men and 80 women, with an age range of 27-89 years and a mean age of 60 years) for the analysis of serum TBIL, IBIL and DBIL. The baseline characteristics of the OSCC patients are explained in Table 1.

3.2. Comparison of pre-therapeutic serum bilirubin levels between OSCC and control cases

First to measured pre-therapeutic serum level of TBIL, DBIL and IBIL of each individual in this study group. Then to compared serum bilirubin level between OSCC cases and healthy controls, and found that mean value of TBIL, IBIL & DBIL were as a 11.64 ± 3.17mmol/L, 8.05 ± 3.65mmol/L & 5.62 ± 1.49 mmol/L respectively as a shown in Table 3. Significantly lower levels of mean serum TBIL and IBIL were detected in study group compared with control individuals ($p = 0.012$ and $p < 0.0035$, respectively, Table 3). Correspondingly, a significantly higher level of DBIL was found in with OSCC cases ($p = 0.008$)

3.3. Correlation between serum bilirubin & clinicopathological prognosis of individuals

To Analysed association between serum bilirubin levels and clinicopathologic parameters in this study. No significant

difference was initiate between serum bilirubin level with age and gender of the OSCC cases. There was a significant difference found between clinical stages, regional lymph node metastasis and histopathological grades (Table 2).

An investigation was carried to determine whether any type of Bilirubin could be a predictor of OS in patients with OSCC. The OS of individuals is as shown in (Figure 1). As projected, regional lymph node metastasis strongly predicted the OS of patients with OSCC (p = 0.001) (Figure 1A). The results of the univariate analysis indicated that DBIL and lymph node metastasis are associated with OS (p = 0.0046 and p = 0.029, respectively; Table 4). Patients with higher DBIL had significantly longer OS than those with lower DBIL (Figure 1A).

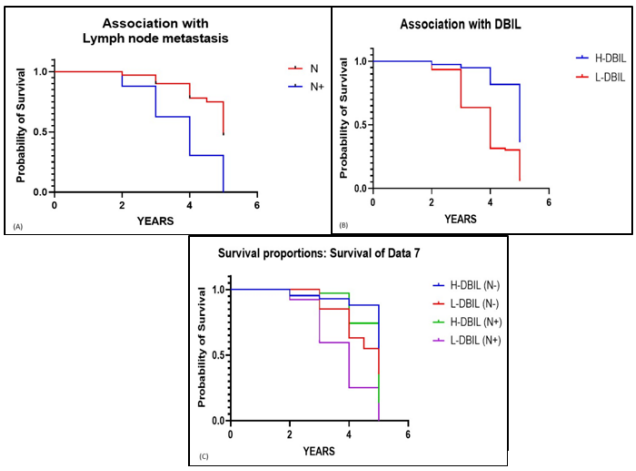
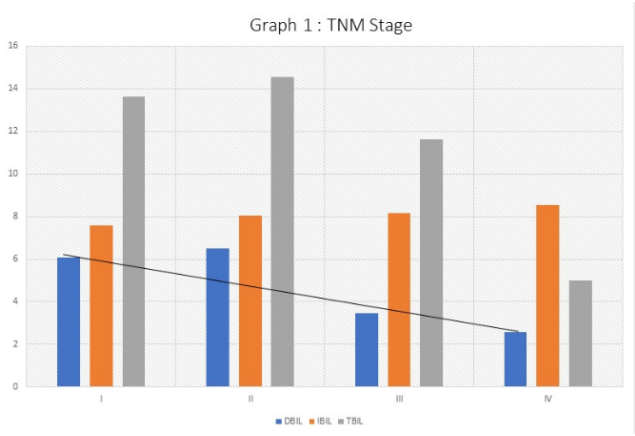


Fig. 1: Kaplan-Meier curves for DBIL and cervical lymph node metastasis (N) status associated with patients' overall survival (OS); **A:** Kaplan- Meier curves of OS in patients with or without cervical lymph node metastasis; **B:** Kaplan-Meier curves of OS in patients with H-DBIL (DBIL≥4.0 μmol/L) or L-DBIL (DBIL <4.0mmol/L); **C:** Kaplan-Meier curves of OS in patients with H-DBIL (DBIL≥4.0 μmol/L) or L-DBIL (DBIL<4.0mmol/L) with or without lymph node metastasis.

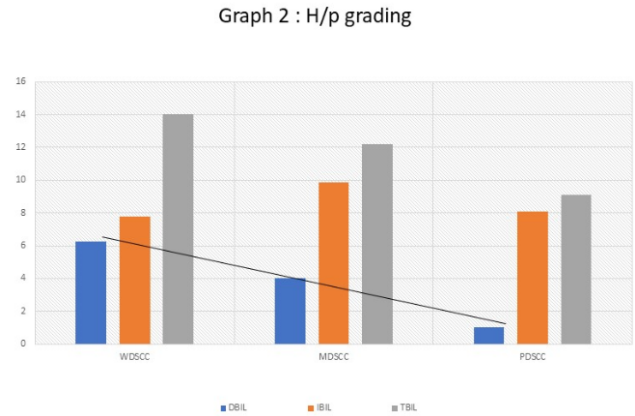
A multivariate analysis was calculated overall survival rate of the patients by considered lymph node metastasis as a cofactor and to determine DBIL was an independent parameter of patient's overall survival (p = 0.011 & p = 0.031, respectively) (Table 4). The poor survival rate was seen in patients with a lymph node metastasis and lower DBIL level, while those individuals having free from lymph node metastasis and higher the DBIL exhibited the good overall survival rate (p = 0.001) (Figure 1C).

4. Discussion

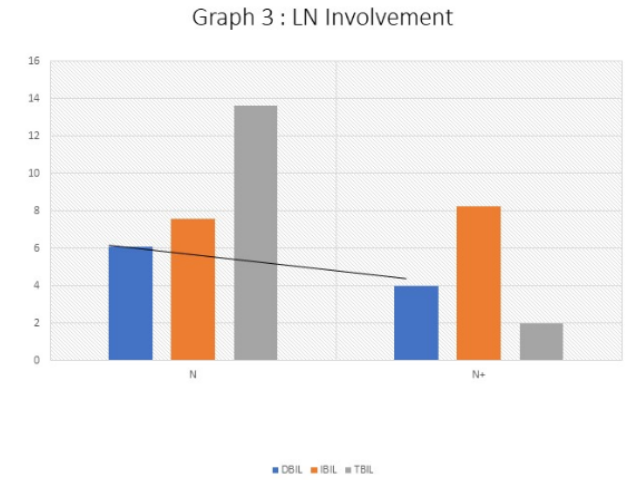
Oropharyngeal cancers is a significant cause of morbidity and mortality. The annual global estimated incidence rate of



Graph 1: Shows association of bilirubin with TNM staging of OSCC.



Graph 2: Shows association of bilirubin with histopathological (h/p) grading of OSCC.



Graph 3: Shows association of bilirubin with lymph node involvement of OSCC. (Significantly difference was noted)

Table 1: Baseline characteristics of OSCC Pts

Characteristics	Number of patients	%
Age		
< 30 Yrs	22	8.87%
30-60yrs	98	39.51%
	129	52.01%
1. Ø		
Gender		
Male	166	67.48%
Female	80	32.52%
Tumor Site		
Buccal mucosa	77	31.30%
Lateral border of tongue	49	19.92%
Floor of mouth	28	11.38%
Lip	10	4.06%
Alveolus	29	11.79%
Ventrum of tongue	04	1.62%
Palate	30	12.2%
Gingiva	15	6.1%
Corner of mouth	04	1.62%
TNM Stage		
I	70	28.45%
II	56	22.76%
III	78	31.70%
IV	42	17.1%
N Classification		
N	70	28.45%
N+	176	71.54%
Histological Differentiation		
WDSCC	97	39.43%
MDSCC	124	50.4%
PDSCC	25	10.16%

about 2,75,000 for oral and 1,30,300 for pharyngeal cancers, excluding salivary gland malignancy, malignant neoplasms of the nasopharynx and pyriform sinus. Amongst $2/3^{rd}$ of the cancers occur in developing countries.[10] The global burden of oral cancers continues to increase because of an increasing acceptance of habits causing cancer, particularly in economically developing countries.[11] In the present study, the age of the OSCC prediction of OS of OSCC cases ranged from 27-65 years with a mean value of 48.24 ± 11.61 years. The peak incidence was seen in 5^{th} & 6^{th} decade. Ribeiro ACP, et al suggest that the diagnosis of OSSC in the young population is made late.[12] In the present study, the statistically significant difference was not found between mean serum bilirubin with age and gender of the OSCC cases.

Rådestad E, at al, shown that oxidative stress may be involved in affecting many tumor behaviours, including survival, proliferation, chemotherapy resistance, radiation resistance, angiogenesis, and distant metastasis.[12] Local recurrence and nodal metastasis was considered as leading to reason for death of OSCC cases. Clinical staging and histopathological grading is valuable for predicting the

Table 2: Comparison of serum bilirubin components between different clinicopathologic sub-groups

Characteristics	DBIL (mmol/L)	IBIL (mmol/L)	TBIL (mmol/L)
Age			
< 30 Yrs	6.05	7.79	14.04
30-60 yrs	4.01	9.18	13.19
	5.64	7.80	9.12
1. Ø			
Gender			
Male	4.18	8.05	12.14
Female	5.41	8.07	13.49
Tumor Site			
Buccal mucosa	4.50	8.32	12.82
Lateral border of tongue	4.30	7.93	12.23
Floor of mouth	3.83	8.01	11.85
Lip	6.37	8.18	14.55
Alveolus	4.58	7.80	12.39
Ventrum of tongue	4.52	8.52	13.05
Palate	5.18	7.86	13.04
Gingiva	4.77	7.99	12.76
Corner of mouth	5.47	7.5	12.97
TNM Stage			
I	6.07 ± 1.65	7.57 ± 1.31	13.64 ± 2.19
II	6.49 ± 4.19	8.06 ± 1.83	14.55 ± 3.89
III	3.45 ± 2.42	8.17 ± 1.49	11.63 ± 2.79
IV	2.59 ± 1.79	8.53 ± 1.97	11.13 ± 2.53
N Classification			
N	6.07 ± 1.65	7.57 ± 1.31	13.64 ± 2.19
N+	3.99 ± 3.22	8.25 ± 1.73	12.24 ± 3.3
Histological Differentiation			
WDSCC	6.24 ± 3.54	7.79 ± 1.52	14.04 ± 3.44
MDSCC	4.01 ± 1.74	8.18 ± 1.85	12.19 ± 2.32
PDSCC	1.04 ± 0.41	8.08 ± 1.74	9.12 ± 1.76

Table 3: Comparison of pre-therapy bilirubin serum level in patients with OSCC and control individuals

	Control	OSCC	P value
TBIL (mmol/L)	12.13 ± 3.06	12.64 ± 3.17	0.012
DBIL (mmol/L)	3.12 ± 1.50	5.62 ± 3.65	0.008
IBIL (mmol/L)	8.96 ± 3.45	8.05 ± 3.65	0.035

Table 4: Univariate and multivariate analysis of clinicopathologic characteristics and survival

	P value	95% CI	Risk ratio
Age (>60 Vs <60)	0.094	1.24	0.57
Gender (Male Vs Female)	0.14	1.26	0.6
Tumor size (>4cm Vs <4cm)	0.29	2.59	1.62
N classification (N+ Vs N-)	0.029	7.35	2.89
DBIL(0.4mmol) (>0.4mmol Vs <0.4mmol)	0.0046	0.8	0.345
IBIL (0.3-0.7mmol) (>0.8mmol Vs <0.2mmol)	0.092	1.13	0.52
TBIL (0.2-1mmol) (>1.1mmol Vs <0.2mmol)	0.26	2.41	1.56

prognosis of OSCC cases.[13] In our present study, we have to ruled out the relationship between serum bilirubin and prognosis of OSCC cases with prediction of OS of OSCC cases.

The down-regulation of serum bilirubin in OSCC patients might reflect its protective effects, including potent anti-inflammatory, antioxidant and antitumor events of cancer cells. Inflammation can facilitate cancer progression and has been considered as the seventh hallmark of cancer.[14] Vogel ME, Zucker SD suggested that bilirubin is able to inhibit inflammatory responses by preventing the migration of leukocytes into target tissues through the disruption of vascular cell adhesion molecule-1 (VCAM-1) dependent cell signalling.[15]

A first line of defence against ROS is protection against their formation and prevention. Another measure is to change the direction of radical function from more sensitive target sites to cellular compartments in which oxidative damage would be less deleterious. Studies have reported lowered antioxidants or antioxidant capacity in blood and tissues of oral precancer and cancer.[16] This could be due to (i) increased utilization of antioxidants to scavenge ROS/RNS, (ii) poor antioxidant defence system in cancerous environment, (iii) inadequate production of antioxidant enzymes, and (iv) increased destruction of antioxidants by reactive oxygen metabolites. Lowered capacity to defence ROS/RNS might be one of the possible mechanisms operating in the progression of oral cancer.

Ollinger R, et al suggested that bilirubin can induced apoptosis and prevent proliferation of tumor cells in various cancers, such as colon cancer & adenocarcinoma.[17] Therefore, it looks that serum bilirubin has a protective and antineoplastic result in many human malignancies. This study has an important clinical application value with low cost and little trauma with predicting prognosis of individual.

In the present study, the mean serum TBIL, DBIL & IBIL level was 11.64 ± 3.17mmol/L, 8.05 ± 3.65mmol/L & 5.62 ± 1.49mmol/L in the OSCC cases which was statistically significantly higher value ($P < 0.001$) in the OSCC cases compared to that in control group. These observations were consistent with Gao et al. used to predict prognosis in Chinese rectal cancer patients.[10] In the present study, the mean serum DBIL concentration was stage I (6.07 ± 1.65), Stage II (6.49 ± 4.19), Stage III (3.45 ± 2.42), and Stage IV (2.59 ± 1.79), respectively. The mean serum bilirubin levels of Stage IV cases were uncovered to be statistically significant difference ($P < 0.01$) and lowered as compared to Stage I, Stage II, and Stage III cases. These observations were consistent with the observations of Rao p et al., Jiang D et al., and Céruse et al. found that serum Bilirubin levels of patients significantly related to tumor stage in various malignancies such as adenocarcinoma, Lung cancer and head and neck SCC (HNSCC), respectively.[4,7] The mean bilirubin concentrations of Stage I and Stage II cases were found similar ($P > 0.05$) to controls group i.e., statistical difference was not created.

In our study 42 cases of stage IV OSCC cases indicated that raised TBIL 11.13 mmol/L and IBIL 8.53 mmol/L were associated with poor OS and lowered level of DBIL was considered an self-determining prognostic factor for OS. To the finest of our knowledge, this study was report as a prognostic role of DBIL in stage IV OSCC individual.

The poor OS rate was detected in cases with a lymph node metastasis and decrease DBIL level. It may propose that more violent therapy should be required and follow-up intervals should be reduced for those OSCC individual with lower DBIL (3.99) and LN metastasis. In addition, the pre-therapeutic serum DBIL, recognized as a simple indicator to predict prognosis of OSCC patients.

5. Conclusion

Bilirubin has a vital part in several clinical applications and promising biological parameter to predict prognosis of the disease. It possesses anti-oxidative, anti-inflammatory and immunosuppressive properties. In fact, these characteristics have been thought to act as a central link in the pathogenesis of many diseases. Hence, we should consider that serum bilirubin is closely related to human health, although the present study ruled out a primary correlation between serum bilirubin and OS rate of OSCC. Diagnosis and treatment of early staged of OSCC are valuable in improving the prognosis of metastatic and locally advanced cases of OSCC. DBIL can be imitated as an independent prognostic biomarker for OS.

6. Conflict of Interest

The authors declare no relevant conflicts of interest.

7. Source of Funding

None.

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