Original Research

Association of Endogenous Metabolic End products & Clinicopathological traits of Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is a widespread cancer and is associated with social and economic consequences. Clinico-pathological correlation improves patient survival and disease management. Our research intends to examine the relationships between OSCC and metabolic processes reflected by final catabolism products.

Methodology: In this cancer comparative retrospective analysis, metabolic byproducts in OSCC patients and healthy controls were compared. One way ANOVA analysis was done to record notable changes in OSCC patients by correlating along with disease's clinico-pathological traits.

Result: Serum Urea and creatinine level were determined in a total of 109 subjects, 55 diagnosed as OSCC and 54 healthy subjects who had been reported to our hospital for the past two years. OSCC and healthy patients revealed blood urea levels with significant correlation (p=0.000). Serum creatnine was insignificant between two groups (p<0.68). Blood urea and creatinine was insignificant with random blood sugar (p<0.205), negative correlation with habits (p<0.953) and significant with metastasis (p=0.000).

Conclusion: OSCC in advanced stages have nearing higher serum urea levels, and is associated with nodal metastases. Deregulation of protein catabolism processes may be associated with invasive characteristics of OSCC, enabling aggressive behaviour in OSCC.

Key words: Urea, Creatinine, OSCC, metastasis

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Introduction

Oral squamous cell carcinoma (OSCC) is a common oral malignancy world wide ranging from sixth to ninth common anatomical location based cancer especially in south east asian region (1). Pan chewing, smoking, drinking, UV exposure, and other variables like HPV (Human Papillomavirus), nutritional deficiency, candidal infection, and hereditary factors are the main predisposing causes for OSCC.(2).

The primary effect of OSCC is the late-stage diagnosis of local invasion and metastases. That affects its therapeutic tactics, especially in light of the patient's post-treatment side effects. Economic stress also has a significant impact in nations with higher incidence (3). There have been efforts made to comprehend the carcinogenic mechanism, tumour growth, and invasion. The use of therapeutic challenges has made it possible to treat all types of cancer. Even though cancer has

been detected earlier, the survival rates for people with advanced stages have not increased. Data on the role of the immune system, genetics, and inflammation in the development of cancer have been published in literature. Additionally, numerous innovative techniques have been used to characterise and treat OSCC (4).

Metabolic end product reprogramming has been identified as a hallmark of cancer. Protein and gene expression show that it is regulated. Additionally, metabolic end products serve as a prognostic biomarker and a tool for illness diagnostics (5). The community has expressed interest in finding out how well metabolic byproducts correlate with oral cancer since amino acids and lactate are involved in the aetiology of cancer. Metabolic processes including aerobic glycolysis or fatty acid oxidation, as well as their endogenous metabolic byproducts such blood urea, uric acid, and creatinine, had an impact on the growth of tumours and metastases (6). Similar research on alterations in urea—the byproduct of amino acid breakdown—has been documented in cases of renal and hepatocellular cancer. As a result, it was suggested that one of the parameters be the identification of first malignancy using ammonia end product. While creatinine has also contributed to the correlation of a prognostic characteristic in a number of

epithelial malignancies (7) (8).

Our research intends to examine the relationship between OSCC's clinicopathological manifestation and the metabolic process expressed in serum analysis. Blood urea and creatinine, the end catabolic products in OSCC, have been assessed for this analysis.

Methodology

Characterization of data collection groups

Patients admitted to the Oral and Maxillofacial Surgery Department at Saveetha Dental College and Hospitals, Simats University, Chennai, between October 2020 and August 2022 with an OSCC diagnosis were included in the study. The mouth floor, buccal mucosa, tongue, palatal mucosa, gingiva, and lips were among the areas affected by the tumour. The patient underwent a preliminary systemic and clinical evaluation, as well as blood and imaging testing. The study included patients who had been diagnosed with OSCC and had data on oral habits, serum urea creatinine, random blood sugar data, Broaders grading, pTNM staging, and nodal metastasis. Patients with other comorbidities other than diabetes mellitus, OSMF that had progressed to OSCC, other malignancies, and patients with incomplete data were excluded from the study.

Diagnosed lesions underwent Neck resection either ipsilateral or both the sidesfor those lesions which have crossed the midline with nodal metastasis including posterior orally located tumors and ulcerated growth pattern of tumour based on the national guideline criteria. Post pathological diagnosis patients also unerwent radiotherapy along with or without chemotherapy based on the

regional spread of cancer.

According to national guidelines, diagnosed lesions that had crossed the midline and had nodal metastases, including posterior orally situated tumours and tumours with an ulcerated growth pattern, received neck resection on either the ipsilateral or contralateral side. Patients who received a post-pathological diagnosis underwent radiotherapy in addition to or instead of chemotherapy depending on the extent of the cancer's local dissemination.

The study was conducted with Ethical approval committee from our hospital (IHEC/SDC/FACULTY/22/GPATH/564)

Data Collection

Data about patients were input using our hospital's computerised database. Patients with OSCC diagnoses had a thorough clinical evaluation that included imaging, systemic analysis, and complete blood analysis, as well as a few other investigations if necessary. Our research sought to identify metabolic byproducts like urea and creatinine.

Statistical analysis

Utilizing SPSS software version 23, statistical analysis was carried out. Non-parametric distribution is analysed using the independent T test. ANOVA was used for comparisons, followed by a post hoc test, and Pearson correlation analysis was used for correlation analysis. We performed pairwise comparisons within and between groups. Correlation analysis was performed in order to compare OSCC with healthy groups and identify any significant biological parameter differences. Depending on the distribution of the data, mean values plus or minus standard deviation and median values for the pertinent parameters were presented.

Statistically significant values were deemed to be p 0.005.

Results

Characteristics of patient groups

In total, 236 patients were hospitalized and OSCC was diagnosed in 182 of them. Only 55 patients met the inclusion criteria, while the remaining 57 were eliminated because of poor data collection, skewed data, or a diagnosis of another malignancy. Table 1 lists the general characteristics of the data from the chosen group. The average age of the selected OSCC group was 51.25 (range from 35 to 76), with men making up the majority of the population—roughly 78% of the population. Depending on the degree of midline involvement, patients who had undergone radical neck dissection had it done either ipsilaterally or bilaterally. More than 57% of the population had been identified with the disease at an advanced stage, according to staging. Twenty patients (36% of the study population) had tumours that invaded nearby structures and were larger than 4 cm in T3 and T4 staging. In 23 individuals (41%), regional cervical spread has been identified. After pathological evaluation, the remaining 32 patient samples presented after surgical dissection showed no nodal involvement. According to histopathological analysis, 71% of the tumour was found to be well-differentiated, 23% to be moderately differentiated, and the remaining 40% to be poorly differentiated. During our mean follow up in our study was 3 months to one year of that five patients have died due to disease progression. Whereas the rest 52 were alive till their last follow up.

In the control group, 54 healthy subjects were included with 43 males and 11 females with mean age of 52.5, ranging between 35-78 years. The two groups were

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insignificant between age and gender characteristics (p= 0.007 for age and p= 0.844 for gender).

Comparison of urea and creatinine levels between study groups

Blood urea levels were significantly different between OSCC and healthy control groups. Blood urea levels in the OSCC group were substantially higher than those in the control healthy group (p=0.00), with a mean value of 26.41 mg/dl in the OSCC group and 19.34 mg/dl in the control healthy group (Figure 1). The mean value of the OSCC was 0.861 mg/dl, while the control healthy group's value was 0.701 mg/dl for creatinine (Figure 2) (Table 2).

Table :1 Characteristics of OSCC group

Catergories	Sub categories	Total number of Population	Percentage (%)
Age	Mean ± SD	**55**	51.25 ±2.28 (ranging between 35-76)
Gender	Males	43	78%
	Females	12	22%
Habits (Smoking, Pan)	Yes	38	68%
	No	12	23%
	May be	5	9%
Alcohol	Yes	5	10%
	No	26	47%
	May be	24	43%
TNM Staging	EarlyTNM (1-2)	29	43%
	Advanced TNM(3-4)	31	57%
Nodal metastasis	Positive	23	41%
	Negative	32	59%
Degree of Differentiation	Well differentiated	39	71%
	Moderately differentiated	13	23%
	Poorly differentiated	3	6%
Survival rate	Alive	50	91%
	Deceased	5	9%

Analysis of Blood urea versus Progression of OSCC

Our research on blood urea showed that there was a significant difference between the groups (p<0.00) when we did comparative study of blood urea with disease development against control healthy groups. OSCC and a regional spread were assessed pairwise, and the results showed a significant difference (p<0.00). Therefore, a significant difference between the control group and those with advanced disease was detected in the increase in serum urea levels. The mean blood urea level in advanced stages was 1.76 (±0.42) mg/dl, compared to 26.4 (10.29) mg/dl for OSCC and 19.34 (±0.2.45) mg/dl for the control group.

Analysis of the relationship between OSCC and pathology and urea. In OSCC, our findings indicated a positive connection between blood urea and age (r=0.08, p<0.000). Urea and smoking were shown to be negatively correlated (r=-0.08, p<0.953). Similar to this, there was a negative connection between nodal metastases and blood urea (r=-0.208, p<0.128). The urea level in OSCC was positively correlated with staging and random blood sugar (r=0.0207, p<0.129; r=0.174, p<0.205). Alcohol consumption and blood urea levels do not correlate when using histological grading.The correlation analysis values were mentioned in Table 3
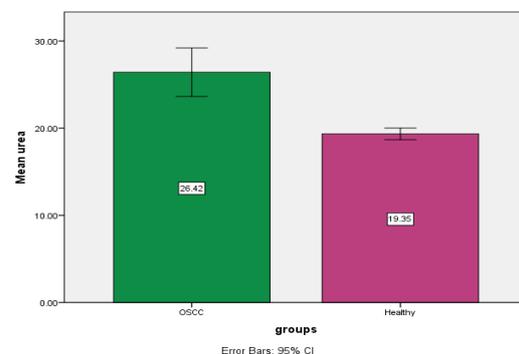

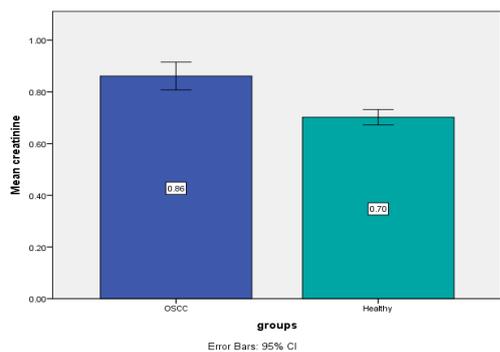**Figure 1:** Comparison of Blood urea level between groups

Figure 2: Comparison of Blood creatinine between groups

Discussion

When proteins are broken down, nitrogen is released in the form of the extremely poisonous toxin ammonia. In order to release dangerous excess metabolites, several metabolic pathways must be activated by ammonia. Ammonia, a water-soluble, less harmful metabolite that is discharged in urine, releases urea. The urea cycle occurs in the liver, and scientific literature has described how the metabolic cycle works (9). Membrane transporters and catalytic enzymes are used in the urea cycle, which converts two nitrogen molecules into one urea molecule. There may be a connection between the urea cycle and OSCC pathogenesis. The only source of endogenous amino acid synthesis in our body is tricarboxylic acid metabolites, which are connected to enzymes in the urea cycle. By changing the metabolism of urea to anabolic chemical, which activates the biosynthesis in cancer cell growth, dysregulation of the urea cycle is immediately implicated in the promotion and proliferation of cancer cells. Through the deflection of nitrogen molecules in the direction of pyramiding synthesis, Dihydrooratase, Aspartate transcarbamylase, and Carbomyl-phosphates Synthetase 2 (CAD enzyme activation) promote the proliferation of

cancer cells in melanoma, ovarian cancer, and hepatocellular carcinoma cell lines(10). The fundamental pathophysiology of cancer was the conversion of extra ammonia into amino acids, which were then used to synthesise lipid and nucleotide macromolecules, which served as a source of energy for cells with low metabolic energy. Instead of elevating urea discharge, inclusion of carbonyl phosphate allowed for the creation of pyramidines. In lung cancer cell lines, the growth and proliferation of cancer cells were accompanied by the activation of mitochondrial enzymes. In colorectal cancer, a negative association between prognosis and therapeutic therapy on patient survival was reported (11).

Table 2: Comparison between Urea and Creatinine levels between groups

	Serum Urea		Serum Creatinine	
	OSCC	Control	OSCC	Control
Total No of patients	55	54	55	54
Mean(mg/dl)	26.41	19.34	0.861	0.701
SD	10.29	0.331	0.198	0.108

According to the findings of our study, patients with OSCC had blood urea levels that were greater than those of healthy, normal people. In later phases of OSCC, a further link between illness development and virtually elevated normal blood urea level was observed. Previous research in the literature found a link between elevated serum urea levels and a good prognosis for individuals with small lung cell carcinoma, with an early death prediction suggesting anabolism in the production of pyramidine. (12).

Table 3: Correlation analysis clinicopathological characteristics and Blood urea in OSCC

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Parameter	Correlation coeffcient	p value
Age	0.08	0.00
Habits	-0.08	0.953
Tumour staging	0.207	0.129
Nodal invasion	-0.208	0.128
Random blood sugar	0.174	0.205

The concept of homogeneity in our study group has been supported by changes in blood urea levels that are connected to ageing, which have been confirmed in our group, where the mean age of the group varied beyond fifty years. Our assertion that there is a correlation between ageing and an increase in serum urea level was validated by earlier research (13). Despite the fact that in our study group, the data showed a negative association between habits and blood urea levels. The results, however, were consistent with earlier studies that found that the urea level was lower in non-smokers. Although the precise mechanism underpinning the findings is unknown, it has been suggested that aspartate, an intermediary metabolite in the urea cycle, may have a role in habitats (14) (15).

Blood urea levels may be influenced by protein intake. Previous studies revealed that functional disability and diet may play a role in urea level in OSCC patients (16). Our study's findings, however, did not support this criterion. In OSCC individuals, urea level and initial tumour size were found to be negatively correlated. However, nodal invasion and metastasis were significant, and our study group's elevated urea level was nearly normal. Increased blood urea levels, which had a strong link with nodal metastasis, indicate a poor prognosis for the two thirds of patients who had advanced tumour stages. Head and neck malignancies will have an impact on changes in the body's fluids' production of metabolic byproducts. Due to shifts in the synthesis of byproducts, similar outcomes

have been observed in other cancers with an underlying complicated metabolic pathway (17). Changes in the urea cycle and altered immune responses that cause tumour cells to metastasize can be blamed for the spread of tumour cells to nearby or distant areas. Tumor infiltration was caused by Arginase II, a urea cycle enzyme change found in OSCC, as well as CD11+ dendritic cells of myeloid and T cells (18). According to research on prostate and ovarian cancer, abnormal immune responses may contribute to changes in the urea cycle enzymes. Although the epithelial-mesenchymal transition can also be facilitated by cancer cell plasticity, this enables the cancer cells to spread locally as well as distantly (19).

Aspartate is the primary substrate for the synthesis of pyrimidines, and aspargine synthase is a biosynthetic intermediate product in the urea cycle. Alterations to this product's function in malignancies also show urea cycle deviation. (20).

The study investigation report in OSCC have expressed metabolic changes associated with the disposal of ammonia and aspartate as nitrogen. Yet in depth analysis of the same is needed to confirm whether the above findings have influenced the OSCC disease progression. Previous study literature have results concordance with urea findings in colon cancer , lung cancer (21)and hepatocellular carcinoma (22). Our study's shortcomings include the small sample size, population homogeneity, a lack of information on nutrition habits, and any underlying illnesses or genetic results. Additionally, there was a dearth of research describing the results for urea and creatinine and how they related to OSCC. Further research and in vitro studies were required to reveal the underlying mechanisms relating to the pathogenesis of OSCC and metabolic changes in order to clear the way for the strategic deployment

of therapeutics against oral cancer in the future.

Conclusion

Urea levels in OSCC have been approaching normal levels in advanced stages of the tumour, which was linked to nodal invasion and interconnected with food metabolic dysregulation, which was linked to the aggressive tumour behaviour. Additionally, the environment, immunity, underlying systemic, and genetic abnormalities were taken into account for the progression of tumours. Finding the evident causes for the linkages between metabolic alterations and the pathophysiology of OSCC requires extensive investigation.

Acknowledgements

Conflict of interest

The author declares that there were no conflicts of interests in the present study.

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