ASSOCIATION OF HYPOXIA RESPONSIVENESS AND CELL TURNOVER IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT
Introduction: Oral cancer is one of the major health problems in India and Indian subcontinent countries. During the past few years, the association between cancer and raised uric acid has drawn a lot of attention. High cell turnover can lead to hyperuricemia and tumorigenesis implicating an underlying link between purine metabolism disorders and cancer. Objective: To evaluate serum levels of uric acid and hypoxia induced factor 1 alpha in oral cancer patients and compare them with those of healthy controls. Material and methods: 50 confirmed oral squamous cell cancer cases and controls had serum uric acid levels measured using timed end point method given by Fossati and Berti (1980) on spectrophotometer and HIF 1 alpha was measured by performing Sandwich ELISA. The data obtained was analyzed using the Statistical Package for the Social Sciences, version 20.0 (SPSS 20). Statistical significance was determined at p < 0.05. Results: Significant variation was observed in mean value of HIF 1 α in cases and control with normal (P value <0.05) as well as high (p value <0.001) uric acid levels. Conclusion: As hypoxia is a common feature of many cancers. HIF-1alpha is an essential component in changing the transcriptional response of tumours under hypoxia. Elevated uric acid may be a true risk factor for cancer incidence and mortality and mechanisms by which uric acid may contribute to cancer pathogenesis by increasing the expression of gene coding for HIF-1alpha.

KEYWORDS: Oral squamous cell carcinoma, HIF-1alpha, Uric acid, Hypoxia, Tumour, Antioxidant.
Uric acid is a product of the metabolic breakdown of purine nucleotides. The ability of urate to scavenge oxygen radicals and protect the erythrocyte membrane from lipid oxidation was originally described by Kellogg and Fridovich and was characterized further by Ames et al. Uric acid can become a pro-oxidant by forming radicals in reactions with other oxidants, and these radicals seem to target predominantly lipids (LDL and membranes) rather than other cellular components. At the same time, the hydrophobic environment created by lipids is unfavorable for the antioxidant effects of uric acid and oxidized lipids can even convert uric acid into an oxidant.[7]

The aim of this study was to investigate the correlation between the expression of hypoxia-inducible factor 1 alpha (HIF-1 alpha) and uric acid levels in patients with oral cancer and healthy controls.

MATERIAL AND METHOD
Hospital based Descriptive Observational Case control study. Study was conducted in the Department of Biochemistry in collaboration with the Department of Dental, Lady Hardinge Medical College & Smt. Sucheta Kriplani Hospital, New Delhi. The participants were enrolled after informed and written consent and the study protocol was in agreement with the guidelines of the institutional ethics committee.

INCLUSION CRITERIA
Samples will be selected from the patients attending the Dental & Oromaxillofacial surgery department fulfilling the following criteria.

The study subjects were divided into two (2) groups.

CASES: 50 biopsy proven oral squamous cell carcinoma patients

CONTROLS: Healthy person from department of biochemistry and dental.

EXCLUSION CRITERIA
CASES
Past history of any ischemic disease ex: cerebral ischemia, myocardial infarction etc.
Chemotherapy or radiotherapy started for oral cancer.

CONTROLS
1. Past history of any malignancy.
2. Apparently unhealthy oral healthy cavity on inspection.
3. History of acute / chronic illness.

Sample collection and analysis
Ten ml of venous blood sample was collected from the subjects under sterile conditions. The blood samples were processed immediately for separation of serum, and then serum were stored in aliquot at -40°C.

For other routine investigations sample were immediately analyzed.

METHODOLOGY
SERUM URIC ACID – was estimated by timed end point method given by Fossati and Berti (1980)
HIF-1α levels estimation by Sandwich ELISA

STATISTICAL METHODS
All analyses were performed with the SPSS software programme version 20. For comparison of variables with a normal distribution unpaired, 2-tailed Student’s t-test and Pearson’s correlation were used.

RESULTS
Table no 1 depicts the demographic characteristics of the study population. The cases and controls were age and sex matched. A high incidence of the risk factors for oral cancer (intake of alcohol, khaini, tobacco, sharp tooth, regurgitation of food) is seen in the cases with oral cancer.

<table>
<thead>
<tr>
<th>TABLE NO.1: DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n=50)</td>
</tr>
<tr>
<td>Age (Yrs) (Mean ±SD)</td>
</tr>
<tr>
<td>Sex m/f</td>
</tr>
<tr>
<td>Tobacco Chewing</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>Khani</td>
</tr>
<tr>
<td>H/O Hypertension</td>
</tr>
<tr>
<td>H/O DM</td>
</tr>
<tr>
<td>H/O Hot Spicy Food</td>
</tr>
<tr>
<td>H/O Sharp Tooth</td>
</tr>
<tr>
<td>H/O Regurgitation of food</td>
</tr>
<tr>
<td>family h/o cancer</td>
</tr>
</tbody>
</table>
TABLE NO. 2: Showing number of cases and control having low, normal and high uric acid levels.

<table>
<thead>
<tr>
<th>URIC ACID LEVELS</th>
<th>CASES N (%)</th>
<th>CONTROL N(%)</th>
<th>TOTAL N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW(3mg/dl)</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>N(3-6 mg/dl)</td>
<td>7</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>HIGH(&gt;6 mg/dl)</td>
<td>41</td>
<td>24</td>
<td>65</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

FIGURE NO.2: Showing number of cases and control having low, normal and high uric acid levels.

TABLE 3: showing mean value of HIF1α in cases and control at low, normal and high uric acid levels

<table>
<thead>
<tr>
<th></th>
<th>CASES MEAN VALUE OF HIF</th>
<th>CONTROL MEAN VALUE OF HIF</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW UA</td>
<td>295</td>
<td>380</td>
<td>0.245</td>
</tr>
<tr>
<td>NORMAL UA</td>
<td>413</td>
<td>412.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HIGH UA</td>
<td>789</td>
<td>426</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DISCUSSION
Cancer is a multifactorial disease which results from a complex interplay between environmental and genetic factors. It has become one of the most challenging health issues today. Oral squamous cell carcinoma (OSCC) is a cancer of the oral cavity that is increasingly becoming a public health problem due to its late detection and increased incidence of recurrence.

Hypoxia of cancer tissue is a hallmark of solid cancer. Intra-tumoral hypoxia is a common feature in solid tumors. HIF-1α is a transcription factor that initiates the expression of genes involved in neo-vascularization and glucose metabolism that confers survival advantage. Studies have shown the synergistic influence of tobacco and betel quid on HIF-1 α expression.

Strasak et al. Levine et al. Bengston et al. and Petterson et al. found that high serum uric acid levels was associated with higher risk of cancer mortality. This could be possibly due to high turnover in cancer leading to high uric acid levels. In our study also more number of cases (41) were having high serum uric acid levels. On the contrary, Mazza et al., in a study in Italy, found that serum uric acid could protect against cancer. This was corroborated by Bozkir et al. who reported significantly lower serum uric acid levels in lung cancer patients as compared to healthy controls. They explained that uric acid is a known antioxidant, and thus may prevent cancer by mopping up free radicals that may cause cellular and genetic injury. Uric acid is involved in a complex reaction with several oxidants and may have some protective effects under certain conditions.
On the other hand, uric acid cannot scavenge all radicals, with superoxide as an example. Uric acid is an antioxidant only in the hydrophilic environment, which is probably a major limitation of the antioxidant function of uric acid. Reactions of uric acid with oxidants may also produce other radicals that might propagate radical chain reaction and oxidative damage to cells.

In addition, uric acid itself and downstream radicals can engage, as a biologically active proinflammatory factor, intracellular oxidant production via the ubiquitous NADPH oxidase-dependent pathway resulting in redox-dependent intracellular signaling and in some conditions, oxidative stress.

The difference in mean value HIF-1α levels were found to be statistically significant (p<0.05) in cases and control with normal uric acid levels and highly significant (p<0.001) among cases and control with high uric acid levels. It could possibly be due to the increased demand of oxygen by cancer cells and proinflammatory action of uric acid.

HIF-1α is a key transcription factor that regulates cellular reaction to hypoxia and is over expressed in most solid tumors in response to low oxygen concentrations. It has the ability to influence metabolic reprogramming, angiogenesis, cell survival, and energy metabolism.

Zhu et al. reported that HIF1α level was significantly associated with T stage, lymph node involvement, histologic differentiation and microvessel density. Patients with positive HIF1α nuclear staining had a significantly worse overall survival and disease-free survival.

Kang et al. also found that HIF1α overexpression was significantly associated with poor overall and disease-free survival rates, independent of T stage and lymphatic metastasis. The Cox proportional hazards regression model demonstrated that the level of HIF1α expression may be an independent prognostic factor for tongue SCC.

Eckert et al. demonstrated that increased HIF1α expression, alone or in combination with a low CAIX (carbonic anhydrase IX) expression, was significantly correlated with a poor prognosis of OSCC.

In our study we found that cases and controls having high uric acid levels had high mean HIF1α values clarifying that in tumour progression the uric acid level rises may be due to cell lysis or cell division and due to tissue hypoxia because of increasing tumour growth the HIF1α level increases. At the same time high uric acid level can act proinflammatory and can lead to tumour progression which in turn lead to rise in HIF1α levels.

Further a study with more population under study should be investigated in this regard to reach a more valid conclusion.

**CONCLUSION**

HIF1α has an important role to play in pathophysiology of oral cancer both under normal and hypoxic conditions. High HIF1α value in cases with high uric acid levels explains that after certain cutoff value of uric acid start acting as proinflammatory. To determine the cutoff value of uric acid to switch its role from antioxidant to pro-oxidant more extensive study is required.

**REFERENCES**

12. Mazza A, Pessina AC, Pavei A, Scarpa R, Tikhonoff V, Casiglia E. Predictors of stroke mortality in


