ejpmr, 2017,4(1), 417-421

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

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

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Article Received on 10/11/2016 **Article Revised on 30/11/2016** **Article Accepted on 20/12/2016**

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.

INTRODUCTION

Oral cancer is high in India because of cultural, ethnic, geographic factors and the popularity of addictive habits. However, if detected early, the probability of survival from oral cancer is remarkably better than for most other cancers. Risk factors like tobacco and tobacco elated products, alcohol, genetic predisposition and hormonal factors are suspected as possible causative factors.[4]

Cancer of buccal mucosa, lower alveolus and the retro molar trigone are grouped together as cancers of gingivo-buccal complex constitute 60% of all oral cancers in India. Tongue and the floor of the mouth cancers form the bulk of oral cancers in the west.[5] In state of tumor growth, cancer cells continue to proliferate near supplying blood vessel and ultimately massive growth of the tumor renders its distant cells in a state of hypoxia. This activates the hypoxia inducible factor-1 (HIF-1) signaling cascade.

HIF-1 is overexpressed in many human cancers and plays a key role in promoting tumor growth and metastasis through its role in initiating angiogenesis and regulating cellular metabolism to overcome hypoxia. Hypoxia leads to cell death in normal as well as tumour tissue. In tumour, state of hypoxia develops and in tumour microenvironment along with genetic alterations overexpression of HIF1 alpha gene occurs.[6]

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 -40^0C.

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 1depicts 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 NO.1: DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

	Cases (n=50)	**Controls (n=50)**
Age (Yrs) (Mean ±SD)	50.32±8.04	50.15 ±8.03
Sex m/f	41/9	41/9
Tobacco Chewing	40	3
Smoking	30	12
Alcohol	24	10
Khani	11	0
H/O Hypertension	9	2
H/O DM	1	0
H/O Hot Spicy Food	8	5
H/O Sharp Tooth	3	0
H/O Regurgitation of food	12	5
family h/o cancer	1	0

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

URID ACID LEVELS	CASES N (%)	CONTROL N(%)	TOTAL N(%)
LOW(3mg/dl)	2	2	4
N(3-6 mg/dl)	7	24	31
HIGH(>6 mg/dl)	41	24	65
TOTAL	50	50	100

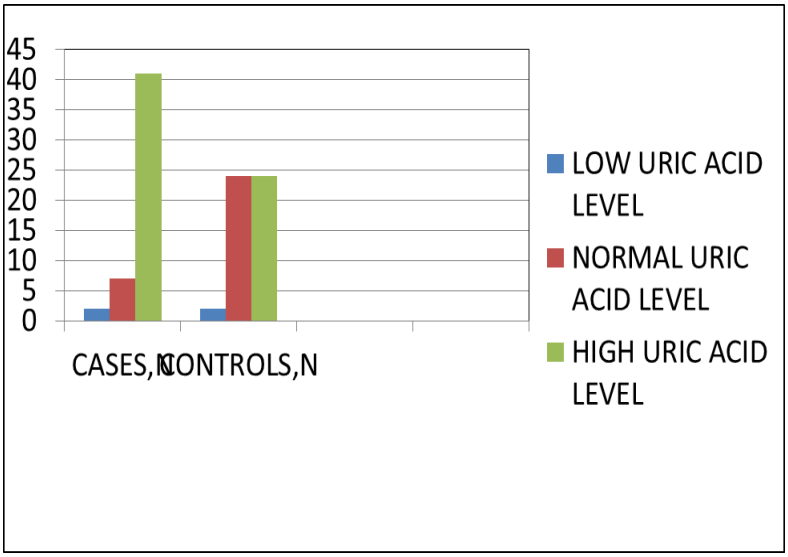

FIGURE NO.2: Showing number of cases and control having low, normal and high uric acid levels.

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

	CASES MEAN VALUE OF HIF	CONTROL MEAN VALUE OF HIF	P VALUE
LOW UA	295	380	0.245
NORMAL UA	413	412.5	<0.05
HIGH UA	789	426	<0.001

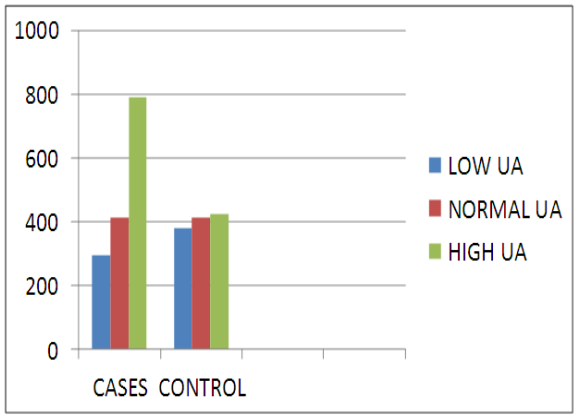

FIGURE 3: showing mean value of HIF1α in cases and control at low, normal and high uric acid levels

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.*[8], Levine *et al.*[9] Bengston *et al*[10] and Petterson *et al*[11] 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*[12], in a study in Italy, found that serum uric acid could protect against cancer. This was corroborated by Bozkir *et al.*[13] 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-1 α 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.[14] also 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.[15] 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.[16] 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

HIF-1alpha has an important role to play in pathophysiology of oral cancer both under normal and hypoxic conditions. High HIF-1alpha 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.

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