REVIEW ARTICLE

Zinc α-2 Glycoprotein (ZAG) a Novel Biomarker for the Detection of Oral Squamous Cell Carcinoma

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ABSTRACT

Oral cancer, the most challenging and life threatening disease in the field of dentistry, may start as a reactive lesion due to constant stimulus from tobacco consumption, transform into a pre-malignant lesion (dysplastic lesion) and ultimately develop into a cancerous lesion (Invasive carcinoma). There is a fundamental revolution taking place in the analyzing methods; extraction of biological protein from the saliva rather than from tissues or blood. Several of the biomarkers have been studied with pro-carcinogenic effects like Interleukins (ILs), tumor necrosis factor (TNF) and leptin, but only a few have been stated in the literature, which show anti-cancer characteristics like adiponectin and zinc alpha-2 glycoprotein. This review explored the diagnostic and prognostic values of a biomarkers zinc alpha-2 glycoprotein (ZAG) in adults suspected of oral squamous cell carcinoma (OSCC). The PubMed, EMBASE and Google Scholar were searched for scientific studies reported on the potential mechanism of zinc alpha-2 glycoprotein. All the research articles were selected in which ZAG is applied solely or in conjunction with other biomarkers in oral cancer and other cancers. These literatures were carefully assessed to find out and compile the diagnostic and prognostic values and to inquire therapeutic action of ZAG in the process of carcinogenesis.

Keywords: Biomarker; Early Diagnosis; Oral Squamous Cell Carcinoma; Zinc-Alpha (2)-Glycoprotein.

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doi.org/10.36283/PJMD9-3/015

INTRODUCTION

Adipokines or adipocytokines are biologically active molecules, which function as a cell signaling protein and usually synthesize in the adipose tissues[1]. These factors can be synthesized at other sites in the human body like saliva, plasma, urine and other fluids in a variety of circumstances. However, its mechanism of action will be different on other sites of secretion as compared to adipose tissue[2]. Adipokines include a variety of pro-inflammatory peptides like tumor necrosis factor (TNF), leptin, resistin, adiponectin and zinc alpha-2 glycoprotein[3]. Several of the adipokines have been studied with pro-carcinogenic effects like ILs, TNF and leptin, but only a few have been stated in the literature, which shows anti-cancer characteristics like adiponectin and zinc alpha-2 glycoprotein[4]. The alteration in the level of such molecules fetches changes in the human cells or tissue, which leads to the sequence of events from chronic inflammatory process to pre-cancerous state to cancerous growth[5]. There up-regulation or down-regulation in contrast to normal ranges will indicates that there must be suspicious changes or diseases undergoing in thehumanbody[6]. These altered levels of biomarkers may be rendered as a valuable candidate for screening, diagnosis, prognosis and therapeutic intervention for specific disease[7-8].

In the field of Dentistry, Oral cancer is the most challenging and life threatening disease which may start as a reactive lesion to a constant stimulus such as tobacco consumption, transform into a pre-malignant lesion (dysplastic lesion) and ultimately develop into a cancerous lesion (Invasive carcinoma)[5]. Oral squamous cell carcinoma (OSCC) is the most commonly occurring epithelial

derived malignant lesion (90-95%) of the oral cavity[9]. Oral squamous cell carcinoma (OSCC) is the most prevalent disease sixth highest globally and ranked second highest in Pakistan or Indian suncontinent[10]. The consumption of high risk factors like smoked or smokeless tobacco is very common amongst our population[11], which brings changes in proteomics and creates a microenvironment for the tumor initiation and proliferation[12,13]. The majority of OSCC cases are diagnosed at an advanced stage along with nodal involvement and distant metastasis, this occurs usually due to patient's negligence to seek early treatment or either due to misdiagnosis of the suspicious lesion by the consultant[14,15]. This ultimately leads to a poor prognosis (<50%) with high morbidity and mortality rate of this lethal disease[16,17].

The research articles were selected in which ZAG is applied solely or in conjunction with other biomarkers in oral cancer and other cancers. There were total 282 research articles available on the search engines out of which only four articles represented the association of zinc alpha-2 glycoprotein with oral squamous cell carcinoma from 1999 to 2017 which were included in this research. The other 85 articles stated the correlation of zinc alpha-2 glycoprotein and other type of cancers, out of which only those article were selected fall under10 year period.

In this study, we have focused and analyzed about the action of zinc alpha 2 glycoprotein (ZAG) in the early diagnosis of OSCC in order to identify such lesions at its initial phase? Can it be useful in predicting the prognosis of OSCC patient and can it be used as a therapeutic molecule for the future researches? Is there any correlation of ZAG expression with the etiological variables, tumor differentiation and different clinical parameters of oral squamous cell carcinoma?

DISCUSSION

This review explored the diagnostic and prognostic values of a biomarker zinc alpha-2 glycoprotein in adults suspected of oral squamous cell carcinoma (OSCC). The searched for scientific studies reported on the novel adipokine zinc alpha-2 glycoprotein (ZAG).

Structural Framework of Zinc α-2 Glycoprotein

Zinc alpha-2 glycoprotein was first identified in the human serum by Burgi and Schmid in 1961 and belongs to a group of macroglobulin, which is associated with multiple functions in the human body[20]. A soluble protein synthesizes in the human tissues and is secreted in the body fluids like serum, plasma, saliva in various metabolic diseases and cancerous processes[20-23]. Human serum ZAG is encoded by the AZGP-1 gene on Chromosome 7q22.1 of the liver[21]. Its molecular weight ranges from 38-41kDa, with a single chain polypeptide. Araki et al. in 1988 had determined the first polypeptide sequence of ZAG consisting of 278 mature amino acids in a polypeptide chain[24]. The Figure 1 represents the ribbon diagram of ZAG showing three domains (α1, α2, and α3) and glycan chains are represented in ball and stick model[20]. The α1-α2 super domains of ZAG adopt a fold that is symmetrical to MHC-1 but lacking binding to β2M is slightly different from that in MHC-1[4].

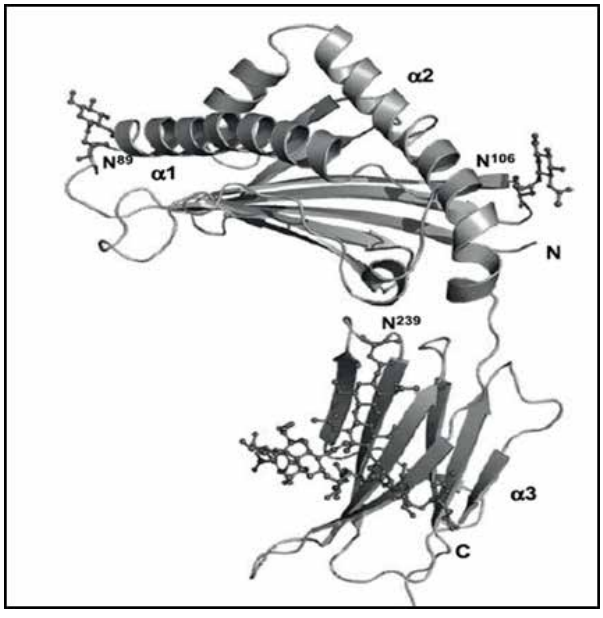

Figure 1: Structural framework of Zinc α-2 glycoprotein (Hassan et al., 2008).

Functional Role of Zinc α-2 Glycoprotein

Zinc alpha-2 glycoprotein functionally resembles a lipid mobilizing factor (LMF), so it has a strong role in cancer cachexia[25]. Its lipolytic effect takes place by activation of the cyclic AMP pathway via β3 adrenoreceptor[26]. Felix et al. have reported elevated levels of zinc α-2 glycoprotein in the serum of pancreatic cancer cachexic patients and suggested that ZAG can be used as a reliable tool for early diagnosis of cancer cachectic patients in the clinical setting[27]. In addition, ZAG possesses a high degree structural and functional similitude with the MHC-1 molecule and a large groove analogous to MHC-I peptide binding groove[20]. The configuration of the binding groove reflects its primary role in immune response and lipid catabolism[23] (Figure 2). The uniqueness of ZAG molecule is exhibited by alteration in the residue of peptide binding groove and because of this alters the molecule binds with different proteins, antigens and ligands[20]. Vidotto et al. reported that zinc α-2 glycoprotein possibly reflects immune responses to tumor antigen for killing the tumor cells as well as mucosal break down through proteolytic actions to prevent tumor invasion in Head and Neck Squamous Cell Carcinoma[28,29].

 doi.org/10.36283/PJMD9-3/015

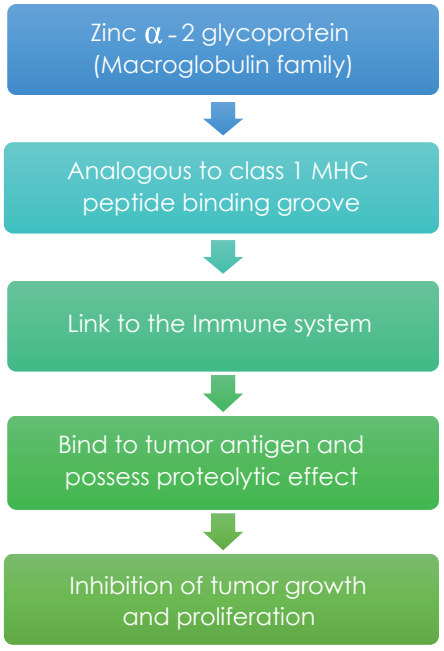

Figure 2: Relationship of ZAG with immune system.

Zinc α-2 Glycoprotein Role in Carcinogenesis

The consumption of smoked or smokeless tobacco is a prevalent factor behind the causation of OSCC[13]. These products trigger an inflammatory reaction in the tissue or body fluids which creates an oxidative stress and activates redox-sensitive transcription factors such as NF-kappaB, which leads to release of Inflammatory cytokines/adipokines such as IL-6, TNF-α and ZAG[18] (Figure 3). The high expression of these factors in the majority of circumstances correlates with the tobacco-related diseases[19]. Thus, may be high or low expression of ZAG can link to the initiation, progression or suppression of OSCC[20]. According to Chukkris et al. betel nut chewing, tobacco use and alcohol consumption were highly correlated with the increased expression of ZAG with statistically significant ($p < 0.001$) outcomes in OSCC cases as compared to cancer free controls[8].

Similar to the above study, Tsai et al. also found a strong association of smoking with increased expression of ZAG protein in smokers as compared to non-smokers ($p < 0.0001$)[21]. Their results showed that smoking can be an independent risk factor causing a variation in ZAG levels in the human plasma. Luisa Airoldi et al. published a research in which there was a 2.5 fold down-regulation of ZAG protein in the urine of chronic smokers as compared to non-smokers[19]. Cigarette smoke elevated levels of TNF-α in the circulation, which might decrease ZAG expression in some body compartments. These statistics indicate towards the role of ZAG in the process of carcinogenesis but through this review, we attempt to summarize its relative part in the oral cancer progression.

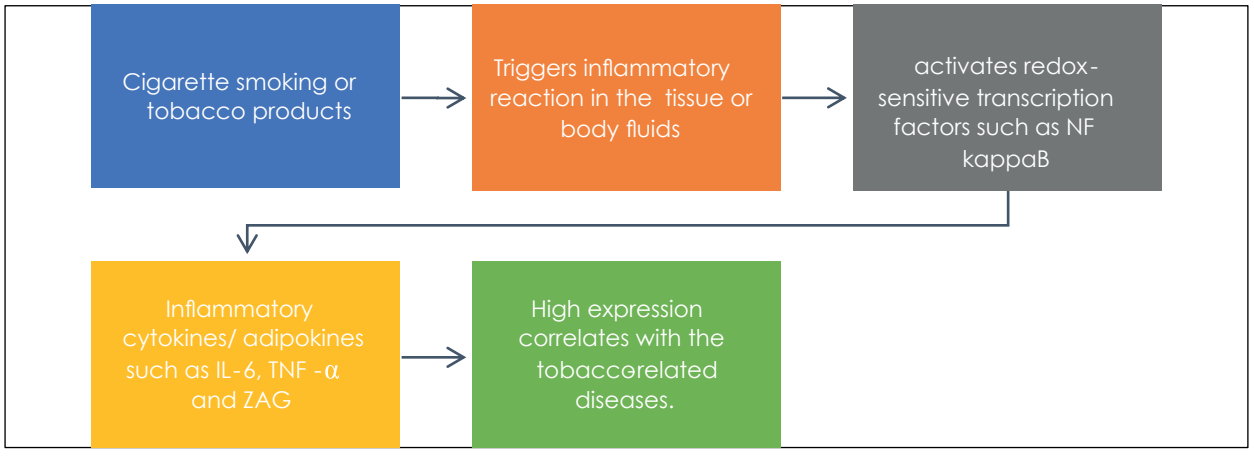

Figure 3: Mechanism of tobacco-related disease instigation.

Zinc α-2 Glycoprotein and Signaling Pathways

Zinc alpha-2 glycoprotein holds anti-cancer effect against the tumor cells as its high expression down-regulates the Cyclin dependent kinase 2 (cdc-2) enzymatic activities in the cell cycle (Figure 4) and RNase activity eliminate the mutated or damaged RNAs ultimately hinder tumor cell growth[30]. Similarly, Brysk et al. reported that zinc α-2 glycoprotein mRNA expression was lower in oral squamous cell carcinomas (Tu-138) cell line than the adjacent non-tumor tissue and Tu-138 cells growth was inhibited on ZAG matrix for the first 2 days and then proliferated at a reduced rate[31]. According to the literature, the high expression of ZAG binds to hydrolases and inhibits the enzyme-mediated tumor invasion indirectly activates apoptosis[32]. Yi Xu et al. described that knockdown of ZAG would promote epithelial-mesenchymal transition, tumor invasion and reduced apoptosis by TGFβ1-ERK2 signaling pathway[33]. It suggests that high expression of ZAG being associated with down-regulation of mesenchymal marker

(N-Cadherin) and up-regulation of epithelial marker (E-Cadherin) by inhibiting the TGF-β 1/ERK 2 signaling pathway (Figure 5). However, they proposed that the up-regulation of ZAG may have an anti-proliferative and pro-apoptotic function in human hepatocellular.

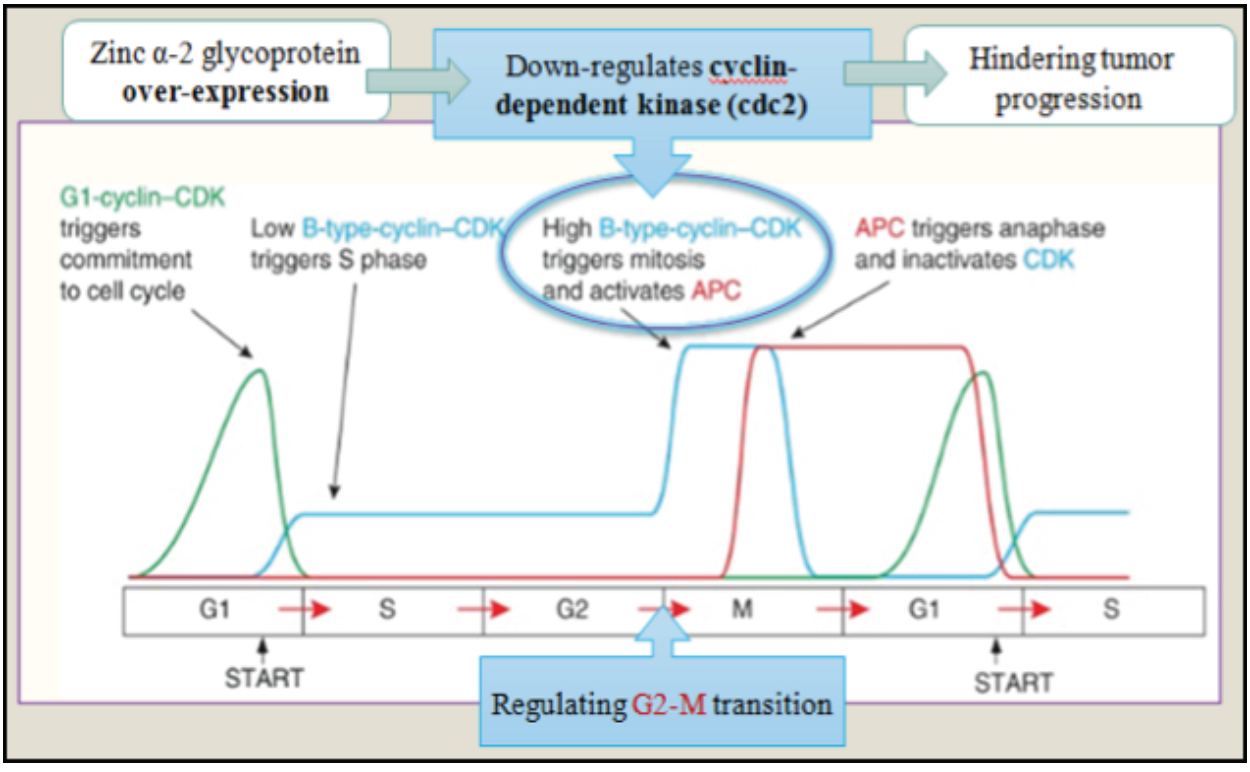

Figure 4: Represents the effect of ZAG on the cell cycle.

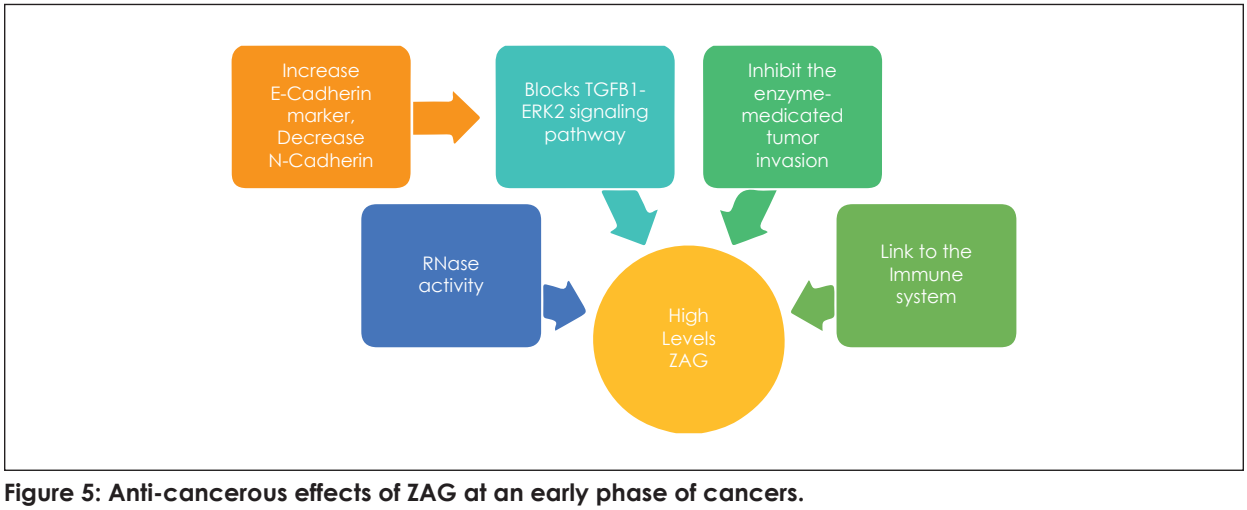

Figure 5: Anti-cancerous effects of ZAG at an early phase of cancers.

Expression of Zinc α-2 Glycoprotein in OSCC

Zinc alpha-2 glycoprotein is a biochemically important protein and gene, which is directly or indirectly involved in various chronic diseases and cancers including oral squamous cell carcinoma (OSCC)[34]. However, very little work has been done to find an association between zinc alpha-2 glycoprotein and oral cancers by using different methods of analysis. Chukkris et al. have reported the significant up-regulation of zinc alpha-2 glycoprotein in the saliva of OSCC patients as compared to controls with relative intensity of <1 in controls and 0-15 in OSCC cases[8]. In this study the expression of ZAG was significantly up-regulated in lesional cells than oral exfoliated cells ($p<0.001$), and this result was consistent with the salivary results. Its increased level in the early phases of OSCC might render as an early diagnostic marker with 85% sensitivity and 100% specificity.

Another study conducted by Chen et al. showed higher levels of zinc α-2 glycoprotein in the saliva samples of OSCC patients at early stages and suggested that it can be used as novel diagnostic biomarkers for the oral cancers[9]. Similarly, Vidotto et al. has also reported an increased expression of zinc alpha-2 glycoprotein in saliva specimens of head and neck cancer (HNSCC) up-to 1.5 fold[28]. They correlated that the up-regulation of ZAG in early stages of oral cancer may reflect an immune response against the tumor cell surface antigen and prevent the enzyme-mediated invasion of tumor cells by binding of macroglobul into hydrolases.

Lei et al. and Brysk et al. have evaluated the role of zinc α-2 glycoprotein in inhibiting proliferation of oral squamous cell carcinoma cell lines they found the down-regulation of Cyclin dependent kinase 2 (cdc2) activities in the cell cycle which is an important rate limiting enzyme to regulate G2-M phase for the growth of cells[30-32]. These statistics recommended that ZAG could be used as a new early diagnostic or therapeutic target for the analysis and its unique behavior in tumorogenesis help to secure a better prognosis for OSCC patients.

Expression of Zinc α-2 Glycoprotein in Other Malignancies

Many studies had been conducted to identify an association between zinc alpha-2 glycoprotein and different type of malignant tumors[35-37]. In this regard, Jung reported that the over-expression of AZGP-1protein was seen in the well-differentiated prostate cancer tissue as compared to a poorly differentiated tumor on immunohistochemistry (IHC)[38]. They also stated that the expression of ZAG decreases as the tumor differentiation increases and higher the expressions better the chances of patient survival. AGZP-1 can be an independent prognostic marker in clinical setting for localized cancer, but it may also be used to stain adjacent cancerous field[37,38]. Similarly Dubois et al found higher expression of ZAG proteins in well-differentiated breast cancer tissue (81%) and even higher in normal tissue immediately adjacent to breast tumor (91%) through IHC, which may indicate its crucial role in the initial stages of carcinogenesis and it might encompass some anti-carcinogenic activity[39].

A study conducted by Hua Xiao et al. showed a significant difference between the expressions of AZGP1 in the saliva of healthy controls and a lung cancer group; controls showed the low relative intensity of ZAG in comparison to cases[40]. However, this study showed no significant association of smoking with the expression of AZGP-1 in the healthy controls and lung cancer cases. The expression ofAZGP-1 mRNA in the cancerous process may be affected by chromatin remodeling by histone acetylation, which regulates gene activity by amending the chromatin conformation[41]. Chun-Yu Huang et al. showed a strong correlation between ZAG levels and histological grade, tumor location and tumor invasion in the gastric tumor[42]. Another study by Hong Tang et al. showed down-regulation of ZAG expression in the poorly differentiated tissues of ESCC and this down regulation of ZAG might indicate ESCC progression, aggressive tumor behavior, and poorer clinical outcome[10].

In a research conducted by Yingming Xue et al., showed high expression of AZGP1 mRNA (57.1%) was seen in the colon cancer tissue with at least a two-fold rise compared to paired normal colonic mucosa tissue[43]. The high levels of AZGP-1 signified amore advanced progression of the disease with worsen clinical outcome in colorectal cancer patients. Fei Wang et al. showed up-regulation of ZAG mRNA and protein expression in Hepatocellular carcinoma and liver cirrhosis compared to normal liver tissue with statistically significant results ($p<0.01$)[44]. This significant expression may result due to modification in the protein levels caused by the infective risk factors for hepatocellular carcinoma, but there is a scope for future research to find an exact relationship between ZAG expression and carcinomas. All the above statistics are conferring that ZAG can be applied as an early diagnostic, tumor suppressive, prognostic marker and promising therapeutic target for oral cancer and other cancers in the clinical capacity[36-45] but we cannot refute that there is also necessitated for further clinical based studies to conform these functions of ZAG in the carcinogenesis.

CONCLUSION

As the time changes, many advancements and evolution are enchanting in the field of research and medicine. However, there is still a vast scope for further clinical experimentation to establish potential biomarkers for the mass screening of high risk individuals of OSCC or other cancers. It is essential to detect such lesion in the initial phase of its malignant transformation to ensure better prognosis of the patient and provide good health. There is a fundamental revolution taking place in the analyzing methods; extraction of biological protein from the saliva rather than from tissues or blood. In this review, some promising mechanism of action of ZAG has been recognized by analyzing the previous researches, but there is requisite for further researches to be conducted on its diagnostic and therapeutic values in the saliva. It is necessary to discover the exact mechanism of zinc α-2 glycoprotein in the pathogenesis of precancerous lesions and oral squamous cell carcinoma.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Afsheen

Maqsood and Dr. Hasan Mehdi for their valuable contribution towards reviewing this article.

CONFLICT OF INTEREST

There was no conflict of interest among the authors.

AUTHORS' CONTRIBUTION

MF did the conceptualization of study, literature search and prepared the write up. MH and SB had done the proof reading. RA did the overall evaluation of the study and proof reading.

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