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UPREGULATION OF LIPID PEROXIDATION PRODUCTS AND THEIR INTERPLAY IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS UNDERWENT SURGICAL PROCEDURE

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is a modern day catastrophe affecting millions of people around the globe, including Pakistan. It has gained worldwide attention owing to low survival rates, late detection, poor prognosis, and escalated mortality rates. Oral cancer affects men more than women as the former are more habitual users of notorious tobacco and tobacco products. Currently, the search for predictive OSCC biomarkers is in demand to maximize survival rates. The present study was done to evaluate the oxidative damage caused by increased lipid-peroxidation in oral cancer subjects. Present research work provides strong evidence that oral cancer patients presented elevated peroxidation of lipids, and their products, enhanced formation of free radicals and reduced antioxidant enzymes in their serum, saliva and urine as compared to their healthy counterparts. Therefore the study strongly approves that lipoxidative biomarkers hold promise for their predictive potential in OSCC. Fifty subjects with diagnosed oral cancer through biopsy and pathological experiments were included in this study and fifty normal healthy individuals were taken as control. Results of current study shows that there were

significantly (p=0.021) elevated levels of MDA were observed in patients of OSCC in their serum, saliva and urine as compared to healthy controls. Levels of isoprostane were also raised in patients among their serum saliva and urine (6.26±0.526, 1.66±0.019 and 1.26±0.426 pg/ml respectively) as compared to healthy controls (1.66±0.015, 0.616±0.017 and 0.166±0.019 pg/ml respectively), whereas the levels of 8-OHdG and 4-HNE were raised significantly (p=0.022 and p=0.019)) in serum, saliva and urine of OSCC patients as compared to healthy controls respectively).

Keyword: Oral squamous cell carcinoma (OSCC), Malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-hydroxynonenal (4-HNE)

INTRODUCTION

Carcinoma an affection pervaded by immoderate multiplication and proliferation of aberrant cells is one of the major cause of deaths due to cancer. Generally cancer formation comprises three stages recruitment, development and progression, different studies demonstrate that reactive oxygen species (ROS) and free radicals play their causative role especially in recruitment and development of carcinoma [1]. Squamous cell carcinoma (SCC) is a fairly slow growing and the second most common form of skin cancer constitute more than 90% cancers of neck and head. Development of oral squamous cell carcinoma (OSCC) often occur after the 50 years of age and reaches to its peak in the 6th decade of life. Oral cancer more than 90-95% are squamous cell carcinoma or one of its variance. ROS and free radical formation play very vital role in both carcinogenesis and pathogenesis [2]. Squamous cell carcinoma of oral cavity worldwide a major form of cancer affecting males is the 6th most common malignancy and a major cause of morbidity and mortality [3]. Etiology of OSCC includes the consumption of tobacco and alcohol and betel quid chewing. These etiological factors along with ROS burst are directly associated with DNA damage and may be responsible to cause cellular DNA damage by producing free radicals and play significant causative role in carcinogenesis [4]. Previous studies reveals that free radicals are produced by the nourishment of oral epidermal carcinoma cells with smokeless tobacco extract and by the production of heat during smoking as well as change in pH occurs during chewing which also favor free radical generation [5]. It has been reported that free radicals development and stabilization is affected by production of heat and change in pH of body

fluid. Furthermore in the saliva of tobacco users and alcohol consumers free radicals are produced by auto-oxidation of AN-polyphenols are associated with increased risk of oral cancer recruitment and promotion [6]. It has been reported that in addition to the direct carcinogenic effect of tobacco smoke on oral mucosal membrane epithelial cell it is indirectly responsible for the formation of H_2O_2 and hydroxyl radicals [7]. Due to consumption of tobacco oral epithelium is exposed to toxic oxygen and free radicals which may affect the defense mechanism of host. Every living cell has their own antioxidant defense system against ROS in the form of different enzymes such as reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and other GSH dependent enzymes i.e GPx and GR. These antioxidant enzymes provide protection for the cell against ROS by preventing its (ROS) accumulation in both cases, during normal cell metabolism and after oxidative insult [8]. Uncontrolled rise in ROS production or reduced antioxidant defense system results in cellular damage which ultimately ends up in malignant transformation [9]. Basically lipid-peroxidation is damaging due the generation of lipid-peroxidation products which results in free radicals reaction spread [10]. Peroxidation of lipids induced by free radicals may be responsible to alter cell membrane structure and function and leads to loss of cell homeostasis [3]. As a result of interaction with cellular DNA, formation of MDA-DNA adducts is the most important characteristics of peroxidation of lipids [11, 12]. Peroxidation of lipids results in the degradation of lipids and formation of a wide array of oxidation products such as MDA, Isoprostanes and other aldehydes such as malondialdehyde and 4 hydroxynonenal [10]. The biological function of MDA and other aldehydes comprises cross-linking with proteins and DNA which may modify the activity of these molecules. Toxicity of tissue have been shown by MDA and 4HNE [13]. Due to short half-life of free radicals the quantification of free radicals is not possible therefore different biomarkers are needed to measure the damage caused by these free radicals. As there is no appropriate technique which is sensitive enough to measure free radicals so there are some different indirect ways which include measurement of damaged products such as lipid-peroxidation results in lipid per oxide formation [5]. A fundamental biomarker 8-OHdG has been used to estimate the endogenous oxidative DNA and damage as a factor of recruitment and progression of cancer development or

growth [14]. In current study to evaluate the extent of lipid-peroxidation the levels of MDA, Isoprostanes, 8OHdG and 4-HNE in serum, saliva and urine were analyzed as the biomarkers of lipid-peroxidation, DNA damage and end product of peroxidation of lipids in OSCC patients as compared to healthy controls.

MATERIALS AND METHODS

Current study was performed to evaluate the role of lipid-peroxidation bio-markers in pathogenesis of oral squamous cell carcinoma. All the patients were selected and screened at Shoukat Khanum Hospital, Lahore. Fifty subjects the having history of any tobacco use and were diagnosed oral cancer through biopsy and pathological experiments were included in this study. Subjects that were on medication, using alcohol or diagnosed with any significant systemic disease were excluded from the study. Informed consent was taken from them before starting this research. Whereas fifty healthy patients that never use tobacco were taken as controls. The protocols that were used to perform this research were approved by the Research Ethical Committee of The Institute of molecular biology and biotechnology, The University of Lahore. Five ml of blood sample was withdrawn from each participant from their cubital vein that was centrifuged within an hour and stored at -70˚C under specific wiles. Saliva and urine sample of all participants were also taken.

RESULTS

Results of present study shows that there were significantly (p=0.021) high levels of MDA were observed in patients of oral cavity cancer in their serum, saliva and urine (1.77±0.094 nmol/ml, 0.046±0.0034 nmol/ml and 0.26±0.065 nmol/ml respectively) as compared to healthy controls (0.85±0.019 nmol/ml, 0.016±0.0056 nmol/ml and 0.015±0.0016 nmol/ml respectively). Levels of isoprostanes were also raised significantly (p=0.000) in patients among their serum saliva and urine (6.26±0.526, 1.66±0.019 and 1.26±0.426 pg/ml respectively) as compared to healthy controls (1.66±0.015, 0.616±0.017 and 0.166±0.019 pg/ml respectively), whereas levels of 8-OHdG were raised significantly (p=0.022) in serum, saliva and urine of oral squamous cell carcinoma patients (1.22±0.14, 0.3±0.0064 and 1.42±0.14 pg/ml respectively) as compared to healthy controls (0.35±0.0162, 0.17±0.0039 and 0.45±0.016 pg/ml respectively). Levels of 4-HNE were raised significantly with the p value of (0.019) in subjects diagnosed with OSCC (3.265±0.124, 0.66±0.15 and 3.35±0.16 µmol/ml respectively) as compared to healthy controls (1.66±0.016, 0.161±0.0091 and 0.064±0.0016 µmol/ml respectively).

Table 1: Upregulation of lipid peroxidation products and their interplay in oral squamous cell carcinoma patients under went surgical procedure

VARIABLES	CONTROL (n=50)			SUBJECT(n=50)			P-VALUE
	Serum	Saliva	Urine	Serum	Saliva	Urine	
MDA (nmol/ml)	0.85±0.019	0.016±0.0056	0.015±0.0016	1.77±0.094	0.046±0.0034	0.26±0.065	0.021
Isoprostanes (pg/ml)	1.66±0.015	0.616±0.017	0.166±0.019	6.26±0.526	1.66±0.019	1.26±0.426	0.000
8-OHdG (pg/ml)	0. 35±0.0162	0. 17±0.0039	0.045±0.016	1.22±0.14	0.3±0.0064	1.42±0.14	0.022
4-HNE (µmol/ml)	1.66±0.016	0.161±0.0091	0.064±0.0016	3.265±0.124	0.66±0.15	3.35±0.16	0.019

DISCUSSION

Significant biomarkers play a key role in the diagnosis of various diseases. Inflammation and oxidative stress are the major contributors in OSCC disease. Oxidative stress and lipid-peroxidation markers such as MDA, Isoprostanes, 8OHdG and 4-HNE have very high demand because they play key role in the proliferation and is a prognostic variables in various diseases. Early diagnosis of OSCC by these lipid-peroxidation and stress markers provides a new hope to overcome this disease through different treatment regimes [15, 16]. Oral squamous cell carcinoma (OSCC), currently a global dilemma, is an aggressive malignant disorder which is increasing at a threatening pace. It has become a serious lethal problem for developing countries because of late diagnosis of this disease and have poor survival rate [17]. Oral cancer is a slow process that composed of metastasis and tumor invasion, this disease results in inflammation and triggering angiogenesis. Carcinogenesis is owing to the interaction between cancerous cells and cells in vicinity. The oral epithelium is bombarded with unlimited free radicals by the consistent use of tobacco, tobacco related products and alcohol. Mitochondria, an ROS generating organelle, through the mitochondrial respiratory chain and mitophagy, leads to the direct and indirect generation of intermediates of reactive nitrogen (RNI) and reactive oxygen (ROI) under aerobic and hypoxic conditions. Moreover it initiates hypoxia induced nitrative stress and fabricates lipid peroxidation byproducts 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). In addition, inflammatory cytokines, chemokines and processed proteins activate a wide range of transcription factors, hence indirectly initiating oxidative stress. In OSCC both oxidative stress and inflammation induce and trigger one another that result in more and more OSCC [18].

Pathogenesis of oral squamous cell carcinoma is associated with increased ROS production. Various studies supposed that

free radical induced damage of cellular material could be responsible to stimulate the transformation of normal cell into cancerous cell [9]. Extent of this damage is also vulnerable to antioxidant mediated defense system of body against free radicals. In malignant cells ROS metabolism is altered which favor increased production of ROS and suppress antioxidant defense system [19]. Lipid peroxidation occurs because of oxidative stress, its markers such as MDA, 4-HNE, Isoprostanes and 8-OHdG describes the damage done to lipid membranes. This highly unstable product of lipid peroxidation has also been known to form DNA-MDA adducts hence causing collateral damage to the cellular DNA. A number of diseases have shown the presence of lipid peroxidation as a key factor in their pathogenesis. Numerous studies on oral cancer individuals have reported elevated MDA and lipid hydroperoxides (LHP) in serum, saliva and urine samples. Present study showed a significantly increased MDA level in serum, saliva and urine of oral cancer patients as compared to healthy controls [20]. Results of present study describes that oral cancer release free radicals that induce cancer on the other hand OSCC tumor itself participate in the generation of free radicals that leads to cause decrease in antioxidants and results in lipid peroxidation. Results of present studies are similar with the studies of Kurtul and Gokpinar, [21]; Agha-Hosseini *et al*., [22]; Srivastava et al., [2]; Shilpasree *et al*., [24]; Ganesan and Kumar, [25]; Rasool *et al*., [26]; Metgud and Bajaj, [27] and Malik *et al*., [28]. To estimate oxidant damage or injury Isoprostane are one of the most accurate and reliable biomarker because the product of Isoprostane pathway have been shown to exert potent biological actions and therefore could be pathophysiologic mediator of carcinogenesis. As isoprostane are chemically stable, specific product of peroxidation which are present in detectable amount in all biologic fluids are markedly elevated in case of oxidative damage [28]. Previous studies revealed that peroxidation of lipids induced by free radicals results in the formation of isoprostane, for this reason in current study the levels of isoprostane was evaluated as a vulnerable marker in serum saliva and urine sample to determine the pathogenesis of oxidative damage. In current study the levels of isoprostane was significantly increased in serum saliva and urine of oral cancer patients as compared to healthy subjects which indicate oxidative damage induced by free radical generation. It has been stated that malignant cells have elevated level of 8-OHdG that can be

estimated to determine the oxidative damage of DNA [29, 30]. In present study increased level of serum, saliva and urinary 8-OHdG was recorded in OSCC patients as compared to healthy individuals. Another most reactive lipid electrophile 4-HNE induced by lipid-peroxidation also associated with the formation of DNA adducts and play central role in carcinogenesis of oral cavity [31]. Significantly increased level of 4-HNE was observed in OSCC patients as compared to normal controls. Current study appears that serum, salivary and urinary MDA, Isoprostane, 8-OHdG and 4-HNE are advantageous biomarker to evaluate the extent of damage caused by lipid-peroxidation induced free radical production and may be used as best prognostic indicator of oral cavity cancer.

CONCLUSION

Current study suggests that patients with oral squamous cell carcinoma are predisposed to increased lipid-peroxidation mediated free radical formation which leads to the formation of DNA adducts and oxidative damage to DNA. To progress a better understanding of the fundamental or pivotal pathophysiology of oral squamous cell carcinoma oxidative DNA damage can be determined by evaluating the levels of MDA, isoprostane, 8-OHdG and 4-HNE.

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CONFLICT OF INTEREST

Authors declares no conflict of interest

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