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Original Research

To evaluate the role of oxidative stress and the microenvironment in the development of oral submucous fibrosis

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ABSTRACT:

Aim: To evaluate the role of oxidative stress and the microenvironment in the development of oral submucous fibrosis.**Methods:** The research included 60 participants who were divided into two groups: those with oral submucous fibrosis with dysplasia (OSFW) (category A) and those who did not (category B). The research eliminated those who had an abnormality. H&E staining was used to confirm the biopsy tissues. Enzyme-linked immunosorbent assay was used to assess the amount of oxidative stress parameters in serum (ELISA).**Results:** FFA levels in the OSF group [266± 50] are significantly higher than in the control group [148± 11]. Ascorbic acid, a powerful antioxidant, protects the cell from oxidative damage. Table 2 shows that the ascorbic acid level in the OSFW group is lower [143± 8.2] than in the control group [235± 11.8]. The serum citrate level in the OSF group is significantly higher [71±6.4] than in the control group [53± 7.9]. Oxaloacetate (OAA) is a key intermediate molecule in the citric acid cycle that contributes to the metabolic cycle. the serum OAA level in the OSFW group [59± 13.1] is significantly higher than the control [33± 3.3]. ROS levels are very important in carcinogenesis. The OSF group had significantly higher levels of 8-OHdG [27±2.0] than the control group [13±1.6]. In our findings, there was a significant positive association between glucose and DNA damage (R2 14± 0.48, $p< 0.05$). Significantly higher amounts of 8-iso PGF2a were found in the OSF group [356± 8.0] compared to the control [271± 28.2].**Conclusion:** we may assume that OS causes metabolic changes in the metabolic system, which aids cancer cell growth. The current research is noteworthy in that it demonstrates a statistically significant positive connection between OS parameters and biochemical parameters for OSF.

Keywords: oxidative stress, microenvironment. oral submucous fibrosis

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INTRODUCTION

OSMF is a chronic debilitating illness of the oral cavity characterized by inflammation and increasing fibrosis of the submucosal tissues. OSMF causes significant stiffness and, eventually, the inability to open the mouth. OSMF is a chronic, progressive, and possibly malignant illness of the oral mucosa. It is distinguished by soft-tissue fibrosis, which causes stiffness and eventual inability to open the mouth. Even after controlling tobacco usage, which is known to play a key role in the disease's development, the condition has been found to be precancerous and has a high relative chance of malignant conversion. [1] It might be hereditary, viral, or caused by environmental or societal factors.

OSMF has an incidence rate of 4 per 1000 adults in India. OSMF affects around 5 million young Indians as a consequence of increased tobacco usage, with tobacco-containing pan chewing being the primary cause; this illness is spreading fast over time. The OSMF starts with a burning feeling or a sensitivity to spicy foods. [2] Areca nut was chewed more often alone in most OSMF patients than in combination with pan, i.e. betel leaf + lime plus betel catechu, with or without tobacco, or had a greater areca nut concentration. [3]

Epidemiological investigations have shown that carcinogenesis is caused by the production of Reactive Oxygen Species (ROS), which operate by beginning lipid peroxidation (LPO). Antioxidants

such as -carotene, Vit E, Superoxide dismutase (SOD), and glutathione peroxidase protect against LPO-mediated damage (GPx). [4,5] Antioxidants are substances that, at very low concentrations, inhibit the oxidation of any molecule. It has been proposed that oxidative stress caused by increased free radicals and decreased antioxidant levels in target cells and tissues plays an important role in carcinogenesis. Antioxidants neutralize the damaging effects of free radicals produced during metabolism. [6] Free radicals are very reactive compounds that either contribute or take electrons from adjacent molecules. [7] Aerobic organisms have a free radical-neutralizing antioxidant defense mechanism. This system contains both enzymes and non-enzymatic antioxidants, both of which are vital in scavenging free radicals. Antioxidants found in cells work to protect cells from the effects of oxidative stress. The two principal enzymatic antioxidant defense systems responsible for scavenging free radicals and nascent oxygen are SOD and GPx. ROS breakdown is catalyzed by antioxidant enzymes. In oxidative stress, redox modulation is seen as discrete variations in the activity of these enzyme systems. As a result, an overall balance between ROS generation and clearance may be more crucial in OSMF and other malignancies, including OSCC. [8]

MATERIAL AND PROCEDURES

The research included 60 participants who were divided into two groups: those with oral submucous fibrosis with dysplasia (OSFW) (category A) and those who did not (category B). The research eliminated those who had an abnormality.

METHODOLOGY

H&E staining was used to confirm the biopsy tissues. Within 30 minutes of the biopsy, all tissue samples were snap-frozen and kept at -80 C for later examination. For 24 hours, all specimens were fixed in 10% neutral buffered formalin. Microtome was used to cut 3 mm thick tissue slices from paraffin-embedded tissue. After that, it was deparaffinized in xylene, hydrated in 100 percent ethanol, and thoroughly washed in deionized water for 10 minutes. The sections were stained using the H and E staining procedures, respectively. 9

Enzyme-linked immunosorbent assay was used to assess the amount of oxidative stress parameters in serum (ELISA). ELISA is an antigen-antibody test that is used to identify and measure biomolecules. In ELISA, an antigen is coated on mi- croplate wells and then reacts with an antibody that is coupled to an enzyme to form a molecule. After incubation with a substrate, the antigen-antibody complex is identified by an enzyme-linked secondary antibody using a colorimetric test. 10 Enzyme-linked immunosorbent assay was used to determine the amount of OS parameters in serum (ELISA). Commercially available ELISA kits were used to measure the concentrations of FAA [EnzyChromTM Free Fatty Acid Assay Kit], Ascorbic acid [EnzyChromTM Ascorbic Acid Assay Kit], Citrate kits [EnzyChromTM Citrate Assay Kit], Oxaloacetate (OAA) [EnzyChromTM Oxaloacetate Assay Kit], Oxidative DNA damage [Ox-iSelectTM], Oxidative lipid peroxidation Data on the standard curves of individual molecules were examined. Blood Biochemical Parameter Estimation The concentrations of serum glucose, total protein, and total cholesterol [TC] were determined using commercially available enzymatic colorimetric diagnostic kits.

STATISTICAL INVESTIGATION

SPSS software was used for statistical analysis (Version 25.0; SPSS, Inc., Chicago, IL). The t-Test was used to assess the data. For two groups, data are given as mean SEM. P 0.05 was used to evaluate statistical significance.

RESULTS

Table 1 displays the patients' gender and age. Table 2 demonstrates that FFA levels in the OSF group [266± 50] are significantly higher than in the control group [148± 11]. Ascorbic acid, a powerful antioxidant, protects the cell from oxidative damage. Table 2 shows that the ascorbic acid level in the OSFW group is lower [143± 8.2] than in the control group [235± 11.8].

Table 2 shows that the serum citrate level in the OSF group is significantly higher [71±6.4] than in the control group [53± 7.9]. Oxaloacetate (OAA) is a key intermediate molecule in the citric acid cycle that contributes to the metabolic cycle. Table 1 shows that the serum OAA level in the OSFW group [59± 13.1] is significantly higher than the control [33± 3.3].

ROS levels are very important in carcinogenesis. The OSF group had significantly higher levels of 8-OHdG [27±2.0] than the control group [13±1.6]. In our findings, there was a significant positive association between glucose and DNA damage (R2 14± 0.48, p< 0.05).

Significantly higher amounts of 8-iso PGF2a were found in the OSF group [356± 8.0] compared to the control [271± 28.2].

Pearson correlation analysis revealed a significantly positive association between total cholesterol and 8-iso PGF2a [R2 14± 0.41, p< 0.01]. The OSFW group had a significantly greater amount of protein carbonyl [16.0±2.3] than the control [8.4± 2.6]. In OSFW, there is a strong connection [R2 14 0.45, p 0.00] between total protein and protein carbonyl.

Table 1: Gender and Age of the patients

Gender	Category B	Category A
Male	18	16
Female	12	14
Mean age	37.25±3.55	39.63±5.63

Table 2: Comparison of serum levels of oxidative stress parameters in control and OSF

Levels of oxidative stress-induced metabolic intermediates in serum	Category B	Category A	P-VALUE
Free Fatty Acid [mM]	148 ± 11.0	266 ±50	0.004
Ascorbic Acid [mM]	235 ±11.8	143 ± 6.2	0.0001
Citrate [mM]	53 ± 7.9	71± 6.4	0.11
Oxaloacetate [mM]	33± 3.3	59 ±13.1	0.0001
Levels of oxidative stress-induced metabolic end products in serum			
8- Hydroxydeoxyguanosine (8OHdG)	13 ± 1.6	27±2.0	0.001
8-iso-Prostaglandin F2a [pg/ml]	271±28.2	356± 8.0	0.001
Protein carbonyl [nm/mg]	8.4±2.6	16.0 ±2.3	0.001

DISCUSSION

Numerous studies have indicated that malignant transformation at the molecular level is the fundamental pathogenic cause for carcinogenesis, often being connected with OS ROS levels in cancer cells are greater than in normal cells and are causative factors for mutation. [11] Many biological processes need oxygen, which produces ROS, and this reaction is begun by metabolic cycle intermediates. So, in our investigation, we focused on certain key intermediate metabolites that play an important part in the metabolic pathway. According to our findings, a rise in blood levels of intermediate molecule FFA in the OSFW group indicates a change in fatty acid metabolism. [12] FAA is thought to be crucial for cell proliferation, division, and metastasis, as well as for cellular synthesis and energy consumption. According to the literature, free fatty acid activates OS. According to the findings, it stimulates many signalling pathways for cell growth and proliferation, suggesting that targeting lipid metabolism may be a new technique for cancer prevention and therapy. [13] According to published research, vitamin C's job is to maintain redox equilibrium (oxide/reduction). The OSFW group also had a low quantity of ascorbic acid. According to the literature, low ascorbic acid levels may cause oxidative damage to cells and tissues. [14] Nonetheless, there are strong and persuasive findings showing blood ascorbic acid concentrations are favourably associated with health and change inversely with illness and death. [15] It has been proposed that blood ascorbic acid concentrations may be a good biomarker of illness because oxidative cell damage may be causing disease development. Furthermore, this molecule may serve as a co-substrate for various enzymes that are essential for metabolic activity. [16] The change in intermediate serum levels in this research suggests that metabolism is made up of highly interwoven pathways involving multiple intermediate components. [17] Alterations at an intermediate level in the metabolic pathway may operate as the primary pathogenic activators for carcinogenesis, resulting to metabolic rewiring and potentially leading to malignant transformation of the illness. [17] As a result, changes in intermediate molecules of pathways may put a strain on cells, causing them to adapt to new circumstances via metabolic rewiring. [11] Because of the changes in intermediate by-products, it is vital to evaluate the oxidative damage induced to biomolecules as well. Increased ROS levels efficiently target guanine bases in DNA and generate 8-OHdG, hence the amount of 8-OHdG is widely recognised as a biomarker of OS mutagenesis. [18] Higher levels of serum 8-OHdG and a strong connection with blood glucose in our research imply that oxidative DNA damage contributes to DNA repair pathways and nutritional requirements in the tumour microenvironment.

In the human body, lipid peroxidation is a well-defined process of cellular destruction. Lipid peroxides, which breakdown to generate more reactive and complicated compounds such as isoprostanes, are markers of OS. 8-iso-Prostaglandin F2a (also known as 8-epi-PGF2a) is an isoprostane that has been described as a marker for oxidative stress evaluation in serum. Our findings reveal that the serum of the OSFW group has an elevated level and a positive connection with total cholesterol, indicating a shift in membranes, lipoproteins, and altered lipid metabolism. As previously described, abnormal lipid metabolism promotes cancer growth, invasion, and metastasis via a variety of signalling mechanisms. [13] As we know, severe OS causes protein carbonyl, an irreversible post-translational modification that plays crucial roles in cancer growth and suppression. [19] illustrates metabolic rewiring as changed intermediate metabolites and biomolecules.

CONCLUSION

We may assume that OS causes metabolic changes in the metabolic system, which aids cancer cell growth.

The current research is noteworthy in that it demonstrates a statistically significant positive connection between OS parameters and biochemical parameters for OSF.

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