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Salivary nitrosamine output voltage levels as an early detection of malignancy in the oral cavity

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the world's sixth malignant disease with multifactorial causes. Nitrosamine compounds can be found in saliva and used as a biomarker for the early detection of oral cancer. Carcinogenic nitrosamines derived from tobacco use contact with normal oral cavity cells. It affects the process of controlling cell growth by interfering with DNA translation. This study aims to determine and compare the output voltage values of nitrosamines in the saliva of nonsmokers, smokers, and patients with oral cancer in Medan.

Methods: This research is a descriptive-analytic study with a cross-sectional approach. The output voltage value of nitrosamines was measured by collecting the saliva of nonsmoking, smoking and cancer patients.

Results: using the One-way ANOVA test, the results of this study showed that the output voltage of nitrosamines in the saliva of nonsmoker patients (standard) was 4.46±0.17 Volt, compared to smoker 4.91±0.14 Volt. Whereas, In the saliva of patients with oral cancer 7, 66±0.48 Volt. This study concludes that there was a significant difference in the output voltage level of salivary nitrosamines between the nonsmokers, smokers, and oral cancer patients ($p<0.05$).

Conclusion: salivary nitrosamine levels can be an early detection biomarker of malignancy in the oral cavity.

Keywords: nitric oxide, nitrosamine output voltage, oral cancer, non-smoker, smoker

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Background

Oral cancer is one of the highest prevalence cancers in developing countries and is one of the world's top 10 causes of death.[1] Oral cancer or oral squamous cell carcinoma (OSCC) is the sixth malignant disease in the world.[2–4] found on the lips and buccal mucosa, gingiva, hard palate, tongue and floor of the mouth.[4] From 2007-2016, the United States reported an increased incidence of oral and pharyngeal cancer.[5] Gracia reported that in 2011-2015 found, 49 cases of OSCC were in the lingual area (53.8%), buccal area (20.9%), and palatal area (12.1%).[3] Oral cancer spreads rapidly and usually are diagnosed at an advanced stage with a high mortality rate. Only half of the diagnosed cases will survive more than five years, but if the cancer is detected early, about 80-90% will survive.[6]

Early detection is vital in determining the success of treatment and the safety of the patient's soul, especially in cases of malignancy in the oral cavity. However, early detection of malignancy in the oral cavity takes work. The evidence is from the low cure rate of patients, as it has been reported that 50% of patients at the time of diagnosis have had regional or distant metastases. Detection of oral cancer at an early stage that is asymptomatic will improve patients' quality of life by minimizing complicated and tiring treatments.[7]

Nitric oxide is a carcinogenic promoter compound because it reacts with amines and amides to form carcinogenic nitrosamines.[8] Nitrosamines derived from tobacco use will come into contact with normal cells of the oral cavity, thereby affecting the control process for cell growth by interfering with DNA translation. Normal cells in the oral cavity become abnormal, such as leukoplakia, leading to cancer cells in the oral cavity.[9] Nitrosamine compounds can be found in saliva and can be used as a biomarker for early detection of oral cancer. This study aims to determine the levels of nitrosamine output voltage in the saliva of nonsmokers, smokers, and oral cancer patients.

Method

This study is a descriptive analytic study with a pilot study approach which aims to determine the output voltage of nitrosamines in the saliva of non-smokers, smokers and patients with oral cancer. Sampling was carried out using purposive sampling method with a sample size of 12 people, of which 5 were normal, 5 were smokers and 2 were patients with oral cancer. Inclusion criteria were no smoking habits, active smokers, and patients with oral cancer. The exclusion criteria were the contaminated saliva of the research sample. This research has been approved by the Health Research Ethics Commission of the USU Faculty of Medicine (No.145/KEP/USU/2021).

The research subjects were taken saliva by spitting method. Next, the saliva is dripped in to the chitosan biosensor using a dropper and look at the nitrosamine output voltage. The measurement of nitrosamine output voltage was repeated 3 times. The results of the study were tested using the One-way Annova test.

Result

From research that has been conducted on 12 research subjects consisting of 5 non-smokers, 5 smokers, and 2 oral cancer patients, the output voltage value of salivary nitrosamines was obtained.

Based on Table 1, the average output voltage of nitrosamine (nitric oxide) in saliva using a chitosan biosensor is obtained, the output voltage of nitric oxide in non-smokers is 4.46±0.17 V and in smokers is 4.91±0.14 V.

Based on Table 2, the average output voltage of nitrosamine (nitric oxide) in saliva using a chitosan biosensor is obtained; the output voltage of nitric oxide in patients with oral cancer is 7.66±0.48 V.

Based on Table 3, it was found that there were significant differences in the average value of nitrosamine (nitric oxide) output voltage in the saliva of non-smokers, smokers, and patients with oral cancer ($p<0.05$).

Table 1 The value of nitrosamine output voltage (nitric oxide) in the saliva of non-smokers and smokers

Subjects	Nitric oxide output (Volt)			
	Non-smokers	Mean ±SD	Smokers	Mean ±SD
1	4,29		4,76	
2	4,30		4,80	
3	4,46	4,46 ±0,17	4,93	4,91 ±0,14
4	4,56		4,98	
5	4,70		5,11	

Table 2 The value of nitrosamine output voltage (nitric oxide) in the saliva of patients with oral cancer

Oral cancer patients	Nitric oxide output (Volt)	Mean ± SD
1	7,32	7,66 ±0,48
2	8,01	

Table 3 Differences in the output voltage value of nitrosamines (nitric oxide) in the saliva of non-smokers, smokers, and patients with oral cancer

Groups	Nitric oxide output (Volt)	p
Non-smokers	4,46 ±0,17	
Smokers	4,91 ±0,14	0,000*
Oral cancer patients	7,66 ±0,48	

Discussion

Oral cavity cancer is a malignancy originating from the epithelium, whether it originates from the mucosa in the oral cavity, organs in the mouth or salivary glands.[10] Oral cancer is one of the ten most common cancers worldwide. One of the most common causes is tobacco. Tobacco plays an essential role in causing OSCC. Tobacco that is chewed or smoked in the form of cigarettes contains other alkaloid compounds and N-nitro sonornicotine (NNN), which are known carcinogenic substances. These nitrite compounds can be found in the saliva.[8] Salivary nitrosamine is a cancer biomarker. This study was conducted on 12 subjects: five nonsmokers, five smokers, and two patients with oral cancer. Detection of nitric oxide or nitrosamine levels in saliva was measured by the output voltage using a biosensor electrode.

The testing of nitrosamine levels in the saliva using a chitosan biosensor.

The examination showed nitrosamine output in the saliva of nonsmoker patients was 4.46±0.17 V. Saliva is an important body fluid and not only has a role in speech, mastication and swallowing but also can be useful for medical diagnosis.[11] In saliva, nitrate can turn into nitrite (NO_2-).[8] This nitrite can bind to the chitosan biosensor so that the voltage or output voltage of nitrosamines in saliva can be known. In several studies, nitric oxide or nitrosamine levels were measured using units of Mol or M.[11,12] In this study; nitrosamine measurements were carried out using a chitosan biosensor which is a Cu electrode that can produce a voltage when detecting the presence of nitric oxide or nitrosamines in the saliva of patients with the unit of output used is Volt. This method is a novelty using electric voltage. It has never been done before, so there is no supporting reference regarding the level or amount of nitrosamine output voltage in the saliva.

In this study, the average value of nitrosamine output voltage in the saliva of smoking patients was 4.91 ± 0.14 V. Cigarettes are one of the causes of cancer, and this is due to the content of the cigarettes. Cigarettes contain alkaloid compounds and N-nitro sonornicotine (NNN), carcinogenic substances. Druckrey I and Prcussmann (1962), cited Shende V (2013), suggested that a possible carcinogen is formed from nicotine, and this alkaloid is a precursor of NNN. Salivary nitrate in the oral cavity is converted to nitrite (NO_2-), which is very important as a promoter of carcinogenesis because it reacts with amines and amides to form carcinogenic nitrosamines.

Smoking causes neoplastic diseases, including cancers of the lung, pharynx, oral cavity, oesophagus, larynx, bladder, kidney, pelvis, and pancreas. The aromatic benzopyrene hydrocarbons and tobacco-specific nitrosamines (TSNs), namely 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosono- nicotine (NNN) are the most dominant carcinogenic factors.[4,8]

Based on research conducted on the saliva of the cancer group, the output voltage of nitrosamines was 7.66 ± 0.48 V. Nitric oxide (NO) is a highly reactive free radical consisting of one nitrogen atom and one oxygen atom. Nitric oxide is synthesized from the amino acid substrate, I-arginine, by the enzyme nitric oxide synthase (NOS). NO reacts with superoxide to form the oxidant peroxynitrite responsible for cell damage. NOS enzymes can produce NO over a more extended period by mediators and immunological cells, including macrophages, T cells, and NK. NOS can be found in the oral mucosa, endothelial cells, and salivary glands.[12]

In this study, there was a significant difference in the output voltage of salivary nitrosamines between nonsmokers, smokers, and patients with oral cancer. The level of nitrosamines in the saliva of oral cancer patients was significantly increased compared to the groups of smokers and nonsmokers. Likewise, the level of nitrosamines in the saliva of smoking patients was higher than that of nonsmokers. The values of each control group were 4.46 ± 0.17 V, the smokers' group 4.91 ± 0.14 V, and the group with oral cancer 7.66 ± 0.48 Voltage.

The increase in reactive nitrogen species (RNS) occurs through the reaction of NO with oxygen or other free radicals that provide many biological effects. NO can be cytotoxic or cytostatic and interacts with several molecular targets in the cell structure. In chronic inflammation, excessive NOS secretion or expression leads to genotoxicity. Nitric oxide causes DNA damage by making RNS, produces carcinogenic nitrosamines, and inhibits damaged DNA's repair or prevents the repair mechanism. Therefore, Nitric Oxide is considered a tumour-inducing factor that can influence the process of cancer formation.[11] The range of effects of NO is quite broad in tumour genesis, which includes its involvement in cell transformation, neoplastic lesion genesis, and mechanisms of cancer initiation and regulation of metastasis.[12]

The results of this study follow several other studies which have shown differences in the levels of NO or nitrosamines in regular patients to patients with oral cancer. Research conducted by Panjawi S et al. (2013) in a group of control with oral lichen planus showed NO levels in the saliva of the control group of 5-13 M and the group of patients with lichen planus of 20-90 M. The NO level in the saliva of patients with oral lichen planus increased significantly compared to the control group.[13] Research conducted by Ghosh R et al. (2021) on saliva and tissues of patients with oral squamous cell carcinoma showed that in the control group, the mean value of NO in saliva was 78.59 M and tissue NO was 87.6315 M, and in the group of patients with oral squamous cell carcinoma the average value of salivary NO was 115.6765 M/L and tissue NO was 172.376 M. NO levels in the saliva of cancer patients were higher than normal patients.[14]

Oral submucous fibrosis and leukoplakia had been classified as potentially malignant oral disorders.[15] Several studies have evaluated

malignant transformation from many conditions and bad habits.[16] The risk of oral potentially malignant disorder might increase with more subjects practised habitual betel quid chewing patterns.[17] Early examination of conditions that can lead to malignancy must be carried out immediately to stop the case of malignancy itself. An easy saliva collection method can be used to see the nitrosamine level.

Accessible saliva collection and inexpensive biomarker examination are important points in this research. Prevention of malignancy is an actual effort to reduce mortality in the community. Further research needs to be done to obtain an effective and efficient biosensor.

Conclusion

There was a significant difference in salivary nitrosamine levels between non-smokers (normal), smokers, and patients with oral cancer ($p<0.05$).

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Conflicts of interest

The author declares no conflicts of interest.

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