Autofluoresence and p53 Level in Saliva Examination as an Early Detection of Premalignant Lesion in Betel Chewer at Papua, Indonesia

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Abstract

Chewing betel nut may cause premalignant lesion in the oral cavity (ie: Oral Lichen Planus (OLP) and Oral Submucous Fibrosis (OSF)). Premalignant lesion can be detected by autofluoresence and P53 levels in saliva. Oral manifestation in the betel nut chewers are not reported yet at Merauke, Papua Province, Indonesia.

The aim of this study to know the correlation autofluoresence examination and p53 levels in saliva. The study was correlational cross-sectional with purposive sampling.

There are 25 samples (treatment group) betel nut chewer and 5 (control group) non-betel nut chewer examined by autofloresence and P53 levels in saliva by ELISA. P53 levels in saliva III sample groups: non-betel nut chewer (group I control), betel nut chewer with tobacco (group II Treatment) and betel nut chewer without tobacco(group III treatment) analyzed by Kruskal Wallis one way ANOVA test obtained p=0.125 (p>0.05). Mann Whitney test found P53 levels in saliva between group II and III p= 0.15 (p>0.05), group I and II p= 0.085 (p>0.05), group I and III p= 0.220 (p>0.05).

Autofluoresence presented positif result. P53 levels in saliva with autofluoresence examination result analyzed by Spearman's test r= 0.120 (p<0.05). No correlation between the P53 levels in saliva with autofluoresence examination result.

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Introduction

In Indonesia, chewing betel nuts is a habit. It has been done by various tribes and there are in quite a lot in the village. This habit is a hereditary practice for most of the village that was initially closely related to local customs. This custom has been done during the tribe ceremony or at events that are religious rituals since the 6th century. The habit has been done done almost throughout the region in Indonesia such as in Sumatra, Java, Kalimantan, Nusa Tenggara, and Papua. The habit of chewing betel nut on the Papua ethnic group is called as menginang known by all Papuans. Each ethnic tribe of Papua has a different composition of chewing betel which is quite diverse such as by mixing *areca catechu L*, *piper Betle L*, *Uncaria Gambir Roxburgh*, and tobacco. The most commonly used parts of betel plant are fruit, troots, stems and leaves[1,2,3,4].

Premalignant lesion in the oral cavity increase because chewing betel nut habit such as Oral Lichen Planus (OLP) and Oral Submucous Fibrosis (OSF)[5]. Data from the Merauke Health District Office in 2013, there are 1049 mucosal diseases. Based on data from Health Center Kuprik Merauke District, several cases of Squamous Cell Carcinoma in many dental clinic from Urumb village have been reported[6].

Premalignant lesions can be detected by auto fluorescence examination (VELscope®). The auto fluorescence tool (VELscope®) is a handheld device that can be used by clinicians to

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scan mucosal changes visually by looking at fluorescence changes. The mechanism of this examination based on the mucosal tissue has a reflective pattern and absorbs light naturally with the involvement of fluorophores in the tissues. The fluorescence changes in the oral mucosal tissue are strongly influenced by changes in structure, metabolic activity, and hemoglobin in the tissues, as well as the inflammation that occurs. Blue light exposure to the auto fluorescence device (400-460 nanometers) can maximize differences in visualization between neoplastic and normal tissue[7].

In neoplastic or malignant tissue suspected that there is p53 gene involvement. The p53 gene or TP53 is a protein tumor gene that acts as a suppressor tumor gene. A non Mutant P53 protein is located in the nucleus of the cell, bound directly to the DNA cell. In humans the non-mutant P53 protein lies on chromosome 17p13.1. When DNA inside the cell is damaged by chemicals, radiation, or ultraviolet (UV), the non-mutant P53 protein will repair or damage the cell by apoptotic mechanism. Non-mutant P53 also has a function to prevent the development of cells towards the tumor or malignancy[8,9].

Previous studies found that from 27 samples of Squamous Cell Carcinoma (SCC) patient, 11 samples had high P53 levels in serum and saliva[10]. Saliva in the oral cavity is whole saliva which is secreted from the salivary glands major or minor. Saliva contains minerals, electrolytes, enzymes, Growth Factors (GFs), immunoglobulin, mucin and glycoprotein[11].

Saliva is a liquid that is in direct contact with the oral mucosa, either in abnormal or normal condition. Saliva can be used to detect an abnormality in the oral cavity[8]. P53 protein autoantibodies can be found in saliva with malignant oral lesions. Saliva can be used as a biomarker because it is easy, noninvasive when compared to blood serum[12].

Nowadays, there is no data on premalignant lesions in the oral cavity at Merauke, Papua, Indonesia. This study was conducted to determine premalignant lesions by using auto fluorescence and non-mutant P53 protein levels in saliva at Urumb village, Semangga district, Merauke, Papua, Indonesia who chewed betel nuts.

Materials and methods

This study has been received approval ethical clearance letter of human subjects from Ethics Research Committee of Ethical Clearance of Health Research, Faculty of Dental Medicine, Surabaya, Indonesia 146/KKEPK.FKG/VII/2016.

This is correlational cross-sectional design with purposive sampling technique study. The population of this study was the urban community of Urumb village, Semangga district, Merauke, Papua Province, Indonesia on August 2016 examined at Health Center of Urumb village. Sample subject criteria are Marind tribe in Urumb village, betel nut chewer ≥ 5 years, age ≥ 21 years old, and agree to join this study by filled the informed consent. There are 25 samples suspected with premalignant lesions, further examination was done with auto fluorescence and whole saliva has been taken as much as 3 ml. The auto fluorescence examined then coded, if any abnormality (giving a dark color image in the auto fluorescence device) is coded (+), no abnormality (green) is coded (-) / normal. The whole saliva centrifuged (Sorvall™ Legend™ XT/XF Centrifuge Series, Thermo Fisher Scientific, Waltham, MA USA) at a speed of 4000x / min for 10 minutes at a temperature of 2-8^{0}C, for subsequently taken supernatant section, then tested by Enzyme-Linked Immunosorbent Assay (ELISA) test (Invitrogen™ Instant ELISA™ Technology, Thermo Fisher Scientific, Waltham, MA USA) with non-mutant anti p53 antibody.

Results

The samples ware 25 betel nut chewers and 5 controls non betel nut chewer. Some of Betel nut chewer sometimes use a mixture of betel leaf, betel fruit and tobacco, but the other do not use tobacco mixtures (Figure 1).

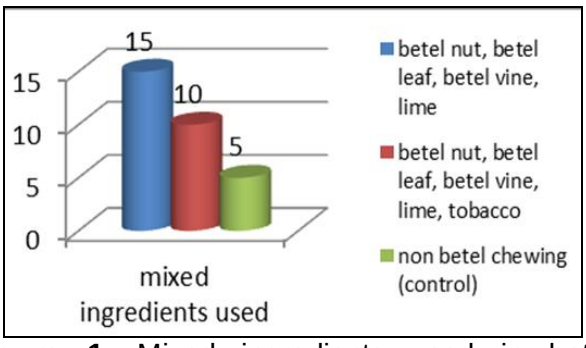

Figure 1. Mixed ingredients used in betel chewing.

Auto fluorescence examination on 25 betel nut chewer, there were positive (dark) results of all samples in oral sites. In 5 samples control group there were negative (no dark spot) (Figure 2 and 3).

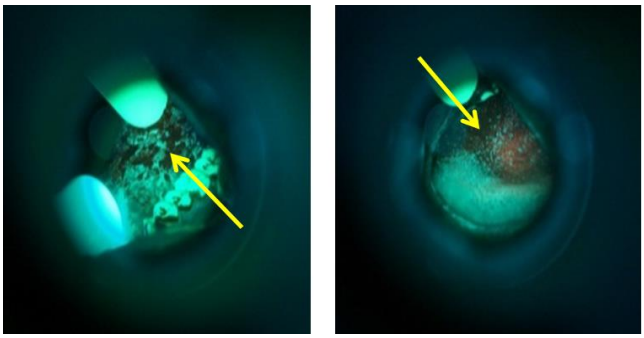

Figure 2. Auto fluorescence (+) / dark / black areas on the dextra buccal mucosa and dorsal tongue.

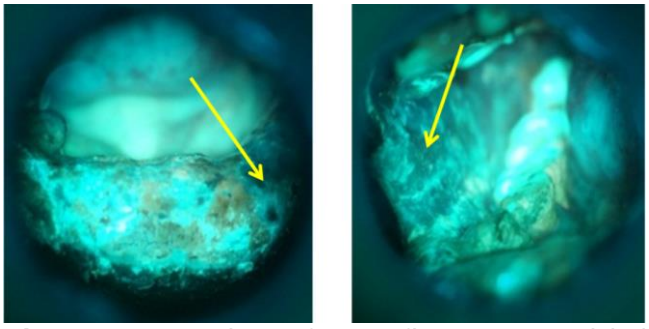

Figure 3. Overview of auto fluorescence (+) / black or dark areas of the dextra buccal and labial mucosa.

In the auto fluorescence results of the samples, 100% (25 samples) of the sample showed a darker picture (+), so it was necessary to continue the Hysto Pathology Anatomy (HPA) examination to see any dysplasia mild or severe. Based on existing clinical features in the form of keratotic tissue, darker color, with desquamation, cannot be scraped, rough surface, unclear boundary, unpainful, the examiner diagnose this lesion as oral submucous fibrosis as many as 24 samples (96%), and 1(4%) suspected OSCC .

P53 non-mutant levels in saliva with ELISA, there are samples with high P53 level in saliva (sample no 19 and 21) can be seen on (Figure 4). The sample data between non-betel nut chewer group (group I), and betel nut chewer with tobacco (group II), betel nut chewer without tobacco (group III) analyzed with *kruskal Wallis one way anova (k sampel)* (Table 1). There was no significant difference between non-mutant P53 levels among the three sample grous p = 0.125 (p> 0.05).

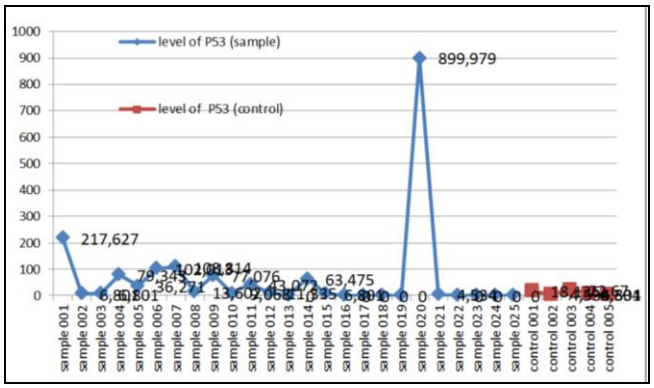

Figure 4. P53 non mutant levels in saliva of research samples.

The *Kruskal Wallis* one way anova (k sample) test only able to know whether there were statistically significant differences without able to know which treatments were different, so between the treatment group we analyzed with Mann Whitney[13].

P53 levels/group	Mean Rank
Group I	9,90
Group II	19,45
Group III	14,73

Table 1. Kruskal wallis test result between three groups toward P53 non mutan level.

Mann Whitney test showed the mean rank of each group III was 11.30 and group II was 15.55. There was no significant difference between non-salt P53 mutant saliva level using group II and III p = 0.157 (p> 0.05). Mean rank of group I was 5.20 and group II was 9.40. There was no significant difference between P53 non-mutant levels in saliva group I and group II p = 0.085 (p> 005). Mean rank of group I was 7.70 and group III was 11.43. There was no significant difference between P53 non-mutant levels in saliva group I and group III p = 0.220 (p> 0.05).

The correlation P53 non-mutant levels in saliva between samples with dark examination results (+) and control with green examination results (-) was performed by the Spearman test method. Spearman correlation test was conducted in this study because Kolmogorov Smirnov test found that the distribution data was

abnormal r = 0.002 (p <0.05). Spearman correlation test obtained r = 0.120 (p <0.05), there was no significant correlation between the auto fluorescence examination result with P53 non mutant level in saliva.

Discussion

The aims of this study to know that P53 non mutant levels in saliva and auto fluorescence examination result can be used an indicator for early detection of premalignant lesion in the oral cavity on betel nut chewer at Urumb Village, Semangga District, Merauke, Papua Province, Indonesia. The village was chosen because almost all population is betel nut chewer. In addition, The premalignant lesion in the oral cavity of papua population have not reported yet[14].

Saliva may be used as early detection of a premalignant / malignant condition in the oral cavity. The presence of P53 non-mutant antibodies in saliva has a correlation with antibodies found in serum, thus become a method of diagnosis of Oral Squamous Cell Carcinoma (OSCC). The selection of P53 (wild type) / non-mutant biomarkers in this study was intended that, P53 is a multifunctional tumor supressor gene and frequently undergoes changes in oral and other cancers[8,16]. Levels of P53 non-mutant in saliva can be used as an indicator of a premaligna / maligna in the oral cavity, whereas the sample of SCC patients obtained 11 samples had high P53 non-mutant levels in serum and saliva[11]. Mutation of P53 gene causes cancer in the oral cavity, due to the accumulation of proteins in the nucleus of the tumor cells. P53 antibodies formed circulate throughout the body including the oral cavity through saliva and Gingival Crevicular Fluid (GCF)[13,17,18,19].

People who use tobacco, whether smokers or chewed, the risk of cancer increase in the oral cavity (especially at smoker ad betel nut chewer)[20]. Malignant or premalignant lesion in the oral cavity can be characterized by an increase in the number of P53 non-mutant levels in saliva or in blood serum[8]. Destruction to cells in betel nut chewer's oral cavity tissues is less severe than betel nut chewer with tobacco. Tobacco consumption is closely related to the accumulation of DNA damage and exposure to chemical carcinogens from tobacco. DNA cells in the cavity of the human oral cavity damaged. The tobacco's active ingredients that are carcinogenic and found in damaged DNA are *Benzi (a) Pyrene (B (a) P)*, *Tobacco Specific N'-nitrosamines (TSNAs), polyciclic aromatic hydrocarbons, nitrosodiethanolamine, nitrosoproline* and *polonium*[20,21,22]. Betel nut chewer with tobacco will increase P53 non mutant levels.

Betel fruit which used by betel nut chewer have potential carcinogenic substances, such as alkaloids, polyphenols, tannins. These materials will bind to Betel Nut Specific Nitrosamine (BNSN) which affects the DNA cell or other targeted cell. DNA cells which bound to the material lead to gene mutation and DNA chain strand broken. Furthermore, P53 gene changed. Proliferation of tumor cells increased that eventually transform to malignancy. If target cells (other than DNA) influenced, then there will be chemical changes and changes in molecular structure, which eventually increased proliferation of tumor cells to malignant[23].

The use of auto fluorescence in this study was to detect early abnormalities of epithelial cells in the oral cavity which transform to premalignant/malignant. It is appropriate that normal cells will reflect the rays emitted by this device, while abnormal cells absorb light, so there will be a visual difference between these two conditions[24].

The action mechanism of auto fluorescence is influenced by the presence of natural fluorophore, which is a component of a tissue that absorbs certain wavelengths and re-radiates longer wavelengths. There are three fluorophores that react to the wavelengths used during irradiation, namely collagen, elastin in connective tissue and Flavin Adenine Dinucleotide (FAD), which is a coenzyme involved in cellular metabolism. During the carcinogenesis these three components decrease, so they do not have the ability to reflect back the received light, which eventually appear darker[25].

Variations of lesions in the oral cavity give a different picture of auto fluoresence depending on the severity. This technology advised to be combined with biopsy method, since auto fluorescence cannot grade the dysplasia transformation of the lesions mild, moderate, or severe dysplasia[26]. P53 non-mutant levels will increase along the severity of lesions.

Conclusions

There was no significant correlation between the auto fluorescence examination results with P53 non mutant level in saliva on betel nut chewer.

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Declaration of Interest

The authors report no conflict of interest.

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