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Mineral characteristics, hydroxyapatite crystal, and mesostructure among heavy smoker teeth: a preliminary report

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SUMMARY

This study aimed to investigate the mineral characteristics, hydroxyapatite crystal, and mesostructure among heavy smoker teeth. Twenty five subjects were divided into two groups including non smoker group (15 subjects) and heavy smoker group (10 subjects). Teeth mineral element was measured using X-Ray Fluorescence. The hydroxyapatite crystal structure was analyzed using X-Ray Diffraction. Mesostructure was determined using Scanning Electron Microscope. The level of Ca, P, Fe, Cu, Zn, S, Si, Cr, Ti, Mn, Ni, In, Re, Ba, K, Mo, V, Sr, Co, Er, Yb, Zr, and Ca/P ratio were not significantly different in the smoker group compared with a non smoker group ($P > 0.05$). The lattice parameter of smoker was different compared with non smoker group. In addition, the crystal size of the smoker was higher compared with control. We also found that mesostructure of the smoker was different compared with non smoker. In conclusion, smoking activity affect the hydroxyapatite crystal and disrupt the remodeling surface of teeth.

Key words: *smoking; dental; structure; surface; remodeling*

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Introduction

Dental enamel is a crystalline latticework composed of various minerals, the principal component of which is a complex calcium phosphate mineral called hydroxyapatite. Chronic exposure to extrinsic/intrinsic acids with a low pH leads to dental erosion.1-5 Dental erosion can be described as the irreversible loss of tooth structure due to the chemical process of acid dissolution which does not involve plaque bacteria. Sources of erosion can be intrinsic such as acid reflux and vomiting or extrinsic, from the ingestion of food, drink, or medication. Lifestyle and occupations can also influence the multifactorial pattern of tooth wear, and erosion frequently coexists with attrition and/or abrasion.6 Enamel erosion is characterized by a centripetal dissolution leaving, a small demineralized zone behind. A substantial number of mineral ions can be removed from hydroxyapatite latticework without destroying its structural integrity.1-5

Cigarette consumption is well established as a major risk factor for periodontal disease, with smokers 2 to 14 times more likely to develop periodontitis than non-smokers7-12 and significant improvements in periodontal health are noted on quitting13,14. The detrimental effects of smoking on periodontal tissues can be observed even in young smokers.9,11 Remineralization of teeth is a process in which minerals are returned to the molecular structure of the tooth itself.15-19 Previous studies showed that Pb and Cd levels in teeth from smokers were significantly greater compared with nonsmokers.20 As far we know, there is no study to compare the mineral elements, hydoryxapatite crystal structure, and mesostructure of heavy smoker dental. Therefore, this study aimed to investigate the mineral characteristics, hydroxyapatite crystal, and mesostructure among heavy smoker teeth compared with non smoker.

Material and methods

Subject

Totally twenty five subjects were divided into two groups, including non smoker group ($n = 15$) and heavy smoker group ($n = 10$). Heavy smoker is a smoker who reports consuming 20 cigarettes or more per day. Under full anesthesia, second primary molars were extracted from these subjects referred for dental treatment. The teeth were extracted because of pain or for orthodontic reasons. The Medical Ethical Committee of the Lambung Mangkurat University approved the study and the patients gave permission to further analyze the teeth.

Analysis of teeth mineral elements

The Ca, P, Fe, Cu, Zn, S, Si, Cr, Ti, Mn, Ni, In, Re, Ba, K, Mo, V, Sr, Co, Er, Yb, Zr, and Ca/P ratio levels was evaluated by X-Ray Fluorescence (XRF). For XRF analysis, the molar teeth inserted in the tube, then put in the proper place in equipment. The processed teeth were then analyzed at 20 kV accelerating voltage by a XRF (PANalytical MiniPAL 4).21

Analysis of teeth hydroxyapatite crystal

Characterization of the X-ray diffraction Results was performed by means of PANanalytical X'Pert PRO-MPD, for smoker and non-smoker tibia. Subsequent analysis was by means of the software programs High Score Plus, Crystal Maker and DDVIEW, complemented with the latest version of PDF2. Diffraction spectra were recorded at an angle of 2θ, from 200 to 60o, with a Cu-K radiation source (wave length = 1.54056 Å, 40 mA, 40 kV) and step size of 0.05o.21

Analysis of teeth mesostructure

Mesostructure analysis was evaluated by Scanning Electron Microscope (SEM). For SEM evaluation, molar teeth from all groups were cut vertically. Then the molar teeth were fixed with phosphate formalin buffer, dehydrated with graded concentration of ethanol and coated with gold and palladium. The processed teeth were then analyzed at 20 kV accelerating voltage by an SEM (FEI Inspect TM S50).21

Ethics

This research has been approved by research ethics committee, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia

Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using Mann Whitney test using SPSS 16.0 statistical package. $p < 0.05$ was considered statistically significant.

Results

The level of Ca, P, Fe, Cu, Zn, S, Si, Cr, Ti, Mn, Ni, In, Re, Ba, K, Mo, V, Sr, Co, Er, Yb, Zr, and Ca/P ratio were not significantly different in the heavy smoker group compared with a non smoker group ($P > 0.05$), as seen in Table 1 .

Figure 1 shows the hydroxyapatite crystal in the heavy smoker group compared with the non smoker group. The lattice parameter and crystal size were different between smoker group (P 6_3/m; a=9.4351; b=9.4351; c=6.8833; crystal size=11.56 nm) than that non smoker group (P 6_3/m;

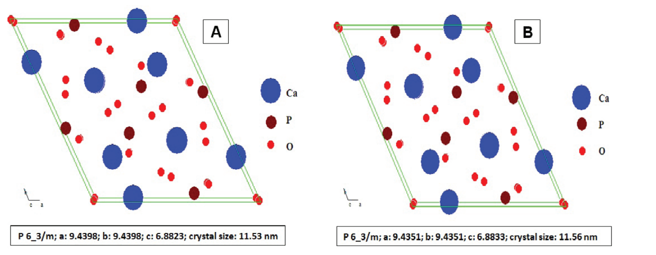

Figure 1. Hydroxyapatite crystal and lattice parameters of molar teeth from heavy smoker and non smoker. The size of the crystal was increased in the smoker group (B) compared to control group (A).

a=9.4398; b= 9.4398; c=6.8823; crystal size=11.53 nm).

The mesostructure of teeth was performed in Figure 2. Non smoker, we showed the sand stone (flaky pattern), regularly surface formation and allignment of the cavity. Mesostructure of heavy smoker showed loss of flaky pattern, irregular surface

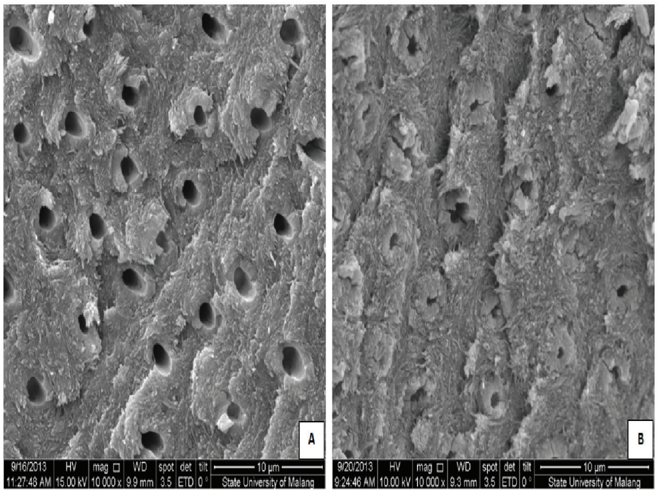

Figure 2. Micrograph illustrating the mesostructure of molar teeth from heavy smoker and non smoker. Non smoker, we showed the sand stone (flaky pattern), regularly surface formation and allignment of the cavity (A). Mesostructure of heavy smoker showed loss of flaky pattern, irregular surface topography, the disregular alignment of the cavity, and crack in surrounding of the cavity (B). (Scanning Electron Microscope; 10.0 KV; Magnification x10000).

topography, the disregular alignment of cavity and crack in surrounding of the cavity.

Table 1. Levels of molar mineral elements in smoker and non smoker group (%)

Level (%)	Non smoker	Smoker
Calcium	78.762 ± 18.981	80.516 ± 7.336
Phosphorus	8.897 ± 2.040	10.480 ± 4.328
Iron	1.016 ± 1.334	1.167 ± 1.169
Copper	0.511 ± 0.889	0.520 ± 0.649
Zinc	0.780 ± 0.738	1.568 ± 3.159
Sulphur	0.518 ± 0.638	0.346 ± 0.662
Silicon	0.286 ± 1.056	0.000 ± 0.000
Chromium	0.183 ± 0.365	0.431 ± 0.838
Titanium	0.373 ± 0.709	0.333 ± 0.323
Mangan	0.013 ± 0.051	0.006 ± 0.020
Nickel	4.643 ± 10.846	3.322 ± 2.697
Indium	0.171 ± 0.459	0.320 ± 0.626
Rhenium	0.232 ± 0.498	0.219 ± 0.290
Barium	0.220 ± 0.558	0.100 ± 0.230
Potassium	0.013 ± 0.051	0.026 ± 0.082
Molybdenum	0.552 ± 1.624	0.715 ± 1.849
Vanadium	0.032 ± 0.102	0.010 ± 0.031
Strontium	0.098 ± 0.205	0.047 ± 0.148
Cobalt	0.009 ± 0.027	0.000 ± 0.000
Erbium	0.013 ± 0.051	0.000 ± 0.000
Ytterbium	0.222 ± 0.256	0.320 ± 0.619
Zirconium	2.573 ± 9.966	0.000 ± 0.000
Calcium/ Phosphorus ratio	9.409 ± 3.058	9.034 ± 3.935

Values are presented as mean ± standard of deviaton.

Discussion

Cigarette smoking affects the oral cavity in a multitude of ways ranging from staining of the teeth to serious diseases such as oral cancer. Besides, smoking has been identified by numerous cross sectional and longitudinal studies as a significant risk factor for periodontal disease, one of the two major causes of tooth loss.22,23 Human tooth enamel is mainly composed of natural carbonated hydroxyapatite (c-HAP). Mature human tooth enamel is unicellular tissue containing of 96 wt.% of c-HAP, 4 wt.% of organic material and from 1 to 6 wt.% of water.24 Previous studies showed that the mineral density, Ca and P weight percent in the outer enamel layer in the older age group were significantly higher than those in the younger age group ($P < 0.05$); however, no age-dependent differences were observed for these properties in the middle and inner enamel layers ($P > 0.05$).25 In this study, the level of Ca, P, Fe, Cu, Zn, S, Si, Cr, Ti, Mn, Ni, In, Re, Ba, K, Mo, V, Sr, Co, Er, Yb, Zr, and Ca/P Ca/P ratio were not significantly different in the heavy smoker group compared with a non smoker group ($P > 0.05$). Our finding indicated that mineralization is an adaptive homeostatic process to compensate the effect of smoking exposure. Although reduced in cell volume, the teeth are normal with respect to mineralization. In addition, substitution of atomic mineral may also contribute to tooth mineralization, a similar mechanism to the bone.26

Two main findings of this study were smoking modifies hydroxyapatitie crystal structure and mesostructure of teeth. The lattice parameter of the smoker was different compared with a non smoker group. This finding indicated that smoker modify the atomic configuration in hydroxyapatite crystal. In addition, the crystal size of smoker was higher compared with control. The pattern of hydroxyapatite crystal will determines the teeth mesostructure. Previous studies found a rough, flaky surface with some smearing product evident in non smoker's teeth.27 We found that mesostructure of the smoker was different compared with non smoker. For non smoker, we showed the sand stone (flaky pattern), regularly surface formation and allignment of the cavity. Mesostructure of heavy smoker showed loss of flaky pattern, irregular surface topography, the disregular alignment of cavity and crack in surrounding of the cavity. This finding showed that heavy smoking activity disrupts the remodeling surface of teeth.

Conclusion

In conclusion, our study suggested that heavy smoking activity affect the hydroxyapatite crystal and disrupt the remodeling surface of teeth.

Competing interests

We declare that we have no conflict of interest.

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