Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 8(1); 74-78
doi: 10.25258/ijcprr.v8i01.9094
ISSN: 0976 822X

Research Article

Experimental Investigation of Human Teeth Demineralization by Three Different Water Treatment

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Available Online: 25th February, 2017

ABSTRACT

In this present study, we try to investigate the effect of three different water to the demineralization of several tooth minerals, such as calcium (Ca), magnesium (Mg), phosphorus (P), and fluor (F). Tooth samples were taken from 24 human maxillary premolars 1 free of caries and defects. All the tooth samples were extracted in Dental Faculty, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia. A water sample is taken from Martapura river, and households that used a tap and well water in Martapura, Banjar District, South Kalimantan, Indonesia with pH value around 5.8, 6.99, and 7.11 respectively. For the control, we used an aquades with the pH value is about 7.2. Tooth samples then divided into four groups with; C served as control; T1 which submerge the tooth in river water; T2 which submerge the tooth in tap water; and T3 which submerge the tooth in a well water. The treatment lasts for 4 days, and each day the demineralization rate constant and regression coefficient of Ca, Mg, P, and F was analyzed. Generally, the tooth submersion in river water increased the demineralization rate constant of all tooth minerals that investigated in this study. The results also show that the treatment shows a negative correlation with demineralization process of all tooth minerals. The results of this present study indicated that all treatment can cause the demineralization of tooth mineral such as, Ca, Mg, P, and F. The highest demineralization rate was found in the treatment with river water than another treatment.

Keywords: Dental Demineralization, tooth, tooth minerals.

INTRODUCTION

The province of South Kalimantan lies on the island of Kalimantan (Borneo) with an area of 3,766,000 ha, situated between latitude 1°21’south to 4°10’ south and longitude 114°19’ east to 116°33’ east[1]. These province areas are dominated by swamp waters and rivers[2]. This makes water is a major determinant of the well-being of the people in Indonesia, especially in South Kalimantan[3].

The people of South Kalimantan known use known to use drinking water through multiple sources. According to previous reports, the people of this province use a river water for drinking water sources and followed by a well and tap water[4]. This can cause various health problems, and one of them is the oral health condition[5]. This is caused by the different characteristics of the water. It is well-known that the river and swamp water is more acidic than tap and well-water[6-7].

The acidic water consumption was suspected to have negative impacts on people’s oral health. The acid condition could make a negative effect like dental demineralization[5]. According to the previous study, chronic exposure to water in acid pH can cause demineralization of the tooth and resulted in a dental erosion[8]. Another previous report also suggested that the exposure of peat swamp water with low pH can cause the demineralization of the tooth minerals, such as Mn, Fe, Mg, and F[9].

Demineralization is the process of loss of the mineral phase of the hard tissues of living organisms.10 In the tooth, the demineralization process balanced with the remineralization process.11 If this balance is interrupted, demineralization will lead to a progressive deterioration of tooth structure[9].

The purpose of this study was to assess the degree of demineralization on human teeth exposed to three different water. These experimental results were compared with several mineral loss such as calcium (Ca), magnesium (Mg), phosphorus (P), and fluor, (F). In addition, the three different water for this study is river water, well water, and tap water.

MATERIAL AND METHODS

Ethics Statements

The study protocol and written consent forms were approved by the Ethics Committee of the Faculty of Medicine of University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

Samples collection

Tooth samples were prepared from 24 extracted human maxillary premolar 1 free of caries and defects. All tooth

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samples were collected from the Dental Faculty of Lambung Mangkurat University, Banjarmasin, Indonesia. After the extraction, the teeth were dried in the oven at 100oC temperature for 2 days. Water sample was taken and collected from Martapura river, and households that used a tap and well water in Martapura, Banjar District, South Kalimantan, Indonesia. The water pH of river, tap, and well water value are about 5.8, 6.99, and 7.11 respectively. For the control, we used an aquades with the pH value is about 7.2.

Experimental models

Samples were divided into 4 groups (1 control group and 3 treatment groups) on 5 sample tooth solution in each. Control (C) group: tooth submerge in aquades; Treatment 1 (T1) group: tooth submerge in river water; Treatment 2 (T2) group: tooth submerge in tap water; and Treatment 3 (T3) group: tooth submerge in a well water. Tooth put in each water for 4 days. The observation was made every day and each day the demineralizationts rate constant and regression coefficient of Ca, Mg, P, and F were examined. In addition, all experimental models and measurement were done in Medical Chemical/Biochemical Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia.

Calcium level analysis

The sample solution was added NH_4OH, HCl (1:4), 0.5 M HCl, and 10 mL of 10% oxalic acid. The solution was heated to boiling and while stirring add 15 mL of NH_4-oxalate. Then filtered and washed with hot water until free of chloride. After that, add 10 mL of H_2SO_4 and heated to boiling and then cooled. Titrate with 0.1 N $KMnO_4$[8].

Magnesium level analysis

Mg content analysis was performed using with complexometric titration with slight modifications[12]. Prepare a sample solution by diluted 250 mg sample with 100 ml aquadest. Add 3 N hydrogen chloride (HCl) to sample solution until the pH of the solution is almost 7 (according to pH indicator paper). Then, add 1 N sodium hydroxide (NaOH), 5 ml buffer ammonia and 0.15 ml eriochrome black T solution. Titrate the sample solution with 0,05 M disodium edetate till the color of the solution changed to blue.

Phosphorus level analysis

A total of 50 mL of the sample solution was added 2 mL of ammonium molybdate and 5 drops of $SnCl_2.2H_2O$. Subsequently, the solution was put in a cuvette and measuring the spectrophotometric with λ = 590 nm[8].

Fluor level analysis

The F content analyses were performed using an ion-meter in conjunction with a combination fluoride electrode12. The instrument calibration was performed using F standard solutions ranging in concentration from 0.05 to 2.0 ppm F- (prepared from 100.0 ppm F- fluoride standard) and TISAB (Total Ionic Strength Adjustor Buffer), a 1M acetate buffer, pH 5.0, 1.0 M NaCl, and 0.4% CDTA (1,2-Cyclohexylenedinitrilotetraacetic acid). The F standard solutions and the TISAB solution were used in the ratio 1:1 (v/v). The standards were chosen based on the desired F concentration in the samples[13]. The samples were analyzed after calibration of the ion-selective electrode coupled with the ion analyzer. The readings of the standards and the samples were performed under stirring using magnetic stirrer (MARTE) and magnet stir bars for one minute for all samples. F concentration in the samples was determined according to the equipment operating procedure, and the accuracy of the results was evaluated using solutions of known concentration (test solutions), added every 3 readings. There was no difference greater than 3%. The data obtained in mV were inserted into a properly calibrated Excel spreadsheet for analysis and comparison of the results. The fluoride concentration results were obtained from linear regression of the calibration curve.

Statistical analysis

The demineralization ts rate constant and regression coefficient of each mineral was analyzed using Microsoft excel software 2010 for windows 10. Then, the value of the demineralization ts rate constant and regression coefficient was compared using descriptive statistic.

RESULTS

This present study which was undertaken to assess the degree of demineralization process. The degree of these process can be seen from the rate of demineralization rate constant and the regression coefficient.

The demineralization rate constant comparison for Ca can be seen in figure 1. According to the figure 1, the highest constant value is in the T1 group compared to the control group. The demineralization rate constant of Ca in T2 and T3 group does not look much different as the control group.

Figure 2 represented the mean values±standard error (mean±SE) of the demineralization rate constant of Mg. The result from figure 1 shows that the highest demineralization rate constant of Mg is also in T1 group compared to control group. The demineralization rate constant in the T2 group seems to be lower, while in T3 group higher compared to the control group.

The demineralization rate constant comparison for P can be seen in figure 3. According to the figure 3, the highest constant value is in the T1 group compared to the control group. The demineralization rate constant of P in T2 and T3 group does not look much different as the control group.

Figure 4 represented the mean values±standard error (mean±SE) of the demineralization rate constant of F. The result from figure 4 shows that the highest demineralization rate constant of F is also in T1 group compared to control group. The demineralization rate constant of F in T2 and T3 group does not look much different as the control group.

Table 1 showed the regression coefficient between minerals in different water treatment. All the data in table 1 shows that there is a negative correlation between treatment and the demineralization process of all tooth minerals. It indicates that the treatment will increase the releasing of all minerals content in the tooth.

DISCUSSION

Enamel is the only tissue of ectodermal origin with an acellular structure, exposed to calcification in the absence

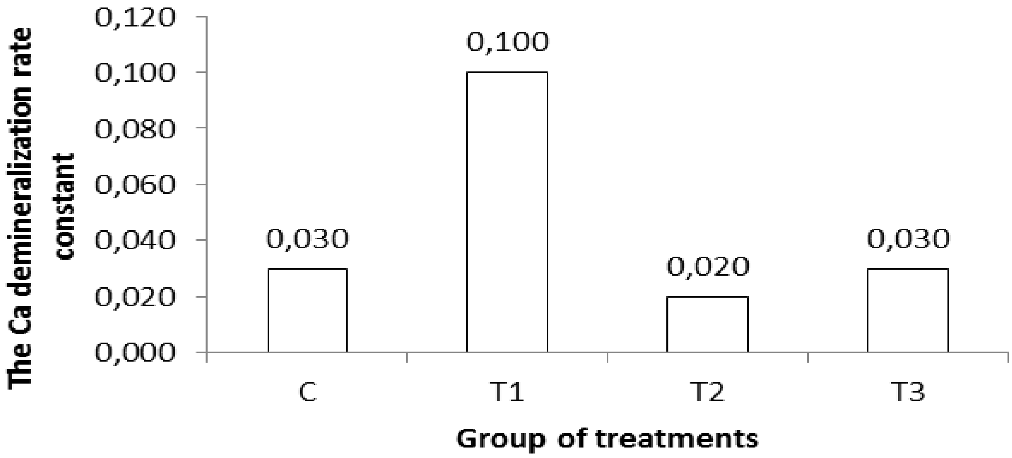

Figure 1: Comparison of Ca demineralization rate constant between group of treatments. C: control group; T1: treatment 1 group (river water); T2: treatment 2 group (tap water); and T3: treatment 3 group (well water).

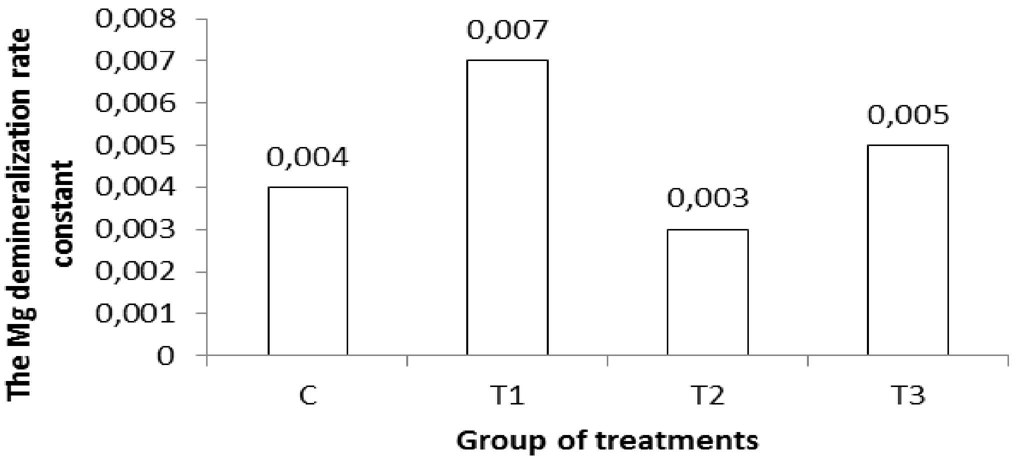

Figure 2: Comparison of Mg demineralization rate constant between group of treatments. C: control group; T1: treatment 1 group (river water); T2: treatment 2 group (tap water); and T3: treatment 3 group (well water).

Table 1: Regression coefficient of several minerals in group of tereatments.

Minerals	Regression Coefficient			
	C	T1	T2	T3
Ca	-0,970	-0,970	-0,950	-0,970
Mg	-0,992	-0,924	-0,910	-0,995
P	-0,990	-0,970	-0,990	-0,990
F	-0,940	-0,940	-0,920	-0,940

Ca: calcium; Mg: magnesium; P: phosphorus; F: fluor; C: control group; T1: treatment 1 group (river water); T2: treatment 2 group (tap water); and T3: treatment 3 group (well water).

of blood vessels and nerves. According to current concepts, tooth enamel is the hardest tissue in the human body due to the high content of inorganic substances (97%)[14]. Tooth enamel is composed of well-packed calcium phosphate hydroxyapatite (HAP) crystals engulfed by a very slim sheath of an organic matrix made mainly of phosphorylated proteins, which has the formula $Ca_{10}(PO_4)_6(OH)_2$[9,15]. However, tooth enamel mineral component contains a lot of carbonate ions (~2-5% w/w) as well as a small percentage of trace elements such as F, Cl, Mg, K, Fe, Sn, Sr, Ni, Co, Cr[15].

Because of its highly mineral content, enamel is extremely strong and can resist the mechanical forces exerted during functioning. However, this hardness makes it easy to break[16]. One of the breaking processes is known as dental erosion, which is caused by the unbalancing of two dynamic processes known as demineralization and remineralization[17]. It has long been recognized that demineralization of dental enamel will occur once the oral environmental pH reaches the critical threshold of 5.5. Acids in the mouth originate from three main sources: produced in situ by acidogenic bacteria, ingested extrinsic

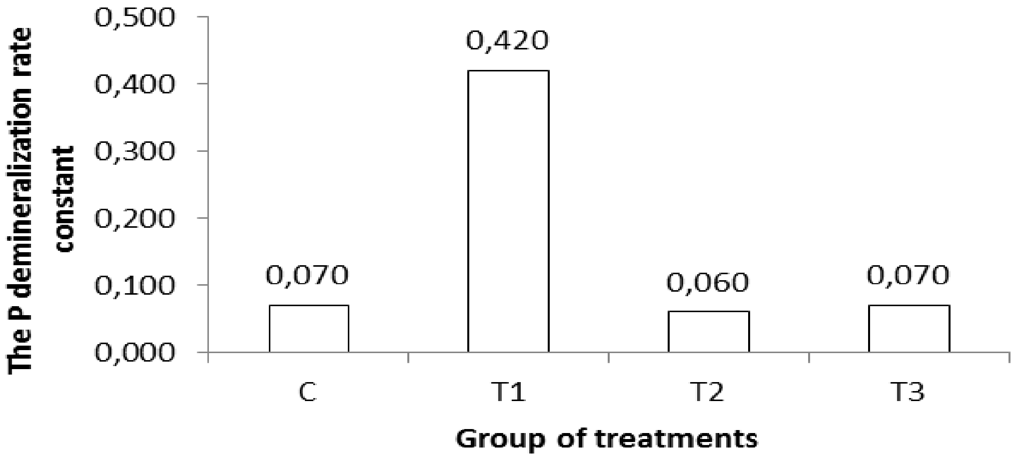

Figure 3: Comparison of P demineralization rate constant between group of treatments. C: control group; T1: treatment 1 group (river water); T2: treatment 2 group (tap water); and T3: treatment 3 group (well water).

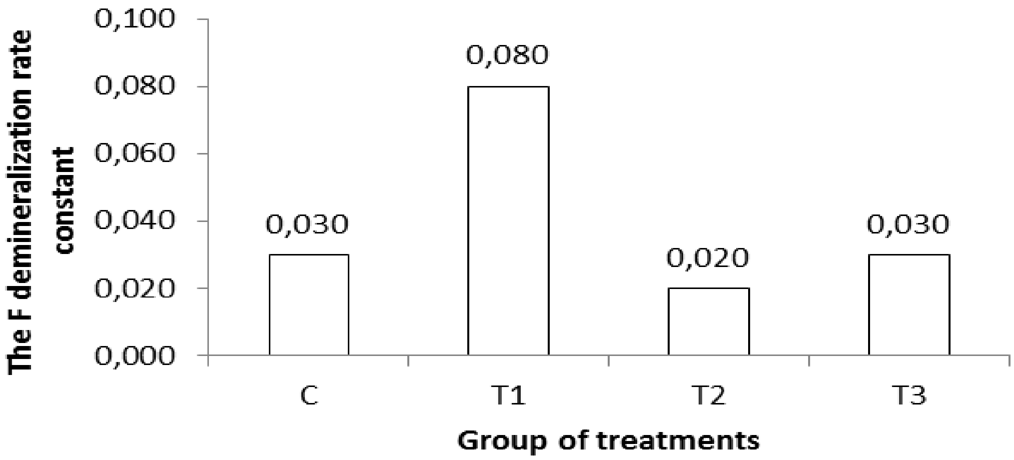

Figure 4: Comparison of F demineralization rate constant between group of treatments. C: control group; T1: treatment 1 group (river water); T2: treatment 2 group (tap water); and T3: treatment 3 group (well water).

acids as dietary components and dislocated intrinsic acids through the backflow of gastric contents[18].

In this present study, we try demonstrated the ingested extrinsic acids factors as mentioned earlier. From this point of view, the extrinsic factors were water treatment. The results show that all minerals that investigated in this study were demineralized from the tooth by water treatment. The highest demineralization was in the river water treatment. The main reason why the highest value was in the river water is because the pH of water river is more acidic than another water that we used in this study.

According to this study, the pH of the water river is 5.8, and the pH of the tap and well water are 6.99 and 7.11, respectively. The pH value of the water river is very close to the critical pH threshold of oral environment, which is known are about 5.5. There is two main reasons for the increased of demineralization of enamel in acid. First, the hydrogen ions remove hydroxyl ions to form water. The product of $[H^+][OH]$ in water always equals 1014 (mol/L). Therefore, as the $[H^+]$ increase in an acid solution, the [OH] must decrease in a reciprocal manner. Second, the inorganic phosphate in any fluid such as saliva or plaque fluid is present in four different forms, namely H_3PO_4, H_2PO_4, HPO_4 and PO_4, and the proportions depend entirely on the pH[10]. This two process – also to maintain neutrality – finally leads to the release of calcium and phosphate from HAP which hard tooth substance, i.e., the tooth dissolves[19].

Another mineral that investigated in this study are Mg and F. The demineralization of Mg by river water in this study might be caused by the chemical bonds breaking in the HAP by river water, which known have a low pH. At low pH, H+ ions are higher. This ion can diffuse into the enamel and dentin that will damage the chemical bonds, including Mg. slightly different from the Mg, F in the tooth can be found as fluoro hydroxyapatites and fluorapatite which occupied in lattice intercrystalline of tooth email prism. The demineralization of F presumably due to pressures of H^+ ions which contained in the river water[9]. This pressure causes the F in fluorapatite are dissolved.

In conclusion, the present study demonstrated that all treatment can cause the demineralization of tooth minerals such as Ca, Mg, P, and F. The highest demineralization rate was found in the treatment with river water than another treatment.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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