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THE EFFECT OF ACIDITY LEVEL AND SUBMERSION DURATION OF TEETH IN PEATLANDS TO DETERMINE BLOOD-GROUP ACCURACY THROUGH DENTAL PULP

Study of postmortem blood-group identification technique through dental pulp

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ABSTRACT

Background: *Indonesia has peatlands which spread all over the state and one of them is in Borneo (Kalimantan). More than three million hectares of peatland spreads in South Borneo. Peatlands have relatively high acidity level with pH range of 3-5. The peatlands in South Borneo is generally used for farming or public cemetery. In certain situation, peatlands is often used as a dumping ground for criminal victims. Sometimes, the authority finds it hard to identify the victim because the body is already decomposed. To identify the victim and to analyse the cause of death, identification process is necessary. Teeth can be used to help the identification process. Biological elements from the teeth namely dental pulp contains antigens that were useful to blood-groups determination by absorption elution method.* ***Purpose:*** *The objective of this research is to discover the effect of peatlands acidity level and teeth submersions durations in determining blood-group accuracy from dental pulp.* ***Method:*** *The method of this research used a quasi-experimental method to discover the effect of peatlands acidity and pre-experimental method to discover the effect of teeth submersions duration. This research used 48 pieces premolar teeth that were divided into 8 groups, control group, group submerged on peatlands with pH 3,0-3,9, pH 4,0-4,9 and pH 5,0-5,9 to discover the effect of peatlands acidity and 1-day, 3-day, 5-day and 7-day groups to discover the effect of teeth submersions time.* ***Result:*** *Fisher's Exact test results showed p value 0,314 (p > 0,05) for the effect of peatlands acidity and p value 0,410 (p > 0,05) for the effect of teeth submersions duration.* ***Conclusion:*** *It could be concluded that there are no effect of the peatlands acidity and teeth submersions time to determine blood-group accuracy from dental pulp.*

Key words*: peatlands acidity, teeth submersions duration, determine blood-group accuracy*

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INTRODUCTION

Indonesia has the largest peatlands among tropical countries. There were approximately twenty-one million hectares of peatlands in Sumatra, Kalimantan and Papua. It is estimated in 2000-2002 that Kalimantan has 5.769.246 ha of peatlands, with 331.629 ha are located in South Kalimantan.[1,2]

Characteristics of peatland is determined by the mineral content, thickness and level of decomposition. The mineral content of peatlands in Indonesia is less than 5%, and the rest of it is an organic compound. The organic compound is

derived from the decomposition of dead plants. The high number of organic compounds made peatlands have a high acidity level with pH range of 3-5. This condition is present in the entire peatlands that were generally utilized by South Kalimantan people as agricultural land, plantation and public cemetery. In certain circumstances peatlands are used as a dumping ground of criminal victims. [2,3]

The use of peatlands as a dumping ground for criminal victims is related to the Autopsy Report from Biddokkes Polda Kalimantan Selatan. In 2013, there were two cases of skeleton discovery, where one of them is found on the surface. In 2016, there's one case of skeleton discovery, and two cases of grave unburial with purpose of upholding the cause of death.[4]

The identification process is necessary to analyse the cause of death, and to provide calmness for the families of the victim, with information of identity certainty. Problems with identifying occurs when the bodies of the victim have undergone advanced decay. Primary identification examination based on fingerprints is difficult to achieve, but it can be replaced by dental examination.[5]

Teeth are the hardest part of the body and is chemically stable. Teeth can last longer than soft tissue and bone, against high temperatures, degradation and decomposition, so it can be used for identification. Teeth postmortem data is used for the identification of the approximate age, sex, race, ancestry, socio-economic background and dietary habits. Biological components in the teeth, namely the dental pulp can be used to examine the blood type. Dental pulp is protected by dentin and enamel, and it is the most protected parts of the teeth in spite of being in extreme environments. Dental pulp contains many blood vessels that have a class of antigens in order to examine the blood type.[6,7,8]

Blood type examination through the dental pulp is done by using the absorption elution method, where the antigen on the walls of red blood cells (erythrocytes) inside the dental pulp, is met with the appropriate antiserum and form the agglutination reaction. The reaction is useful for the identification of blood group of the victims.[9,10]

Human blood groups are classified through several systems, one of which is ABO system that groups the blood types based on different molecules in the cell membranes of erythrocytes. Each cell consists of a base unit of erythrocytes in form of protein and lipid called ceramide, and four types of surface antigen molecules, namely glucose (Glc), galactose (Gal), n-acetylgalactosamine (GlcNAc) and fucose (Fuc).

Blood type A molecules are addition of n-acetylgalactosamine (GlcNAc), blood type B are addition of galactose molecule (Gal), AB blood type have a molecular structure of blood group antigens A and B, and blood type O has no surface antigen molecules.[11]

Ramnarayan et al. (2013) states that the blood group antigens substances are the most abundant in the dental pulp, and increasingly decline in dentin and enamel. Adhani R, dkk. (2015) states that there's a release of calcium and phosphate ions on teeth soaked in water with pH 4 for 4 days, resulting in a white spot on the enamel surface. An irritant acidic media can stimulate odontoblasts in the pulp to form tertiary dentin. This formation of tertiary dentin is expected to occur based on Vavpotič et al. (2009), which states that odontoblasts are still visible in the histopathological examination of the pulp stocks up to 5 days after death. Mahendiratta et al. (2015) also revealed that the histologic changes in the pulp tissue had happened since 48 hours and lasted until 144 days after the death. Srivastava et al. (2008) states that erythrocyte cell surface changes significantly, along with decreased rigidity in blood that's been stored for over than 6 days.[9,12,13,14,15,16]

Based on the description above, the authors consider it is necessary to examine the effect of the acidity levels and teeth submersion duration in peatlands, with the accuracy of blood group determination through the dental pulp.

MATERIALS AND METHODS

This study is conducted by 2 methods, the first one is by using the quasi-experimental method with post-test only with control group design, to discover the effect of peatlands acidity level. The sample of this study was divided into 4 groups, namely: a control group and groups of submersion in peatlands with pH of 3.0 to 3.9, pH 4.0 to 4.9, and pH 5.0 to 5.9.

The second one is by using pre-experimental with one-shot case study design to discover the effect of submersion duration in peatlands. The sample of this study was divided into 4 groups, namely: group 1 day, 3 days, 5 days and 7 days.

This research was conducted at the Laboratory of Clinical Pathology, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin since June to November 2016.

Materials that is used in this research is the post extraction respondent's tooth, respondents blood-grouping, peatlands, antisera α, antisera β, antisera αβ, distilled water, saline and red wax.

The tools that is used in this research is universal pH indicator, lancing device, object glass, test tube 7 ml and 5 ml, test tube rack, micromotor, hand piece, round bur, refrigerator, centrifuge machine, extirpation needle, excavators, handscoon, tweezers, a pasteur pipette and pipette.

This study begins with the selection of the tooth samples in according with the inclusion and exclusion criteria that have been determined by the researcher. Selection of peatlands in Jalan A. Yani Km. 17, Kabupaten Banjar that suitable with the criteria acidity level for each treatment groups, soil depth is 50-300 cm, and the color of the soil is brown to dark brown. The submersion location is determined by an area approximately 50 cm x 50 cm. Soil samples were taken from several points and the measurement level of acidity with pH universal indicator were performed. Preparation of peatland as a place of submersion is done by digging a hole-shaped land 50 cm deep, and teeth that the apical foramen has been covered with a red wax placed in the bottom of the hole and submerged in the appropriate treatment groups.

Blood samples were taken and then the blood groups determination is conducted from respondent's blood. After the teeth submersion was done, the process then continue with taking the pulp. Dental crowns is drilled using the round bur to gain entrance into the pulp chamber. The pulp is then removed with a needle extirpation. Pulps sample are then placed in two tubes with the same quantity. Tubes 1-α antisera are added while the tube 2-β antisera was added until all the ingredients submerged, and then left to stand for 24 hours at a temperature of 4˚C. Antisera remainder is dumped, then added NaCl 0.9% as much as 1 cc. Washing was done as much as 1-2 times by removing the top layer. Last NaCl discarded, and then added 100 microns 0.9% NaCl into the tube and incubated at 56˚C for 25 minutes. Supernatant is taken and transferred into a new tube; the contents of the tube 1 and 2 transferred to tubes 1a and 2a. The tube 1a is added 4% suspension of red blood cells in the tube group A and blood group B in tube 2a is added as much as 50 microns. Tubes were then incubated at 4˚C for 2 hours and mess around with centrifuge machine at 1000 rpm for 1 minute to accelerate agglutination. Determination of the agglutination can be assessed macroscopically and microscopically.

The accuracy of blood group is determined by comparing the results of blood type, through a blood sample with a blood type test results in dental pulp. If the comparison results were identical, then it's categorized as compatibility. If the comparison results were different, then it's categorized as incompatibility.

RESULTS

Results of this research described in diagram and description below

1. The Effect of Peatlands Acidity Level

Table 1. Blood Type Compatibility through dental pulp based on blood type group

Blood Type	Compati-bility	Incompati-bility	Amount
A	**8 (100%)**	-	8
B	**4 (50%)**	4 (50%)	8
AB	7 (87,5%)	1 (12,5%)	8
Amount	19 (79,2%)	5 (20,8%)	24

Table 1 above shows that blood type A has the highest level of compatibility whilst the blood type B has the lowest level of compatibility.

Table 2. The compatibility of blood type through dental pulp based on treatment groups.

Treatment Groups	Compa tibility	In compati bility	Amount
Control	6 (100%)	-	6
Peat land submersion with pH 3,0-3,9	**3 (50%)**	3 (50%)	6
Peat land submersion with pH 4,0-4,9	**5 (83,3%)**	1 (16,7%)	6
Peat land submersion with pH 5,0-5,9	**5 (83,3%)**	1 (16,7%)	6
Amount	19 (79,2%)	5 (20,8%)	24

Table 2 above shows that the submersion of teeth in the peatlands with pH 4.0 to 4.9 and pH 5.0 to 5.9 have the highest level of compatibility whilst

the submersion in the peatlands with pH of 3.0 to 3.9 have the lowest level of compatibility.

The data that has been obtained from each treatment is tested with Chi-square test to determine the differences between the treatment groups. The result show that the data is ineligible for Chi-square test, so alternatively is then tested with Fisher's Exact test. The Fisher's Exact test showed p value 0.314 (p> 0.05), which means there is no significant difference in blood type examination through dental pulp between the treatment groups.

2. The Effect of Teeth Submersion Duration in Peatlands

Table 3. Blood type compatibility through dental pulp based on blood type groups.

Blood Type	Compatibility	Incompatibility	Amount
A	**8 (100%)**	-	8
B	**4 (50%)**	4 (50%)	8
AB	6 (75%)	2 (25%)	8
Amount	18 (75%)	6 (25%)	24

Table 3 above shows that the blood type A has the highest level of compatibility whilst blood type B has the lowest level of campatibility.

Table 4. The compatibility of blood type through dental pulp based on treatment groups.

Groups	Compatibility	Incompatibility	Amount
1 day submersion	**6 (100%)**	-	6
3 days submersion	5 (83,3%)	1 (16,7%)	6
5 days submersion	4 (66,7%)	2 (33,3%)	6
7 days submersion	**3 (50%)**	3 (50%)	6
Amount	**18 (75%)**	**6 (25%)**	**24**

Table 4 above shows that the 1 day submersion group has the highest level of compatibility whilst the 7 days submersion group has the lowest level of compatibility.

The data that has been obtained from each treatment is tested with Chi-square test to determine the differences between the treatment groups. The result show that the data was ineligible for Chi-square test, so alternatively the data is then tested with Fisher's Exact test. Fisher's Exact test showed p value 0.410 (p> 0.05), which means there is no significant difference in blood type examinaton through dental pulp between the treatment groups.

DISCUSSION

Result of this study shows that the blood group A has the highest compatibility whilst blood group B has the lowest compatibility (table 1 and 3). These results are consistent with the research of Rijaldi F (2016) and Fauziah S (2016) which mentions that the compatibility of blood type A is higher than blood group B, after exposure to temperatures of 100˚C to 250˚C for 23 minutes, and soaked in sea water for 3 and 7 days. This condition may be due to differences in antigen molecules on the surface of the cell membranes of erythrocytes of each blood type. Four types of the antigen molecules are glucose (Glc), galactose (Gal), n-acetylgalactosamine (GlcNAc) and fucose (Fuc). Blood type A molecules are adding n-acetylgalactosamine (GlcNAc), blood type B are the addition of galactose molecule (Gal), blood type AB are adding n-acetylgalactosamine molecule (GlcNAc) and galactose (Gal). Hatakeyama et al. (1995) also stated that n-acetylgalactosamine (GlcNAc) has resistance to the process of hemolysis, causing blood groups A and AB to be more resistant to the process of hemolysis compared to blood group B. Molecular galactose (Gal) is an unstable sugar cluster, causing the blood group B to be more prone to hemolysis than blood type A and AB.[11,17,18,19,20]

Result of this study also showed that the submersion in the peatlands with pH of 3.0 to 3.9 has the lowest compatibility (50%), whilst submersion in the peatlands with pH 4.0 to 4.9 and pH 5.0 to 5.9, have the highest compatibility (83.3%). The other result showed that the teeth submerged for 1 day has the highest level of compatibility whilst the 7 days submersion has the lowest level of compatibility (table 2 and 4). This condition can be caused by osmotic pressure from hypertonic acidic media, and also the duration of exposure. Media exposure can destabilize the acid and lowering the fragility of the erythrocyte membrane. Erythrocyte fragility is a reaction from erythrocyte membrane to counter the osmotic pressure from the surrounding media. Erythrocyte that were in the hypertonic medium, will release fluid into plasma, and consequently the erythrocytes become wrinkled (crenation) and than hemolysis occured. Ivanov et al. (1999) also mentions that the acidic media cause the hemolysis process faster. Peatlands with a pH of 3.0 to 3.9

have more hypertonic condition than peatlands with a pH of 4.0 to 4.9 and pH 5.0 to 5.9, which causing more erythrocytes to crenate. The submersion for 7 days allow more erythrocytes to experience crenation than submersion for 1 day. This condition can affect the amount of antigens on the cell surface of erythrocytes. The more antigens together with cell lysis of erythrocytes, will cause the blood type to increasingly provide negative results.[21,22,23]

Analysis of the effect of the acidity level and teeth submersion duration in peatlands, for blood group determination accuracy, showed no differences between the treatment groups. This condition can be caused by ionic calcium and phosphate in the enamel matrix structure, the formation of tertiary dentin, and pulp tissue resistance to decomposition. Enamel contains 96% mineral, 3% water, and 1% of organic matrix that generally contain protein. This high concentration of minerals not only provides strength and hardness, but also brittleness (brittle) on the enamel. The strength and hardness of the enamel is caused by crystals of calcium hydroxyapatite bond or known by the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Acid exposure due to the high level of acidity in peatlands, and duration of teeth submerged in peatlands, can lead to a demineralization process in the crystal structure of hydroxyapatite. The acidic medium can diffuse into the tooth, and damaging the bond between calcium and phosphate ions. Demineralization of enamel occurs when the pH of the media around the enamel is more than pH 5.5. If this condition occurs chronically, it can cause erosion on the teeth. Dental erosion began with the release of calcium ions to the loss of part of the enamel prism. This situation can lead to the formation of enamel porosity that decreasing hardness. The higher level of acidity and the longer the exposure of the media in the enamel surface, the greater the likelihood of calcium and phosphate ions to be dissolved. The research of Adhani R, et al. (2015) mentions the releasing of calcium and phosphate ions on the teeth soaked in water with pH 4 for 4 days. Based on the description above, it is estimated that the teeth submersion in peatlands with the treatment group of pH 3.0 to 3.9, pH 4.0 to 4.9 and pH 5.0 to 5.9, and the treatment group 1 day, 3 days, 5 days and 7 days only formed a white spot. That makes enamel still able to protect the pulps .[12,24,25,26,27]

Dentin is the layer under the enamel and make up most of the tooth. Dentin consists of 70% of hydroxyapatite crystals, the remaining 30% is an organic compound composed of collagen, mucopolysakarida and 10% water. The pulp is a connective tissue composed of cells and intercellular substance composed of blood vessels and nerves. Irritant acidic medium can also stimulate odontoblasts in the pulp to form tertiary dentin. This tertiary dentin formation is expected to occur, based on research of Vavpotič et al. (2009) which states that odontoblasts are still visible in the histopathological examination of the pulp stocks up to 5 days after death. Mahendiratta et al. (2015) also revealed that the histologic changes in the pulp tissue had happened since 48 hours and lasted until 144 days after the death. The structure of enamel and dentin serves as protection for chemical pulp because the tooth is a network of the most stable and the most resistant to degradation and decomposition. Aswath et al. (2012) also concluded that the teeth are the most stable biological material, even in adverse conditions. The stability of the teeth can protect dental pulp antigen in a long time. Based on the above description, it can be concluded that the dental pulp can be used for blood type determination despite having been submerged in peatlands with a pH of 3.0 to 3.9, pH 4.0 to 4.9 and pH 5.0 to 5.9 for 1 day, 3-day, 5-day and 7 days.[6,13,14,15,24,28]

The conclusion of this study is the acidity level and submersion duration of teeth in peatlands have no effect to the accuracy of blood type determination through dental pulp.

The results of this study can be used by the practitioners of dental forensic, to identify the postmortem blood type through the dental pulp, from the body found in peatlands whose identity is yet unknown, as well as to broaden the field of dentistry, especially odontology forensic. This study can also be used as a reference regarding the use of pulp for the blood group determination.

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