Difference in the Number of Polymorphonuclear (PMN) Leukocytes of Gingival Crevicular Fluid (GCF) in Various Blood Types of Patients with Gingivitis

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Abstract: <u>*Background:*</u>*Gingivitis is an inflammatory condition in the gingiva caused by a buildup of plaque and bacteria on the gingiva. It is characterized by gingival redness, gingival enlargement, contour changes, bleeding, and increased gingival crevicular fluid volume. Components in the gingival sulcus include bacteria, desquamation epithelial cells, leukocytes, calcium, and enzymes that migrate through the sulcular epithelium.*Aims of the study were*to determine the differences in the number of polymorphonuclear (PMN) leukocytes of gingival crevicular fluid (GCF) in various blood types of patients with gingivitis.* <u>*Methods*</u> *observational analytic with a study cross-sectional approach was confirmed in this study by checking gingival status based on clinical parameters gingival index (GI), so that 40 respondents were obtained. The data were analyzed using One Way ANOVA followed by Post HocHSD with a significance value 95%.* <u>*Results:*</u> *Based on the calculation of the number of PMN leukocytes using a hematology analyzer, the number of PMN leukocytes was blood type O (385µl), blood group A (368µl), blood group B (345µl), and blood group AB (314µl). The results of data analysis obtained a significance value of 0.000 (p <0.05).The calculation results show there are differences in the number of PMN leukocytes against various blood type of patients with gingivitis.* <u>*Conclussion:*</u> *The highest number of leukocytes is blood type O, followed by blood type A, blood type B, and the lowest number is blood type AB. Gingivitis has various other risk factors such as age, sex, education, place of residence, bad habits, socio-economic, genetic, and smoking habits.*

Keywords: gingivitis, gingival crevicular fluid (GCF), ABO blood type, PMN leukocytes

1. Introduction

Periodontal tissue is a connective tissue that surrounds the roots of teeth and connects to the alveolar bone which can be inflamed just like any other body tissue.[1] Inflammation of the gingiva can occur in one to two teeth or can occur in all teeth. The stage of gingivitis consists of three stages, including Initial Lesion, Early Lesion, and Gingivitis, while the parameters used to assess gingivitis status include bleeding on probing, probing depth, and gingival index.[2] Gingivitis is characterized by redness of the gingiva, enlargement gingival, contour changes, bleeding, and increased gingival crevicular fluid(GCF)[3.]

GCF is a transudate in healthy tissue but will be a fluid exudate excreted by the gums in inflamed tissues found in the gaps located at the point where the gum line meets the teeth, called the gingival sulcus.The concentration of this fluid is usually low butmay increase when there is an inflammatory process in the oral cavity.[4] There are various components in the GCF including bacteria, desquamated cells, and PMN leukocytes, lymphocytes, monocytes/macrophages), which migrate through the sulcular epithelium.[1]

Leukocytes are commonly found in the healthy gingival crevicular fluid, migrate freely, and the majority of cells have phagocytic properties and protective mechanisms against bacterial colonies of plaque and gingival sulcus. Most of the leukocytes found were PMN. Clinically, the number of PMN leukocytes in the healthy gingival sulcus was 91.2-91.5%, while the rest (8.5-8.8%) were mononuclear leukocytes. The inflammation rate is 95-97%, with 1-2% consisting of lymphocytes and 2-3% consisting of monocytes.[5]

Blood is an important element in the human body that is pumped by the heart through a network of arteries and veins. The blood works to provide oxygen and nutrients throughout the body and takes carbon dioxide and metabolism from the tissues. According to Landsteiner, the blood grouping system is divided into four types including blood type A which has A and anti-B antigens, B blood group that has B and anti-A antigens, AB blood group that has antigens but does not have antibodies, and blood type O that has antibodies but does not have antigens.[6]

Clinically, several blood types are known to have systemic disease effects in some populations. Blood type A is more susceptible to gallstones, colitis, and tumors of the salivary glands, pancreas, and ovaries, while blood types A and O are more susceptible to cardiovascular disease.[7] Based on the research by Gautam., et al,[6] gingivitis is more common in blood type O followed by blood type A and periodontitis is more common in blood group B, while blood type AB shows the prevalence of being the most susceptible to contracting the periodontal disease.

2. Materials and Methods

An analytic observational using a cross-sectional study was carried out in this study. The samples were divided into 4 types, namely blood types A, B, AB, and O, totaling 40 specimens with 10 samples each. Patients with mild gingivitis in this study will be subject to taking GCF. Gingival crevicular fluid is used to count the number of leukocytes in patients with mild gingivitis contained in it. The leukocytes to be studied are PMN leukocytes or the so-called neutrophils because these leukocytes are predominantly found in the GCF. Gingival index (GI) examination was confirmed to determine the gingivitis severity status of patients used as research subjects. GCF was taken using a microsyringe inserted into the interdental anterior teeth to touch the bottom of the sulcus (1mm depth), and left for 3 minutes until ±3µl of GCF was taken and put into an Eppendorf tube. After that, it was given 3 ml of distilled water and then stored in the icebox before being calculated.

The samples were collected in the icebox and were calculated by a hematology analyzer. Data were analyzed by one way Anova followed by Post Hoc HSD with significant analysis 95%.

3. Results

It has been examination about PMN leocyte of GCF in various blood types of patients with gingivitis using a hematology analyzer. Data was shown at table 1.

Table 1: The mean and standard deviation of the PMN leukocyte (µl)

Group	n	$\overline{x}$	SD
Blood Group A	10	368	±8,755
Blood Group B	10	345	±8,164
Blood Group AB	10	314	± 8,755
Blood Group O	10	385	± 10,801

Note: n (number of sample), $\overline{x}$ (mean), SD(standarddeviation).

Table 1. showed O blood type has a higher mean PMN leukocyte count (385µl) than other blood types.

Table 2: Shapiro-wilk normality test

Group	N	Sig.
Blood Group A	10	0,191
Blood Group B	10	0,258
Blood Group AB	10	0,124
Blood Group O	10	0,258

Note: n (number of sample), $\overline{x}$ (mean), Sig. (significance value).

The results of the Shapiro-Wilk data for normality test in the four blood types showed the data were normally distributed (p>0.05). The same results was also confirmed for homogenity test using Levene's test.

Table 3: Levene's test homogeneity test

Levene Statistic	Sig.
0,163	0,921

Note: Sig. (significance value).

The results of homogeneity test in Table 3 showed the significance value obtained is 0.921 (p>0.05), therefore the data showed homogeneous results. Based on these data, then data were analyzed using the one way ANOVA followed by Post Hoc HSD..

Table 4: One Way ANOVA test result

Group	Sig.
Between Group	0,000
Within Group	
Total	

Note: Sig. (significance value).

The results of the One Way ANOVA test data show that the significance is p = 0.000 (p <0.05). These results indicate that there are significant differences between various blood types.

Based on the table 5, it can be appeared the number of leukocyte of GCF between blood types.

Table 5: Result Post Hoc HSD test between blood types.

Group	Blood type A	Blood type B	Blood type AB	Blood type O
Blood type A		0,000*	0,000*	0,007*
Blood type B	0,000*		0,000*	0,000*
Blood type AB	0,000*	0,000*		0,000*
Blood type O	0,007*	0,000*	0,000*	

Note: (*) : Significance p<0,05

The results of the Post Hoc HSD test in table 5 showed that all groups have a sign (*, p<0.05), indicating all groups have a significant difference. These results indicate the number of PMN leukocytes in the gingival crevicular fluid has significant differences in various blood groups and between blood groups. Blood type O has the highest number of PMN leukocytes in the gingival crevicular fluid in patients with mild gingivitis, followed by blood type A, B, and AB.

4. Discussion

Blood types is a special characteristic of the blood of an individual because of the different types of carbohydrates and proteins on the surface of red blood cells. In other words, blood type is determined by the number of substances (antigens) contained in red blood cells. There are many systems in blood grouping, one of which is often used is the ABO blood typing system which was discovered by Landsteiner in 1990. Type O blood is the most common blood type in Indonesia and even in the world, and blood typeO is the most common blood type. This is very different from other blood types in that blood type O does not have antigens on the surface of red blood cells (carbohydrates and proteins) which function as a source of energy in cell metabolism.[8]

Leukocytes are the main components in human blood that function to maintain the body's defenses. PMN leukocytes in the gingival crevicular fluid originate from the connective tissue blood vessels at the base of the sulcus that exit through the epithelium to the gingival sulcus. GCF diffuses

through the basement membrane, entering the gingival sulcus from the wide intracellular spaces of the junctional epithelium. The discharge of GCF begins with changes in vascularization, then capillary vasodilation and an increase in blood supply. Inflammation of the gingiva causes changes in tissue permeability and increases the osmotic pressure of the capillaries, thereby making it easier for intracellular fluid to escape through the capillary walls and penetrate the gingival epithelium in relatively larger amounts than usual. Since the discovery of blood types in humans by Landsteier in 1990, the most famous is the ABO blood type. Blood type is a special characteristic of the blood of an individual because of the different types of carbohydrates and proteins on the surface of red blood cells. There are different characteristics in each blood group, this can make a difference for each blood type to respond to a disease.[7]

In this study, the number of leukocytes in blood type O occupies the highest number compared to other blood types, this is following research that has been carried out showing that blood type O is the blood type that has the most GCF[9], as is the case with studies that have been conducted. The results showed that there was the highest prevalence of gingivitis in blood group O followed by blood type A and periodontitis in blood group B, while blood group AB showed the least prevalence of the periodontal disease.[10]

Blood types have abnormalities in the blood that can understand blood clots when inflammation or hemostasis occurs, therefore why individuals with blood type O who experience inflammation are difficult to improve or heal, for example, inflammation of the gingiva such as gingivitis. This is because type O erythrocytes do not have agglutinogens so they cannot react with either anti-A or anti-B serum. Antigens are carbohydrates and proteins that attach to the surface of cell membranes foreign substances to the body that cause an immune response. The immune response occurs when antibodies in the form of proteins in the immune system appear to attack antigens. Surface antigens can attach to the surface of blood cells (especially the plasma membrane that surrounds cells) or proteins or lipids anywhere in the body.[6] In addition to having the highest number of leukocytes among other blood types, individuals with this blood type have a lower risk affected by coronary heart disease and circulatory disease.[11]

The existence of different characteristics in each blood group makes individuals have differences in responding to disease. From this study, blood type A ranks second with the highest number of leukocytes in the gingival crevicular fluid. Type A blood has red blood cells with A antigens on the surface of their cell membranes and produces antibodies against B antigens in their blood serum. People with blood type A are prone to cancer, malaria infection, heart disease, and type 2 diabetes mellitus.[12] Blood type A is also very susceptible to stress because it has higher levels of the hormone cortisol than other blood types, this will make individuals with blood type A is faster to feel anxious.[11]

The next blood types that has a high number of leukocytes in the GCF after blood type A is blood group B. Blood type B is formed from a combination of antigen B and antibody A, this blood type is more susceptible to agglutination than other blood types. Blood type B has a higher risk of high blood pressure (hypertension), coronary heart disease, and coronary artery disease, while blood type AB is the blood group with the lowest number of leukocytes in the GCF. This blood type is the rarest blood group in the world.[13] Individuals with blood type AB have a higher risk of heart disease. Blood type B is a blood type that has factors A and B in red blood cells which results in increased levels of von Willerand, this increase in levels can trigger very fast blood clots, blood clots can cause a person to have difficulty breathing, even high pain, even blood clots. can result in death if the blood supply is interrupted.[7]

Based on these studies, blood type was increasing the number of PMN leukocytes in the GCF. Blood type O occupies the highest number of PMN leukocytes cells in the GCF, followed by blood group A, blood type B, and the lowest number of leukocyte cells in the GCF is blood group AB. However, a periodontal disease also has various other risk factors such as age, gender, education, place of residence, bad habits, socioeconomic, genetics, and smoking habits.[13] ABO blood group factor.This can also be a factor in increasing the number of leukocytes in the GCF in addition to the ABO blood types factor.

In conclusion, the number of PMNleucocytes of GCF in various blood types in patients with gingivitis was significantly different.The highest number of PMN leukocytes was found in blood type O, while the lowest is in blood group AB.

5. Acknowledgments

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