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RESEARCH ARTICLE

Saliva Accuracy Analysis as a Non-Invasive Method for Determining Blood Type

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ABSTRACT

Background: *A phobia is an excessive, irrational, and persistent fear of something that makes it difficult for someone to carry out certain activities. Psychological disorders, such as a phobia of blood and injections, indicate the need for forensic identification methods that do not require blood samples. Blood type identification plays an important role in forensic cases, especially in matching the blood type of the evidence of victims or perpetrators.* ***Objective:*** *To analyze the accuracy of saliva as a noninvasive method for determining blood type in students of the Faculty of Dentistry, Baiturrahmah University, class of 2021.* ***Method:*** *This was a quantitative observational analytic study with a cross-sectional design. The study population comprised 80 students selected using a simple random sampling technique. Saliva samples were analyzed using the absorption inhibition method and compared with available blood type data. The data were analyzed using SPSS version 23.0, and Fisher's exact test was performed as an alternative to the chi-square test.* ***Results:*** *Blood types A, B, and AB have 100% compatibility in secretor individuals, while blood type O has 0% compatibility because there are no antigens A and B in the saliva. Statistical tests showed a significant level of accuracy between blood type examination through saliva and blood type in the data (p=0.000).* ***Conclusion:*** *Overall, saliva blood type examination has the same level of accuracy as the conventional method.*

Keywords: *Blood type, forensic identification, phobia, saliva*

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INTRODUCTION

Psychological disorders are defined and treated as medical problems; they are a collection of abnormal conditions involving physical or mental.[1] Anxiety and fear that is irrational, excessive, and persistent towards something, so that a person is unable to do any activity, is called a phobia.[2] The word "phobia" comes from the Greek term "phobos," which means to run (fight), fear, and panic (panic-fear), and great fear or terror.[3] Blood phobia is a fear or avoidance of situations directly or indirectly related to blood, wounds, and so on. Injection phobia is a fear or avoidance of various types of injections, such as taking samples by injecting into certain parts of the body. The significant impact of psychological disorders such as blood phobia and injection phobia shows the importance of developing forensic identification methods that do not involve blood sampling.[2]

Forensic identification methods are divided into primary examinations, namely, dactyloscopy and Deoxyribonucleic Acid (DNA) and secondary examinations, namely medical characteristics, photography, and the victim's property.[4] Blood type identification is very important in several forensic cases, especially in relation to blood type matching of evidence from victims or perpetrators.[5] Blood type is a genetic substance that is inherited.[6] Blood type identification is generally done based on the ABO blood type system.[7] Human blood types in the ABO system are divided into four types, namely A, B, O, and AB.[8] In general, blood type determination using causesausamoamong fear among individuals, especially children. In addition to blood, blood type antigens are also secreted in various other body secretions, such as saliva, which can determine blood type.[9] Saliva is a complex biological fluid secreted by the major and minor salivary glands.[10]

Blood type examination through saliva can only be performed on individuals with secretor status. Secretor groups are individuals who secrete blood type antigens identical to their red blood cells in body fluids, such as saliva. Conversely, the non-secretor type is an individual with blood type A, B, or AB who does not secrete ABO blood type antigens in the body fluids.[11,12] To find out whether someone is a secretor or a non-secretor, can be determined through a secretor test.[12] Blood type examination using the inhibition absorption method is a procedure to determine blood type indirectly, which utilizes materials other than blood, such as saliva, sperm, gastric fluid, and other body fluids.[9] Blood type examination using the inhibition absorption method has the same principle as the ABO blood type examination, which is commonly performed, but uses a different method.[13]

Research from Lakade's (2018) in India showed that 100% of subjects from saliva samples were secretors, and ABO blood type determined from saliva swab samples showed 100% correlation with blood type determined from extraction socket blood. The results of research from Kimberly Alsbrooks & Klaus Hoerauf's (2022) in the United States, showed that the prevalence rate of needle fear was 20-30% in the 20-40 year age group and of participants who experienced injection phobia, 52.2% stated that they avoided blood collection, followed by 49% who refused blood donation, and 33.1% who refused vaccination. Therefore, this can encourage the use of saliva as a non-invasive method in routine blood tests, especially in children who suffer from blood and injection phobias.[9,14]

Based on the background description above, researchers are interested in analyzing the accuracy of using saliva as a non-invasive method for determining blood type. This research was conducted on students of the Faculty of Dentistry, Baiturrahmah University, class of 2021, who had never previously conducted research on determining blood type through saliva, and several students suffered from symptoms of blood and injection phobia.

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This research is expected to broaden the scientific horizons of dentistry, particularly forensic odontology, by utilizing saliva as a non-invasive means of blood typing. Practically, this study underpins the development of safer, more efficient, and more comfortable diagnostic procedures for patients who struggle with conventional sampling methods.

MATERIALS AND METHODS

This type of research is a quantitative observational analytic approach with a cross-sectional design. This study was conducted on 80 students of the Faculty of Dentistry, Baiturrahmah University, class of 2021, consisting of 71 women and 9 men. The inclusion criteria for this study were 2021 Faculty of Dentistry students who were willing to participate in the study, had signed an informed consent form, and knew their blood type. The exclusion criteria included subjects with diseases affecting saliva (e.g., xerostomia and Sjögren's syndrome).

The minimum sample size for this study was 80, determined using the Slovin formula. Slovin's formula was employed to obtain a representative sample with a 5% error margin. This method was chosen because the subject population size was predetermined, ensuring efficient and scientifically valid saliva. The dependent variable was the blood type. This study was conducted in May until December 2024 at the LLDIKTI Laboratory, Region X, West Sumatra. Participants who consented to participate in this study were individuals who already knew their blood type. This research was approved from the Baiturrahmah University Ethics Committee (No.A.004/KEPKFKGUNBRAH/XI/2024).

The research procedure began with preparing the tools and materials, namely an oven, test tube rack, test tube, centrifuge, hot plate, dropper, beaker, measuring cup, incubator, saliva, antisera reagent (Anti A, Anti B, Anti D), distilled water, 5% erythrocyte suspension, sterile gauze-wrapped cotton (as handscounbe cover), handscoon, face mask, and plastic clip (as a test tube container when collecting saliva).

All tools and materials used in this study were sterilized. Saliva samples were collected in clean and dry test tubes, labeled (Name-Blood Type), and diluted with distilled water (1:2). The samples were heated in a boiling water bath for 10 minutes, cooled, centrifuged at 3000 rpm for 10 minutes, and then the supernatant (the upper centrifugation result which is a liquid separated from the sediment) was discarded. Antisera reagents (Anti-A, Anti-B, Anti-D) in a 1:4 dilution were added to each test tube (1 drop/tube) according to blood type. The sample was shaken well and incubated at 37°C for 10 min. The subject population size was predetermined to ensure an efficient and scientifically valid sampling process. Sampling used a simple random sampling technique according to the calculation results using the Slovin formula. The independent variable in this study was shaken well, incubated at 37°C for 10 minutes, then 5% erythrocyte suspension (1 drop/tube) was added, shaken well, and incubated again for 15 minutes at the same temperature (37°C). Furthermore, the samples were examined for agglutination. If one of the test tubes did not exhibit agglutination, this indicated a positive secretor status, whereas agglutination in all test tubes indicated a negative secretor status. Tubes that did not experience agglutination were considered to show the ABO blood type.[9,13,15] Data were analyzed using SPSS to describe variable characteristics, followed by Fisher's exact test as an alternative to the chi-square test to assess the accuracy between saliva-based and conventional blood type results.

The findings were processed and presented in tables and percentages.

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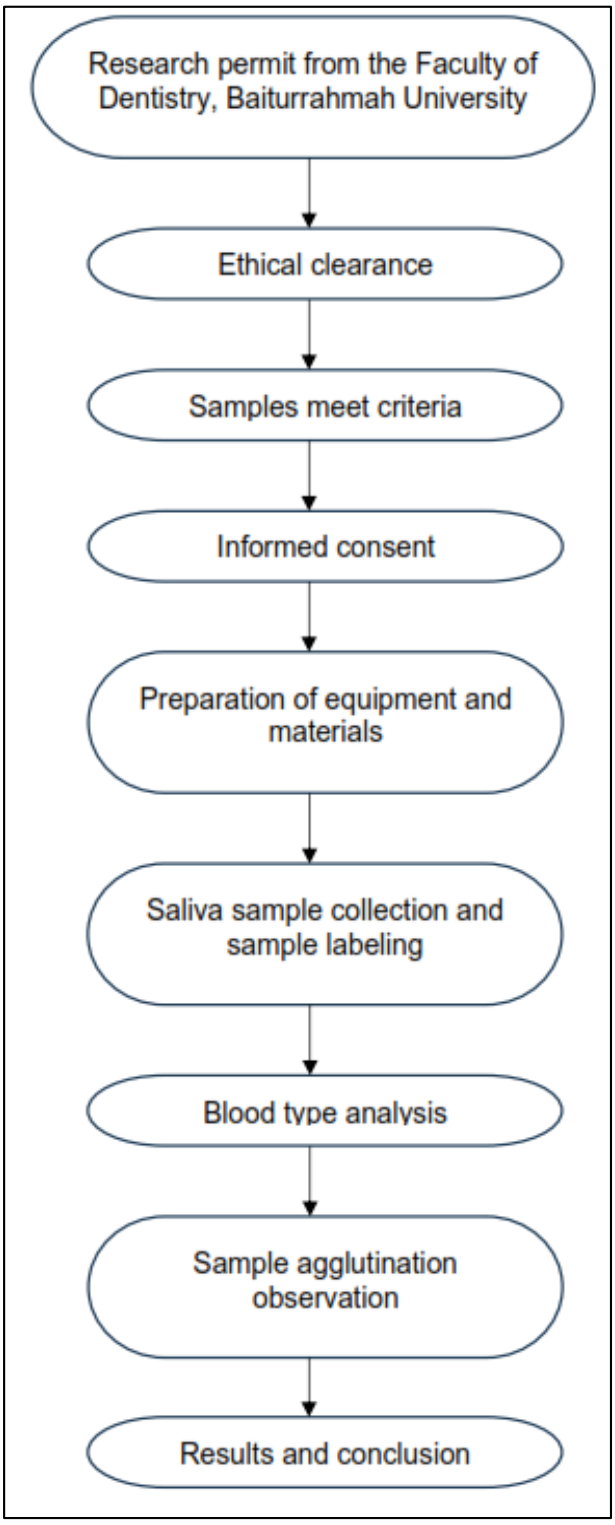

Figure 1. Flowchart Research Methods

RESULTS

Table 1. Characteristics of Research Samples

Characteristics of Research Samples	*f*	%
Age		
20 years old	3	3.75
21 years old	48	60
22 years old	25	31.25
23 years old	2	2.5
24 years old	2	2.5
Gender		
Male	9	11.25
Female	71	88.75
Blood type		
A	18	22.5
B	23	28.75
AB	10	12.5
O	29	36.25
Total	80	100

Table 1 shows that from the 80 research samples, the most dominant age is 21 years old, which is 48 people (60%), while the least age is 23 and 24 years old, with only two people (2.5%) each. The most dominant gender is female, which is 71 people (88.75%), while the least gender is male, which is 9 people (11.25%). The most dominant blood type was O (29 people, 36.25%), while the least dominant was AB (10 people, 12.5%).

Table 2. Status of Secretor and Non-Secretor Groups

Groups	*f*	%
Secretor	49	61.25
Non-Secretor	31	38.75
Total	80	100

Table 2 shows that among the 2021 intake of students of the Faculty of Dentistry, Baiturrahmah University, the majority were included in the secretor group (49 people, 61.25%), while the rest were included in the non-secretor group (31 people, 38.75%).

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Tabel 3. Secretor Status Based on Blood Type

Blood Type	Secretor Status		Total	%
	Yes	No		
A	18	-	18	100
B	22	1	23	100
AB	9	1	10	100
O	-	29	29	0
Total	49	31	80	

Table 3 shows the secretor status based on blood type of the students of the Faculty of Dentistry, Baiturrahmah University, class of 2021. For blood type A, all samples had secretor status, totaling 18 individuals (100%). In blood type B, 22 people (100%) had secretor status, while one person was a non-secretor. Samples with blood type AB mostly have secretor status, namely 9 people (100%), while 1 person is a non-secretor. Conversely, all samples with blood type O were classified as non-secretors, totaling 29 people (0%).

Table 4. Blood Type Test Conformity Results

Blood Type	Blood Type Data	Blood Type Results (Saliva)		
		Secretor	*Non-secretor*	%
A	18	18	-	100%
B	23	22	1	100%
AB	10	9	1	100%
O	29	-	29	0%
Total	80	49	31	

Table 4 shows the results of blood type examination suitability using saliva, which has a good level of accuracy, the same as the available blood type data (conventional method). Blood types A, B, and AB have a 100% suitability rate in secretor individuals. In contrast, blood type O had a 0% suitability rate, where all blood type O samples were non-secretors and were not detected in the saliva.

Table 5. The results of statistical tests using Fisher's exact test

Saliva	Blood Type Data				Total	p-value
	A	B	AB	O		
A	18	0	0	0	18	
B	0	22	0	0	22	
AB	0	0	9	0	9	0.000
Not detected	0	1	1	29	31	
Total	18	23	10	29	80	

Table 5 shows the results of statistical tests using the Fisher's exact test obtained a *p value* of 0.000 ($p<0.05$), which means that there is a significant relationship between saliva and blood type data in students of the Faculty of Dentistry, Baiturrahmah University, class of 2021.

DISCUSSION

This study was conducted on students of the Faculty of Dentistry, Baiturrahmah University, a 2021 class with diverse characteristics. Blood type can be identified from various body fluids, which are then determined by the individual's status as a secretor or a non-secretor. Secretors are individuals who secrete fluids, such as saliva, tears, and sweat. Conversely, non-secretor individuals are individuals who do not secrete blood type antigens in their body fluids.[15]

The results of the study in Table 2 are in line with the study conducted by Alqadri (2016) in Padang City. From 54 samples, it was found that the secretor status was present in 42 samples (78%), while the non-secretor status was present in 12 samples (22%). Similar to a study conducted by Tejasvi (2021) in India, from 60 samples, 52 samples (86.66%) were included in the study.

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The secretor group and 8 samples (13.33%) were included in the non-secretor group. Overall, the results of several studies emphasize that the secretor group tends to be more dominant than the non-secretor group because the distribution of the secretor and non-secretor groups in the human population is 85% in the secretor group and 15% in the non-secretor group, which means that most of the human population has the SeSe or Sese (secretor) gene that encodes a certain fucosyltransferase enzyme found in the epithelium of secretory tissues, such as saliva, tears, and sweat. This enzyme allows body fluids to produce the same blood group antigen as the antigen on red blood cells, but in a water-soluble form. Meanwhile, the non-secretor group has the se gene, which is unable to produce antigens in soluble form, so there are no blood group antigens in their body fluids.[11,15]

This study only calculated the secretor group, while the non-secretor groups were not included. Based on the data presented in Table 3, blood groups A, B, and AB have a 100% compatibility rate in secretor individuals.

Meanwhile, in blood group O, all samples had a non-secretor status, which means that there were no blood group O subjects with secretor status. Research conducted by Klarmann (2010), quoted from Saboor (2014) showed that in blood group O, the limitations of this method are caused by low antigen concentrations, such as vWF Ag (von Willebrand factor antigen) and FVIII Ag (Factor VIII antigen) levels, which play a role in hemostatic function.[16] The low levels of vWF Ag and FVIII Ag in body fluids, which are influenced by genetic factors, make it difficult to detect secretor status in blood group O. The results of this study make it clear that each blood group has a different antigen distribution pattern, so it is important to understand the biochemical characteristics of each blood group in order to improve the effectiveness of saliva as a non-invasive method.[17]

Table 4 shows that blood type examination using the saliva method has a good level of accuracy, similar to that of the conventional method. Blood types A, B, and AB in secretor individuals had 100% conformity with conventional method blood type data, while blood type O had 0% conformity with conventional method blood type data because all blood type O samples were non-secretors that were not detected using the saliva method. The results of this study differ from those of the study conducted by Rajawat et al. (2023) in India, in which, out of 300 samples, the majority of those with secretor status were blood types A and AB, with accuracy levels of 87.14% and 94.28%, respectively. Blood type B had an accuracy level of 73.3%, and blood type O had an accuracy level of 85.04%. Likewise, the study conducted by Lakade (2018) in India showed that all samples were secretors (100%), and the ABO blood type determined from dried saliva samples showed a 100% correlation with the blood type determined from the extraction socket blood.[9,13]

The results of this study were influenced by the absence of antigens A and B in body fluids, including saliva. Most individuals with blood type O have 0% compatibility because blood type O does not have antigen A or B on the surface of red blood cells, so they cannot be secretors of antigen A or B.[18] In addition, secretor status is also associated with various medical conditions, such as ankylosing spondylitis (inflammation of the spine), gastric ulcers, ovarian cysts, and several types of cancer, including squamous cell carcinoma.[9,19]

The results of the statistical test using Fisher's exact test as an alternative to the chi-square test obtained a p-value of 0.000 ($p<0.05$), which means there is a significant level of accuracy between blood type testing through saliva and blood type data in students of the Faculty of Dentistry, Baiturrahmah University, class of 2021. These results are in line with the study by Rajawat et al. (2023) in India, which showed a significant relationship between ABO

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blood type, Rh factor, and secretor status. These findings confirm that blood type testing using saliva has good potential to detect blood type accurately, especially in secretor individuals.[13,20]

CONCLUSION

Blood type examination using saliva has an accuracy level as good as the conventional method. Blood types A, B, and AB have 100% compatibility in secretor individuals, while blood type O is not detected by the saliva method because there are no antigens A and B in the saliva. Statistical tests showed a significant level of accuracy between blood type examination through saliva and blood type in the data.

Further research is recommended to develop a more sensitive detection method for blood type O, for example, by using anti-H reagents, considering the limitations of saliva as a non-invasive method for detecting the secretor status of this blood type. In addition, studies with a wider population are needed to understand the prevalence and characteristics of secretors and non-secretors, because determining ABO blood type through saliva in individuals, this method has limitations and can only be examined using conventional methods. Examination using conventional methods is also recommended to increase the accuracy of the available blood type data.

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