ORIGINAL ARTICLE

A comparison of absorption inhibition and absorption elution methods for estimation of ABO blood groups in saliva

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Abstract

Background: ABO blood groups are the primary, most common, conspicuous, and easily detectable groups. These blood group specific antigens are not the exclusive domain of the erythrocyte, but abundantly present in many other bodily secretions such as sweat, semen and even saliva. Absorption inhibition and absorption elution are the main methods to detect salivary blood group antigens. The aim of this study is to detect the secretor status of 100 samples of saliva and to compare the diagnostic efficacy of absorption elution method and absorption inhibition methods in detecting the ABO (H) blood group antigens in secretors.

Materials and Methods: Totally 100 un-stimulated whole saliva samples were analyzed for the presence of ABO (H) blood group specific antigens by absorption inhibition and absorption elution methods. Initially, secretor status was ascertained by detecting the H antigen in saliva, and both methods were used to detect the blood groups from saliva and the results were compared with that of blood which were statistically analyzed.

Results: Of the 100 samples, 77 secretors and 23 non-secretors. Using absorption inhibition method 62 out of 77 samples gave results that matched with blood. Using absorption elution 70 out of 77 samples gave matching results. Absorption elution depicted better sensitivity, more true positive matches with blood as well as a higher Kappa coefficient.

Conclusion: Absorption elution gave better results than absorption inhibition.

Introduction

Karl Landsteiner discovered ABO blood groups in blood in early 1900, and it still remains the mainstay of blood group investigations in forensics. The reasons for this are that it is the primary, most common, conspicuous, and easily detectable groups.[1] Later on it was found that these blood group specific antigens were not the exclusive domain of the erythrocyte but abundantly present in many other bodily secretions like sweat, semen and even saliva. In 1930, it was found that individuals could be classified as "secretors" and "non-secretors" according to their ability to secrete ABO blood group antigens in saliva.[2]

About 80% of the population secretes soluble blood group substances with A, B, and O (H) specificities corresponding to each individual's blood type; the remaining 20%, the "non-secretors," do not.[3]

The secretor status is determined by detecting the presence of the H antigen in saliva. In secretors, the H antigen can be detected from saliva while in non-secretors H antigen will be absent in saliva. The advantages of detecting the blood groups from saliva, as well as the secretor status, are plentiful.

In addition to ABO blood group applications in blood transfusion and forensic medicine, numerous studies have found strong relations between individuals' susceptibility to some diseases and their ABO blood groups[4] as well as their secretor status.[5] Recent studies suggest that blood group antigen diversity may provide a mechanism of the pathogen evasion whereby distinct ABO (H) antigen structures may reduce pathogen attachment and therefore infectivity.[6]

Absorption inhibition method was the first devised method to detect the blood group substances from body fluids. Absorption elution was later created.

Many studies have detected the presence of ABO blood groups in saliva by using both of the methods. In the present study, an attempt was made to determine the better method to detect the blood group antigens from saliva of the secretors.

Materials and Methods

Un-stimulated while saliva was collected from 100 diseases free individuals in autoclaved collection bottles. Saliva was transferred from the collection bottles to sterile test tubes and kept in boiling hot water bath to denature both salivary as well as the bacterial enzymes.

The saliva was initially assessed for the secretor status by detecting the H antigen in saliva using the anti H antibody. All the secretors and non-secretors were segregated on the basis of the presence or absence of the H antigen in saliva. The blood group specific antigens are present only in the secretors. They are absent in the non-secretors.

All the samples of secretors were subjected to absorption inhibition and absorption elution method. And the results were analyzed statistically.

ABO blood group determination from blood

Permission was obtained from the institutional ethical committee, and informed consent was taken from the patient to participate in the study. The capillary blood was drawn from the patient by a prick on the ring finger by a sterile lancet, and ABO blood group of the patient was assessed by slide agglutination method. A drop of blood was placed on two glass slides on to which monoclonal A and B anti-sera were added respectively mixed and viewed for agglutination in a lighted agglutination viewer. The results were recorded accurately and the blood group detected from blood was considered as standard against which the salivary blood was compared.

ABO blood group determination from saliva

Absorption elution

In this method, the saliva was taken in two test tubes and labeled A and B to which anti-sera A and B were added respectively. The test tubes were thoroughly shaken and allowed to incubate for 5 h for adequate antigen-antibody reaction to occur. Following the incubation the excess antibody was removed by cold saline washes that were repeated 5 times. Then the test tubes were heated in a hot water bath maintained at 56° to elute or break the bond between the antigen and the antibodies. A single drop of freshly prepared pooled red blood cells (RBC) of known group was added to the respective test tubes and shaken well and further incubated for 15 min at 37°. Both the test tubes were then centrifuged for 1 min at 2000 rpm. The presence of agglutination was noted microscopically and the presence of agglutination was considered a positive result.

Absorption inhibition

In this method, the denatured saliva after cooling was taken in two test tubes. A and B anti-sera in the dilution of 1:10 was added to each test tubes, and the test tubes were respectively labeled. A singe drop of saliva was added to both the test tubes thoroughly shaken and incubated at 37° for 10 min. After 10 min a single drop of freshly prepared pooled RBC of known group was added to the respective test tubes and shaken well and further incubated for 15 min at 37° and checked for agglutination. In this test, the absence of agglutination was considered a positive result for a particular blood group.

Both the results were compared to that obtained from blood, and the results were statistically analyzed.

Results

Out of the 100 samples there were 77 secretors and 23 non-secretors. All the 77 secretors were subjected to both the tests. Using absorption inhibition method 62 out of 77 samples gave results that matched with blood [Figure 1, Table 1]. Using absorption elution 70 out of 77 samples gave matching results [Figure 2, Table 2]. The sensitivity of absorption inhibition method was 81% while for absorption elution it was 86%. Using kappa coefficient to quantify the measure of agreement absorption inhibition gave 0.77 while absorption elution gave 0.84. Absorption elution detected 6% more cases than absorption inhibition and the sensitivity were 5% more than absorption inhibition.

Discussion

Indirect blood grouping especially from saliva has been critically important in establishing the identity of criminals in many a crime scene. Apart from forensics there are other implications also. Many studies have correlated the secretor status and many systemic illnesses like ankylosing spondylitis, peptic ulcer, ovarian cysts and even squamous cell carcinoma. Even though the lack secretor status as a risk factor has been established for these ailments it still is not a cause for alarm. The blood groups are a result of many years of evolution and probably influence more characteristics in the human body than that is known to literature. The secretion of these antigens into the body fluids widen the scope of their consequences. Hence, the determination of the best method to ascertain the secretor status as well as to detect the secreted antigens attains paramount importance.

In the present study, 100 samples were included, and they were initially checked for the secretor status by detecting the H antigen in saliva. There were 77 secretors and 23 non-secretors in the study group. Out of the 77 secretors using absorption inhibition method there were 62 matches with that of blood (80.5%). The sensitivity was 81% while the measure of agreement was 0.77. B and O groups gave the most number of true positive matches while AB group gave the least true positive matches most probably due to the small number of samples that were in the study [Figure 1, Table 1]. Using absorption elution in 70 cases the blood group established from saliva matched with that of blood (90.9%). Group B gave 100% matching results while AB group gave 66.66% again probably because of the limited sample size [Figure 2, Table 2]. The overall sensitivity for absorption elution was 86% with a measure of agreement of 0.84.

In 1960, Kind conducted a study on ABO grouping on

dried blood smear by using the absorption elution method. He observed that no predetermined dilutions of anti-sera was required, unlike the absorption inhibition method. This was the primary advantage of the method.[7]

In 1963, Outride compared the two techniques and concluded that absorption elution is the method of choice. The reason was that it was much sensitive than absorption inhibition method.[8]

Aye in 1977 conducted a similar study comparing the two methods but on sweat. In this study, too, more positive results were obtained by absorption elution method when compared to absorption inhibition method.[9]

Kaur and Sharma also conducted a similar study to compare absorption elution and inhibition to detect ABO antigens from sweat and concluded that absorption elution was more suitable and sensitive than absorption inhibition method.[10]

Denault *et al*. also stated that among A the many methods available for blood group determination of antigens in dried stains and found that absorption elution is the most widely used and markedly sensitive than absorption inhibition method.[11]

The present study is in agreement with all the studies because better results were obtained in terms of measure of agreement, sensitivity as well as a percentage of true positive matches by using absorption elution method.

The present study attributes the better results obtained from absorption elution method to the removal of predetermined dilution of anti-sera as suggested by Kind. Also due to the presence of repeated saline washes the excess anti-serum responsible for the false results are also eliminated.

In the present study, the secretors were secretors were segregated initially because it's only in the secretors that the blood group antigens are found in saliva. And from the saliva of the secretors both the methods were used to detect the blood group antigens.

If the secretors are not separated initially it will cause incorrect results as the non-secretors will be incorrectly be typed as O group. This arises from the fact that in non-secretors there is an inherent absence of ABO (H) antigens meaning the typed O group denotes the unavailability of the blood group antigens for typing and it does not denote the mere absence of A and B antigens.

This methodology imparts greater validity to the results. If they were not separated, the prevalence of O group would increase as all the non-secretors would be typed as O group.

Conclusion

The present study concludes that to detect ABO blood group antigens from saliva absorption elution method is better suited. Compared to absorption inhibition method it detects more true positive cases, has better sensitivity and shows greater measure of agreement with that of blood. The segregation of secretors and non-secretors also play a significant role in determining the accuracy of the tests. On hindsight, this segregation is a must before detecting secreted blood group antigen from any body fluid.

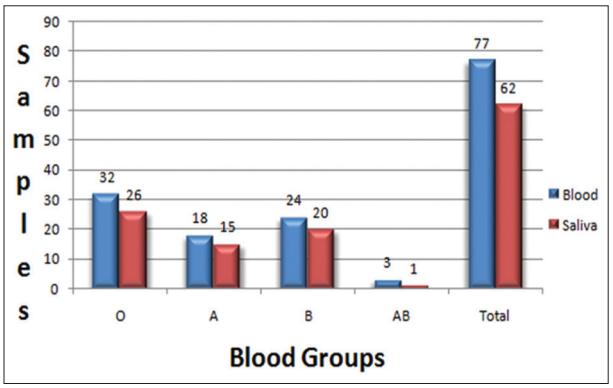

Figure 1: Individual blood group wise and total matches of salivary blood groups with that of blood using absorption inhibition

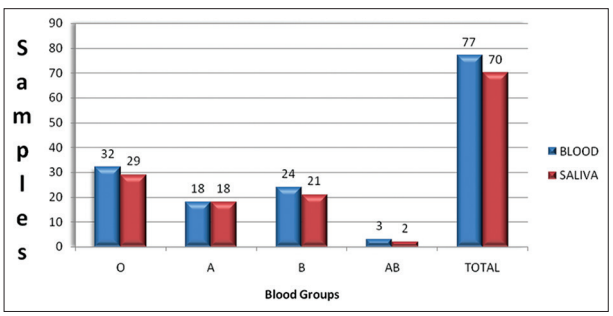

Figure 2: Individual blood group wise and total matches of salivary blood groups with that of blood using absorption elution

Table 1: The percentage of positive matches between blood group in saliva and blood using absorption inhibition

Blood group	From blood	Match in saliva	Percentage
A	32	26	81.25
B	18	15	83.33
AB	3	1	33.33
O	24	20	83.33
Total	77	62	80.51

Table 2: The percentage of positive matches between blood group in saliva and blood using absorption elution

Blood group	From blood	Match in saliva	Percentage
A	32	29	90.62
B	18	18	100
AB	3	2	66.66
O	24	21	87.5
Total	77	70	90.90

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