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ORIGINAL ARTICLE

A Comparison of Absorption Inhibition and Absorption Elution Techniques for Detection of ABO Blood Groups in Saliva in Meitei Population: A Cross Sectional Study

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Abstract :

The ABO blood group system is the significant element for forensic serological examination of blood and body fluids. ABO blood groups are the primary, most common, conspicuous, and easily detectable groups. These blood group specific antigens are abundantly present in many other bodily secretions such as sweat, semen and even saliva. Many studies have detected the presence of ABO blood groups in saliva by using both the absorption inhibition and absorption elution methods. This study of blood group ABO antigens in saliva was carried out using both the techniques in a tertiary care centre in Imphal, Manipur.

Using absorption elution method, out of the 90 secretors subjects, positive results were obtained in 82 (91.1%) subjects and using absorption inhibition method, positive results were detected in 71 (78.9%) subjects. On statistical analysis, the mean age in completed years of the participants is calculated as 22.12 years with a standard deviation of 3.04 years. Further, comparative analysis showed that the positive rate of absorption elution is more than that of absorption inhibition. Therefore, absorption elution method remains an important tool for determination of blood group of an individual from saliva and it is an aid in forensic identification and in solving medico-legal cases.

Keywords : Blood group; Secretor status; Absorption inhibition technique; Absorption elution technique.

Introduction :

Forensic identification by its nature is a multi-disciplinary approach relying on positive identification methodology as well as presumptive or exclusionary methodologies. This is a branch, which deals with identification and has many maxims, the best known of which, is that every contact leaves its trace. Typically, this effect involves the cooperation and co-ordination of law enforcement officials, forensic medicine experts, serologists and criminologists.[1]

The purpose of human identification is one of the major fields of study and research in forensic science. While DNA profiling has become the principal technique for individualization of biological evidences, ABO blood grouping is still a useful test method in the identification process and of crime investigation.[2]

The ABO blood group system was first described by Karl Landsteiner in 1900 and is the primary, most common, conspicuous, and easily detectable group.[3] The ABO blood group antigens were later found to be present not only in the erythrocytes but they are in abundance in many other bodily secretions including the saliva. In 1930, Putkonen[4] noted that individuals could be classified as "secretors" and "non-secretors" according to their genetic ability to secrete ABO blood group antigens in secretions. The advantages of detecting the blood groups from saliva, as well as the secretors status, are plentiful.[5]

Absorption inhibition method was the first devised method to detect the blood group substances from body fluids. Absorption elution was later devised by Siracusa in 1923 and has been refined by Kind.[6]

Many studies have detected the presence of ABO blood groups in saliva by using both the methods, but there is no known study being done in the Meitei population. In the present study, an attempt was made to compare between the two techniques and to find out which one is more sensitive for detection of ABO blood groups in saliva of Meitei population in the state of Manipur.

Material and Methods :

This cross-sectional study was conducted in the Department of Forensic Medicine and Toxicology, Regional Institute of Medical Sciences Imphal on the subjects brought for medicolegal examination, during the period from September 2019 to August 2021, after obtaining approval of the research ethics board of the institute. Taking Prevalence, p = 87.50 (from the study conducted by Kaur G and Sharma VK[7]) sample size is calculated using the formula: $n = \frac{4pq}{L^2}$ Where, n: sample size, p: Prevalence = 87.50, q: 100-p = 12.5, L: absolute allowable error = 7%. The calculated sample size was rounded to 90. The sampling method was a consecutive sampling technique up to a total of 90 individuals.

Procedures and Data collection:

To maintain confidentiality of the participants, coding was used

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for collection of data. For the collection of saliva sample, each participant was asked to rinse their mouth with water and then 4 - 5 ml of whole unstimulated saliva was collected from the individual in a sterile container. The collected saliva was then transferred from the collection bottles to sterile test tubes using pipette, which was kept in boiling hot water bath for 10 -15 minutes for denaturing 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. The presence of agglutination was noted microscopically and the presence of agglutination indicates a positive result. The samples of all the secretors were subjected to absorption inhibition and absorption elution method.

ABO blood group determination from blood:

For determination of participant blood group, capillary blood was taken from left ring finger under aseptic precautions by using a sterile lancet, and ABO blood group of the participant was assessed by slide agglutination method. The results were recorded accordingly and the blood group detected from blood was considered as standard for comparison with saliva findings.

Absorption elution technique:

In this method, 2-3 drops of saliva were taken in two test tubes and labeled as A and B to which anti-sera A and B were added respectively. The test tubes were shaken thoroughly and then incubated for 5 hours for adequate antigen-antibody reaction to occur. Following the incubation, the excess antibody was removed by cold saline washes for 5 times. Then the test tubes were heated in a hot water bath maintained at 56°C for 5-10 minutes 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 were added to the respective test tubes and shaken well. The test tubes were further incubated for 15 minutes at 37°C and then they were centrifuge for 1 minute at 2000 rpm. The presence of agglutination was noted microscopically and the presence of agglutination indicates a positive result.

Absorption inhibition technique:

In this method, the denatured saliva (2-3 drops) after cooling at room temperature for about 4-5 minutes was taken in two test tubes which were labeled as A and B respectively. Anti-sera A and B in the dilution (with normal saline) of 1:10 were added to each test tube. Further, a single drop of saliva was added to both the test tubes and was shaken thoroughly and incubated at 37°C for 10 minutes. After 10 minutes a single drop of freshly prepared pooled RBC of known group was added to the respective test tubes and again after shaking well, it was further incubated for 15 minutes at 37°C and checked for agglutination. In this test, the absence of agglutination was considered a positive result for a particular blood group.

The results were then analyzed statistically.

Results :

In the present study, the secretor status of 178 participants male and female was analysed base on the presence or absence of the H antigen in the saliva, from which the first 90 secretors segregated were selected and their blood groups from saliva were studied using absorption elution and absorption inhibition techniques. Out of the 90 secretor subjects selected, 48 (53.33%) were males and 42 (46.67%) were females. The age distribution of the 90 selected secretor subjects ranged from 18 years to 32 years. On statistical analysis, the mean age in completed years of the

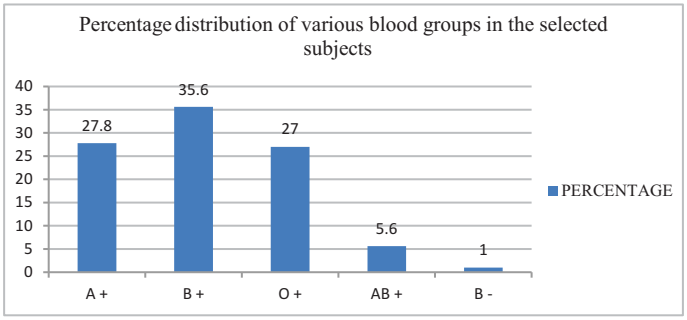

Figure -1: Percentage blood group distribution.

Table 1 : Distribution of various blood groups detected from saliva based on absorption elution technique.

Blood group	Absorption Elution				Total	
	Test positive	%	Test negative	%	subjects	%
'A' positive	21	23.33	4	4.44	25	27.8
'B' positive	30	33.33	2	2.22	32	35.6
'O' positive	26	28.89	1	1.11	27	30
'AB' positive	4	4.44	1	1.11	5	5.6
'B' negative	1	1.11	0	0	1	1
Total	82	91.11	8	8.89	90	100

Table 2 : Distribution of various blood groups detected from saliva based on absorption inhibition technique.

Blood group	Absorption Inhibition				Total	
	Test positive	%	Test negative	%	subjects	%
'A' positive	19	21.1	6	6.7	25	27.8
'B' positive	27	30	5	5.6	32	35.6
'O' positive	21	23.3	6	6.7	27	30
'AB' positive	4	4.4	1	1.1	5	5.6
'B' negative	0	0	1	1.1	1	1
Total	71	78.8	8	21.2	90	100

Table 3 : Comparison of blood grouping through absorption elution and absorption inhibition.

Absorption Elution	Absorption inhibition		'p'value
	Positive	Negative	
Positive	66	16	.027
Negative	5	3	

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participants is calculated as 22.12 years with a standard deviation of 3.04 years.

Blood group Distribution:

In the present study, out of the 90 subjects, the predominant blood group detected was 'B' positive (35.6%) followed by 'O' positive (30%), 'A' positive (27.8%) and 'AB' positive (5.6%) respectively. The least dominant blood group detected was 'B' negative (1.1%) as shown in Fig-1. Further the other blood groups like A - ve, AB - ve, O - ve were not detected in any of the 90 subjects.

Distribution of blood groups detected from saliva based on absorption elution technique:

In this study using absorption elution technique, out of the 90 secretor subjects, blood group could be detected from saliva sample in 82 (91.1%) subjects while in 8 (8.9%) subjects it could not be detected. The predominant blood group detected was found to be 'B' positive (33.33%) and the least was 'B' negative (1.11%). Further, blood grouping could not be detected from 'A' positive (4.44%), 'B' positive (2.22%), 'O' positive (1.11%) and 'AB' positive (1.11%) as shown in Table -1.

Distribution of blood groups detected from saliva based on absorption inhibition technique:

In this study, using absorption inhibition technique, out of the 90 secretor subjects, blood group could be detected from saliva sample in only 71 (78.9%) subjects while in 19 (21.1%) subjects it could not be detected. The predominant blood group detected was found to be 'B' positive (30%) and the least was 'AB' positive (4.4%). Further, blood grouping could not be detected from 'A' positive (6.7%), 'B' positive (5.6%), 'O' positive (6.7%), 'AB' positive (1.1%) and 'B' negative (1.1%) as shown in Table-2.

On comparison of positivity of the two techniques, the absorption elution is found to be more sensitive than absorption inhibition technique. The 'p' value obtained is 0.27 and it is statistically significant as shown in Table-3.

Discussion:

Although ABO and Rhesus factor in serum is the gold standard method, in some situations blood may be absent at the scene.[8] In such circumstances indirect blood grouping especially from saliva has been critically important in identification of a person, especially from the evidences recovered at the crime scene.

Out of the 90 selected subjects 53.3% were males and 46.7% were females which are similar with the study of Harshada R[9] which shows that the ability to secrete ABO blood group antigens in saliva was more common in males than in females.

In the present study the predominant blood group was 'B' positive (35.6%), followed by 'O' positive (30%), 'A' positive (27.8%), 'AB' positive (5.6%) and least detected group was 'B' negative (1.1%). This is similar with the study done by Saboor M et al.[10] where they evaluate the ABH blood group among 101 healthy adult students and concluded that the frequency was highest in blood group B which was in accordance with our study. According to study conducted by Emeribe AO et al.[11] frequency of secretor was common in blood group O followed by A, B and AB. Their findings are different from our findings.

The sensitivity of absorption elution is calculated as 91.1% and that of absorption inhibition as 78.8%. Further, on comparison of blood grouping through absorption elution and absorption inhibition respectively the 'p' value obtained is .027 which is statistically significant. This shows that absorption elution is much more sensitive and reliable than absorption inhibition. The finding is consistent with the findings of various other researchers like Kind SS.[7]

Conclusion :

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 including saliva. Absorption inhibition and absorption elution are the main methods to detect salivary blood group antigens. It is observed that blood groups can be successfully detected from saliva using absorption elution and absorption inhibition techniques. However, in comparison between the two techniques used, the positivity is more by using absorption elution technique. Hence, Absorption Elution technique may be favourably used for detection of ABO blood group from saliva of Meitei population and can be used as an aid in forensic identification and in solving medico-legal cases.

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