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RESEARCH ARTICLE

Efficacy and Accuracy of ABO Blood Group Determination from Saliva

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ABSTRACT

Objectives: The saliva was first analyzed for the presence of anti-A and anti-B hemagglutinins in 1928. However, it was not been utilized as evidence in criminal investigations because of insufficient techniques available at that time. Hence, the present study was designed to evaluate feasibility of ABO group in medicolegal cases by determining the efficacy and accuracy of ABO blood group determination from saliva and to determine the pattern of antigen secretion in saliva in family members.

Methods: A total of 200 subjects (101 males and 99 females) studied. Out of these, 100 subjects were taken at random (sample I) and 100 subjects were taken from 25 families (sample II). The blood group of each subject was determined from saliva by absorption-inhibition method.

Results: The present study showed that 83% subjects are secretors of antigen in saliva. If the subject was secretor, one of his/her parents was essentially a secretor. If both parents were secretor both the children in the family were secretors.

Conclusion: Results can be utilized for identification of subjects, ruling out identification, determination of inheritance in medicolegal cases.

Keywords: ABO blood group, Absorption-inhibition method.

INTRODUCTION

Among the many biological evidences in the medicolegal cases, blood is one of the important evidence because once the blood group is established it remains unchanged throughout the life.[1] Therefore, blood group is one of the very important factors for identification of an individual or for ruling out the identity of suspected unknown individuals.

In 1900, Landsteiner first described ABO grouping of blood. After that many investigators have described other types of blood groups, i.e. MN, Rh, P, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Yt, Ii, Xg, Dombrock but only the ABO and Rh groups are in practice for clinical and forensic use. Other blood groups are of less importance because the antigens are weak and corresponding antibodies are not normally present and appear only after multiple transfusions.[2]

At present, the most common method for ABO blood typing is from a drop of blood obtained by pricking the finger. Besides blood, the antigens are also secreted in various other body secretions, such as saliva, semen, gastric juice, nasal secretions, vaginal secretions, sweat, tear, urine, etc. from which blood groups can be determined.[1]

However, the presence of blood group substances in various body secretions depend on whether the individual is a secretor or nonsecretor which can be determined by the presence of Lewis antigen.[3]

In some medicolegal cases like rape, robbery and hanging, blood stains may not be found but saliva may be found in some form or the other and which may be for at most important in detecting blood group of a victim or suspected culprit. In such cases, saliva may be obtained in wet and dry form from bite marks, cigarette ends, envelope flaps, dental appliances, toothpicks or other articles contaminated with saliva.

The saliva was first analyzed for the presence of anti-A and anti-B hemagglutinins in 1928.[4] However, it was not been utilized as evidence in criminal investigations because of insufficient techniques available at that time.

In past few years, many modified techniques have been introduced and many investigations have shown up to 100% accuracy in detecting ABO blood groups from saliva.

There are mainly two methods for detecting ABO blood group substances from saliva.[5]

1. Absorption-inhibition method
2. Absorption-elution method.

Thus, the present study was conducted to determine the efficacy and accuracy of the ABO blood group determination from saliva by absorption-inhibition method and to determine

the pattern of antigen secretion from saliva in family members for its feasibility in medicolegal cases.

METHODS

The study was conducted in the Department of Oral Medicine and Radiology, CSMSS Dental College and Hospital, Aurangabad after the approval of ethical committee. Informed consent was obtained from all the subjects who were included in the study.

Double dilution series of anti-A (256), anti-B (512) and anti-H (64) was prepared. One percent suspension of red cells was prepared by adding 10 ml of normal saline in 0.1 ml of washed red blood cells.

METHODS OF COLLECTING THE SALIVA SAMPLES

Fresh Saliva

The individuals were asked to rinse their mouth thoroughly to remove debris and asked to spit in clean and dry test tubes. Quantity of this saliva on an average was about 0.5 ml in each case. The distilled water was added as to make the total volume of solution up to approximately 2 ml referred as 'Stock'.

These test tubes were then kept in boiling water bath for 10 minutes. The supernatant was transferred to other test tube for using in further procedures. The sediment was discarded.

Dried Saliva

In medicolegal cases, usually, saliva is in dried form; therefore, the first step is to determine whether dried stains are of saliva stains or the available stains are due to some other materials. If it is due to saliva stains, there will be positive amylase activity test. Only those samples can be used for determination of blood groups, where saliva is present. For this purpose, the procedure in the study was modified as follows.

A small piece of gauze was given to each individual who was asked to wet it with his/her own saliva. The wet gauze pieces were dried at room temperature. The procedure for testing the presence of the amylase activity was carried out for each dried sample as follows:

1. Each gauze piece was kept in separate test tube containing 3 to 4 ml of distilled water and was kept for overnight. The resultant liquid is labeled as an 'Extract'.
2. From these extract, only 0.5 ml was taken in test tube and 3.5 ml of buffered starch was added.
3. It was kept in the incubator at 37°C for half an hour.
4. Again 0.5 ml extract was added after removal from the incubator.
5. 0.75 ml of sulfuric acid (H_2SO_4) and 0.25 ml of sodium tungstate were added.
6. This solution was centrifuged for 10 minutes.
7. Two milliliter of supernatant was taken in another test tube and 2 ml of copper sulfate ($CuSO_4$) alkaline was added to it.
8. This solution was kept for 10 minutes in boiling water bath.
9. After cooling, 2 ml of phosphomolybdic acid was added. Turning of the color of solution into blue indicates the presence of amylase.
10. If the amylase is positive, the remaining extract was used as fresh saliva. 'Stock' and all further procedures are done accordingly.

The A, B and O blood groups were determined from saliva by using absorption-inhibition method.

OBSERVATIONS

All control samples showed clumping as there was no antigen present. The agglutination was not seen in some cases (Fig. 5) indicating that antigen-antibody reaction had taken between the saliva and antisera, and there was no antibody left for RBC to react, indicating the presence of blood group.

On the contrary, some samples showed agglutination (Figs 1 to 4), either macroscopically or microscopically that indicated there was no antigen present in the saliva which could react (neutralize) the antisera. Therefore, antisera reacted with added RBCs, indicating absence of any blood group in the saliva.

The blood group of each individual was confirmed by the routine method also.

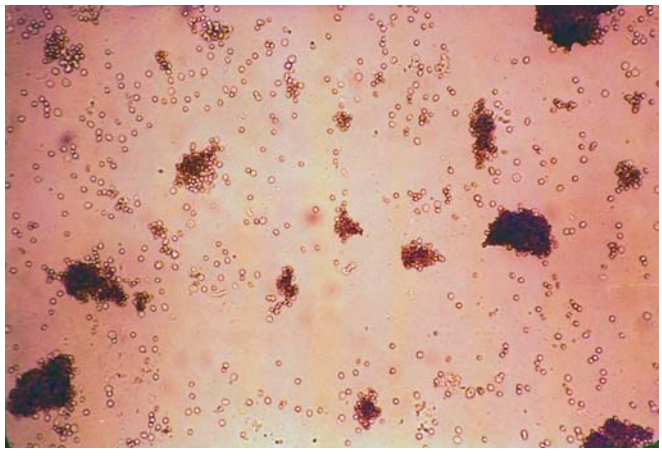

Fig. 1: Photomicrograph showing large masses of agglutinates (3+, visual)

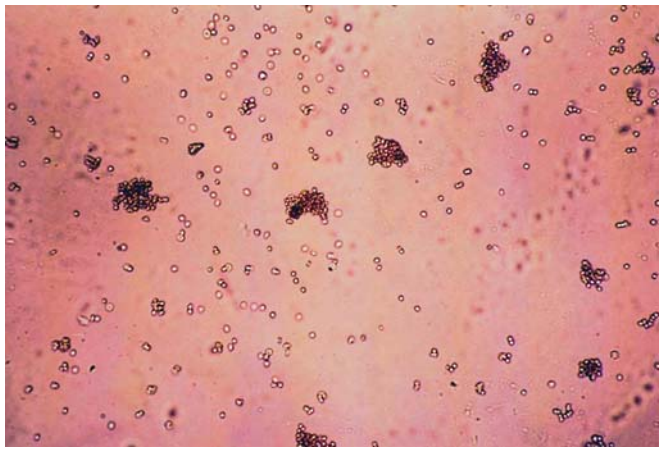

Fig. 2: Photomicrograph showing small masses of agglutinates (2+, double plus)

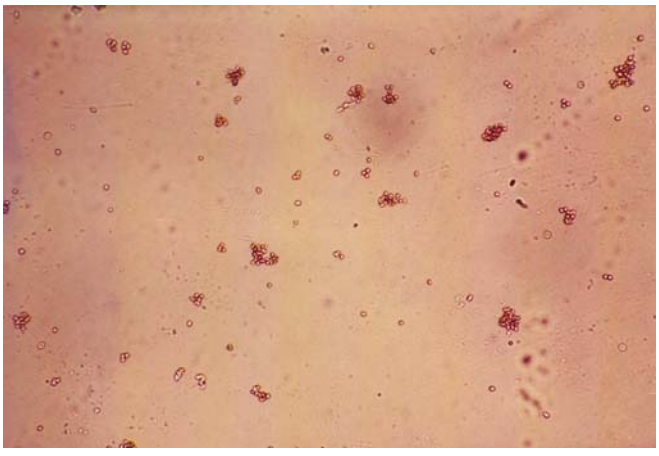

Fig. 3: Photomicrograph showing clumps of more than 20 cells (plus)

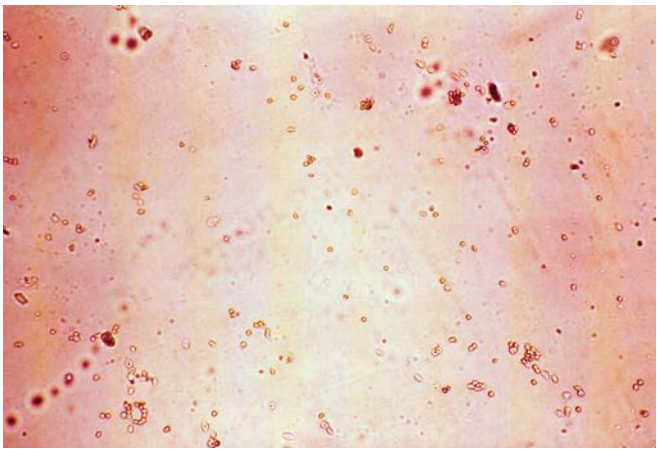

Fig. 4: Photomicrograph showing clumps of 8 to 12 cells (good weak)

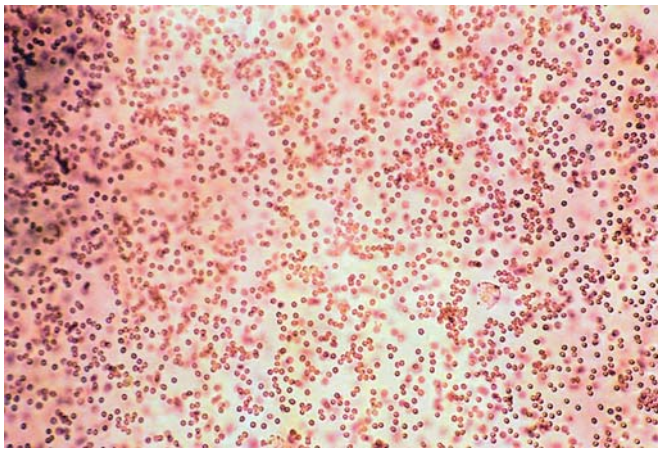

Fig. 5: Photomicrograph showing all free cells (negative)

Determination of Blood Groups

If any sample showed negative response to any antisera, it indicated that the corresponding blood group is present. However, if there was agglutination that means blood group was absent. Determination of blood group was done as shown in Tables 1A and 1B.

RESULTS

In this study, 200 subjects were studied for the determination of blood group from saliva; out of these, 100 subjects were taken at random (sample I) and 100 subjects were taken from 25 families (sample II). Out of 200 subjects, 101 were males and 99 were females ranging from 10 to 60 years of age group.

Blood groups would be determined with 100% accuracy from the saliva of those subjects who were secretors of antigen in the saliva, but the blood group could not be determined from the saliva of those subjects who were nonsecretors. The present study showed that 83% subjects are secretors of antigen in saliva (Table 2).

The sample I showed 82% of antigen secretors in saliva (Table 3). The sample II showed 84% secretors in saliva (Table 4). It was observed that in both the samples as well as in the study, maximum percentage of secretors were in the blood group AB, followed by the blood group 'A', blood group 'O' and minimum percentage was in the blood group 'B'.

Out of 200 subjects, slightly higher percentage of antigen secretors in saliva were observed in females (83.8%) than in males (81%) (Table 5). Sample I showed that female saliva secretors were 82% and male saliva secretors were 81% (Table 6). Sample II showed that the saliva secretors in the males were 81% and in the females were 85% (Table 7). It was also observed that in sample II, out of 25 families, in 17 families (where both the parents were secretors) both the children were secretors. In the remaining eight families, where one of the parents was secretor; out of two children in the family only one was secretor. Thus, it was concluded that there was no effect on sex of the secretor parents on the children and irrespective of sex of secretor parent, male or female child could be secretor.

DISCUSSION

The determination of blood groups is useful for identification of the subjects or ruling out the identity or lineage especially in medicolegal cases because blood groups never change.

Finding of 83% secretor in this study is higher than the studies conducted by Pereira M and Martin PD,[6] Dacie JV and Lewis SM,[3] Kimura A, Matsumura F, Sodesaki K and Tsuji T,[7] Parekh P, Sansare K, Malwankar AG and Gore PG.[8]

The results of the present study are similar with study conducted by Emberibe AD and Igwe CA. They studied 176 subjects for the presence of ABH antigen in saliva using absorption-inhibition method and their results showed 84.90% secretors.[9]

Dacie JV and Lewis SM found 80% secretors in white people,[3] Kimura A, Matsumura F, Sodesaki K and Tsuji T determined the ABO blood groups from mixed body fluids including saliva by sandwich method, using monoclonal antibodies. They concluded that 71% subjects were secretors.[7] Parekh P, Sansare K, Malwankar AG, Gore PG studied 100 subjects for the presence of ABH antigen in saliva using absorption-elution method. They concluded that 79% subjects were secretors.[8]

Table 1A: Scoring of results

4 + complete	One complete mass of agglutinates, easily visible on the slide before microscopic examination
3 + visual	Large separate masses of agglutinates, easily visible on slide, very few unagglutinated cells
2 + double plus	Small agglutinates, still easily visible on slide
1 + plus	A granular appearance, just visible on slide before microscopic examination, big clumps more than 20 cells
Half plus	Smaller clumps (12-20 cells), only detectable microscopically
gw: good weak	Clumps or 8-12 cells, only detectable microscopically
w: weak*	Small clumps of 4-6 cells
– : negative	All cells free, evenly distributed

*Weak reactions (w) are the minimum findings that were considered definitely positive

Table 1B: Grouping of saliva by absorption-inhibition method

	Anti-A					*Anti-B*					*Anti-H*					*Blood group*
Dilution	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32	
Series																
Control	Vis	Vg 3+	2+	+	+	4+	3+	2+	g 2+	+	Vis	g 3+	3+	2+	+	– ve
I	–	–	–	–	–	Vis	4+	g 3+	g 2+	g+	4+	Vg 3+	2+	+	w+	A
II	Vis	Vg 3+	g 2+	g+	+	–	–	–	–	–	4+	g 3+	2+	+	w+	B
III	–	–	–	–	–	–	–	–	–	–	4+	Vg 3+	3+	2+	+	AB
IV	Vis	Vg 3+	2+	+	W+	Vis	4+	3+	2 +	+	–	–	–	–	–	O

- Vis: visible clumps, Vg: very good, g: good, w: weak, – : negative agglutination, – ve: negative

Table 2: Number of subjects of different groups as determined from saliva and blood in 200 subjects

Blood groups	*Blood group determined*		*Percentage*
	From saliva	*From blood*	
A	44	49	89.5
B	61	81	75.3
AB	19	20	95.0
O	42	50	84.0
Total	166	200	83.0

Table 3: Number of subjects of different groups as determined from saliva and blood in 100 subjects at random

Blood groups	*Blood group determined*		*Percentage*
	From saliva	*From blood*	
A	22	25	88.0
B	29	37	78.0
AB	09	10	90.0
O	22	28	78.0
Total	82	100	82.0

Table 4: Number of subjects of different groups as determined from saliva and blood in 100 subjects belonging to 25 families

Blood groups	*Blood group determined*		*Percentage*
	From saliva	*From blood*	
A	22	24	91.0
B	32	44	72.0
AB	10	10	100.0
O	20	22	86.0
Total	84	100	84.0

Table 5: Sexwise distribution of total 200 subjects

Blood groups	*Male*			*Female*		
	Saliva	*Blood*	*Percentage*	*Saliva*	*Blood*	*Percentage*
A	17	20	85.0	27	29	93.0
B	36	47	76.0	25	34	73.0
AB	11	11	100.0	08	09	88.0
O	18	23	78.0	23	27	85.0
Total	82	101	81.0	83	99	83.8

Table 6: Sexwise distribution of total 100 subjects at random

Blood groups	*Male*			*Female*		
	Saliva	*Blood*	*Percentage*	*Saliva*	*Blood*	*Percentage*
A	08	10	80.0	14	15	93.0
B	16	20	80.0	13	17	76.0
AB	05	05	100.0	04	05	80.0
O	10	13	76.0	12	15	80.0
Total	39	48	81.0	43	52	82.0

Table 7: Sexwise distribution of total 100 subjects in 25 families

Blood groups	*Male*			*Female*		
	Saliva	*Blood*	*Percentage*	*Saliva*	*Blood*	*Percentage*
A	09	10	90.0	13	14	92.0
B	20	27	74.0	12	17	70.0
AB	06	06	100.0	04	04	100.0
O	09	10	80.0	11	12	91.0
Total	44	53	81.0	40	47	85.0

The present study showed higher percentage of secretors than other studies that used different methods for determination of secretors. It was concluded that absorption-inhibition method is better method for determination of secretors and more number of Indians are secretors of antigen in saliva as compared with other races. This study may be useful in resolving the claims of parentage in estate or immigration or kidnapped children, disputed paternity and for identification of mass disasters.

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