Study of Secretory Status of ABO Blood Groups Antigens and Rh Typing in Dried Salivary Samples of Normal Individuals

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Abstract

***Background and aim**: saliva is a biological fluid that not only has valuable function in oral cavity but also important in forensic medicine for determining blood group in many crime scenes. Saliva in crime scenes mostly obtained in dried form and there are studies that show secretory status of blood group antigens in dried saliva. In this study also the aim is to observe the secretory status of ABO blood group and Rh typing in dried saliva.*

***Material and method**: This cross-sectional study was conducted in Biochemistry Department of Kabul University of Medical Science. Salivary and blood samples were obtained from 400 normal subject with 42.5% female and 57.5% male who were registered in blood donation centers of Kabul. For Blood grouping agglutination method was performed by commercial antisera. Agglutination inhibition method was used for blood grouping and Rh typing in dried salivary samples. For analyzing relation of secretory status of antigens and Rh typing chi square test was performed.*

***Results**: in this study 170 (42.5%) female and 230 (57.5%) male were participated. The mean age was 37 years with ranges between 20 and 65 years. Totally 70.25% were secretors. the highest percentage of secretors were in blood group A. Percentage of Rh factor secretors (44.8%) compared to nonsecretors was significantly low . In this study relation of gender, age with secretory status of antigen A, B and Rh typing were not statistically significant.*

***Conclusion**: Most of the secretors were belonged to blood group A. Secretory status is independent of age and gender. Result of Rh typing in saliva was not significant positive.*

***Key words**: saliva, ABO blood group, Secretor, nonsecretor, agglutination-inhibition method*

Introduction

Biochemical analysis of different body fluid in the field of forensic medicine has its significant value for different purposes and widely used for interpretation of different concepts. Blood is one of the biological fluids that used widely in forensic medicine as by blood grouping suspected cases can be investigated [1].

Blood groups are antigenic determinants presents on the surface of the RBC, these antigens not only present in blood but also secreted in urine, saliva, tears, nasal secretions, gastric juice and etc. the ABO Blood grouping was described in 1900 and later other types of blood grouping was recognized but in practice and forensic cases just the ABO and Rh group is important for identification of suspected individuals. However it is only possible when blood can be find in crime scenes. In some cases such as rape, child abuse, robbery and murders blood may be absent but fingerprint, semen, hair samples and saliva may be present, saliva can be obtained in dry or wet form from plastic casings, cigarette ends, bite marks etc. For the first time in 1928 saliva was used for determination of antigen A and B. In 1930 it was recognized that there are two groups, secretor and non-secretor for blood antigens that are presents in saliva [1, 2]. The secretor groups are those that beside blood have the antigen in other body fluid such as saliva and the non-secretors are those groups that does not secret the corresponding antigen in other body fluid as saliva [3, 4]. Therefore saliva is not only a protective fluid, important for oral pH maintenance and have antifungal, antiviral system in oral cavity but considering its secretory status of blood antigens it is valuable and significant for blood group determination in crime cases[1]. Mainly there are two methods for determination of related antigens in saliva, the absorption-inhibition method and the absorption elution method [2, 5]. In this study the secretory status of ABO and Rh antigens were considered by absorption- inhibition method.

Material and Method

This is a cross sectional study considered on 400 normal individuals .This study was conducted on Biochemistry Department of Kabul University of Medical sciences (KUMS) on 25 may up to 31 Nov 2019 after approval by KUMS research committee. The study group consists of 170 female and 230 male who were all volunteers with consent, normal individuals that were accepted for blood donation by different blood donation centers of Kabul.

Blood collection and grouping: For blood grouping one milliliter of venous blood was taken by syringe and transferred to EDTA containing tubes. Immediately after collection, blood group and Rh typing was determined by agglutination method using Commercial antisera.

Saliva collection and analysis: A small piece of sterile gauze were given for all individuals to wet it with their saliva after that the wet gauze pieces were collected in a sterile container and kept in room temperature for drying. After that the samples were carried to a tube, diluted with 3 to 4 milliliter distilled water. In order to inactivate the enzymes that might be destroy the blood antigens the saliva samples were kept in boiling water bath for 10 minutes and cooled. After that centrifuged for 5 minutes in 1500rpm for removing debris. Finally it utilized for blood grouping by inhibition agglutination method.

In inhibition agglutination method the antisera was used in dilution of 1:8 and saliva (in this study 100 microliter were used) was mixed with diluted antisera (in this study 100microliter diluted antisera) and kept for 15 to 20 minutes. If the individual were secretor then the blood group antigen in saliva will react with antibodies in the antisera. After 15 to 20 minutes the next step of this method was performed, the blood of the selected sample was added. If there were agglutination of blood with antibodies then it means that the individual was not secretor of antigens in the saliva. Therefore, the presence of a negative result showed the positive secretion status.

Data analysis: Data was analyzed by SPSS version 21 with CI=95% and α=0.05 concerned. Percentage of secretors in each blood group was shown and for finding dependency of secretory status with gender, age and different blood groups, chi square test was performed. All individuals categorized according to age in two groups less and equal to 40 years and more than 40 years and dependency of age were considered with secretory status. In addition, Rh positive samples considered and among them gender, age and blood group relationship were considered with Rh secretor status.

Result

The study group of this research was 400 normal individuals. There were 170 (42.5%) female and 230 (57.5%) male. The mean age was 37 years with ranges between 20 and 65 years. Totally there was 138 (34.5%) blood group A, 116(29%) blood group B, 40 (10%) blood group AB

and 106(26.5%) blood group O individuals. Percentage of gender in different blood groups was shown in Figure 1 that statistically was not significant. Percentage of secretor and non-secretor was show in Figure 2. Number of different blood groups considered with Rh factor was shown in Table1. Of all individuals considered in this study 31 (7.8%) were Rh negative and 369 (92.3%) were Rh positive. Secretory status of A and B antigen in saliva were observed according to gender and chi square test was performed, but there was no relation between gender and secretory status of antigen A and antigen B in saliva (p=0.75)

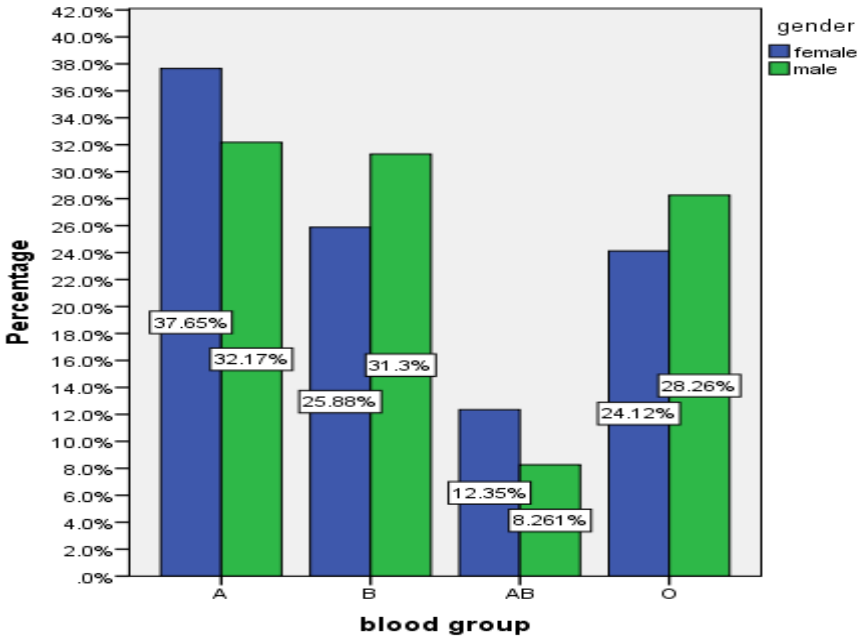

Figure 1. Percentage of different gender in all four blood groups

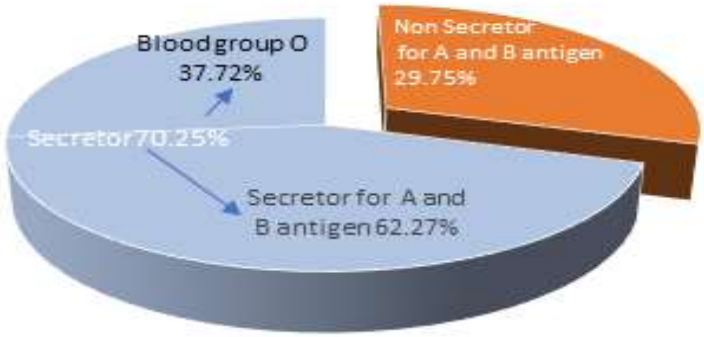

Figure 2. Percentage of secretor and non-secretor of A and B antigen

Non secretors in Age groups more than 40 and 40 years were just 29.7% and in age groups more than 40 years was 29.9% that was statistically not significant so there was no relation between age and secretory status (P>0.05).

Table1. Number and percentage of positive Rh factor in different blood groups

			Rh factor		Total
			positive	Negative	
Blood group	A	Count	124	14	138
		% within blood group	89.9%	10.1%	100.0%
	B	Count	107	9	116
		% within blood group	92.2%	7.8%	100.0%
	AB	Count	39	1	40
		% within blood group	97.5%	2.5%	100.0%
	O	Count	99	7	106
		% within blood group	93.4%	6.6%	100.0%
Total		Count	369	31	400
		% within blood group	92.3%	7.8%	100.0%

It should be mentioned that blood group O shows 100 percent positive secretory status because this group does not have the antigen A and B so relation of secretory status of antigen A and B with blood groups considered in remaining three other blood groups (A, B, AB) , as seen in Table 2 the relation between secretory status and blood groups were statistically significant (p<0.05). The highest percentage of secretors were among blood group A (67.4%) that is 53.1% of all secretors in three groups (A, B, AB) and statistically significant (p>0.05).

In this study the Rh secretory status was also considered according to age and gender. All positive Rh factor individuals (92.3%) were considered for this purpose. The percentage of secretors for Rh were 44.75% and the non-secretor for Rh was 55.25% and this difference was significant (p=0.036), means that Rh activity mostly could not determine by this study. Beside this there was no relationship between secretory status of Rh factor, blood groups, age and gender (p>0.05).

Table 2. Secretory status of A and B antigen in A, B and AB blood group

			Secretory status of A and B antigen		Total
			positive	negative	
Blood group	A	Count	93	45	138
		Expected Count	82.1	55.9	138.0
		% within blood group	67.4%	32.6%	100.0%
		% within secretory status	53.1%	37.8%	46.9%
	B	Count	63	53	116
		Expected Count	69.0	47.0	116.0
		% within blood group	54.3%	45.7%	100.0%
		% within secretory status	36.0%	44.5%	39.5%
	AB	Count	19	21	40
		Expected Count	23.8	16.2	40.0
		% within blood group	47.5%	52.5%	100.0%
		% within secretory status	10.9%	17.6%	13.6%
Total		Count	175	119	294
		Expected Count	175.0	119.0	294.0
		% within blood group	59.5%	40.5%	100.0%
		% within secretory status	100.0%	100.0%	100.0%
P value		0.027			

Discussion

Blood group determination is used for identity of individuals. Blood grouping from saliva has been considered very important in many crime scenes where blood is not present and instead saliva can be obtained in dry or wet form [(1)].

There are many studies that show different results on secretory status of antigens of A and B in saliva. Result of study of Pryiam R Velani et al conducted on 47 subjects was concluded 100 percent secretors [(2)] study conducted by Rashmi Metgut et al shows 83% secretors in 80 subjects [(3)] similarly the result of study conducted by Suha T. Abd in 30 individuals and study of Pawan Motghare which was conducted on 200 subjects were presence of 83% secretors of blood antigen in saliva, in Pawan study mostly females were secretors [(4, 5)]. The study of Pragati Rai et al conducted on 45 controls and 45 oral potentially malignant disorder subjects, 32 controls were observed for secretory status of antigen A and B the remaining O blood group was excluded and among the 32 individuals, 84% were secretor. In this study the result is 70.25% secretors and less than the mentioned studies and not shows any dependency to

gender it may be because of differences in sample size of mentioned studies with current study. This differences may be belong to racial variation among people [4] the similarity of this study with Metgu et al study was detection of high percentage of secretory status in blood group A. it should be mentioned that in Pryiam R Velani et al study the Rh activity in saliva was not well detected, similarly in current study the non-secretory status of Rh was statistically more predominant than secretory status, So observation of Rh activity in saliva is not significantly predominant by the method applied in studies.

Finally it can be assumed that among all groups the highest percentage of secretors are blood group A individuals. Secretory status of blood antigens are not dependent to gender and age.. For more clarification studies should be conducted that the sample size in each category of blood groups should be the same and similarity of gender number should be considered in all groups. Also racial differences should be noticed. For observation of Rh activity in saliva more studies with sensitive methods should be applied.

Conflict of interest: The authors declare that there is no conflict of interest

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