DOI: 10.7860/JCDR/2016/11598.7161

Original Article

Dentistry Section

Evaluation of the Secretor Status of ABO Blood Group Antigens in Saliva among Southern Rajasthan Population Using Absorption Inhibition Method

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ABSTRACT

Introduction: The ABO blood group system was the significant element for forensic serological examination of blood and body fluids in the past before the wide adaptation of DNA typing. A significant proportion of individuals (80%) are secretors, meaning that antigens present in the blood are also found in other body fluids such as saliva. Absorption inhibition is one such method that works by reducing strength of an antiserum based on type and amount of antigen present in the stains.

Aim: To check the efficacy of identifying the blood group antigens in saliva and to know the secretor status using absorption inhibition method among southern Rajasthan population.

Materials and Methods: Blood and saliva samples were collected from 80 individuals comprising 20 individuals in each blood group. The absorption inhibition method was used to determine the blood group antigens in the saliva and then the results were correlated with the blood group of the collected blood sample. The compiled data was statistically analysed using chi-square test.

Results: Blood groups A & O revealed 100% secretor status for both males and females. While blood groups B and AB revealed 95% secretor status.

Conclusion: Secretor status evaluation of the ABO blood group antigen in saliva using absorption inhibition method can be a useful tool in forensic examination.

Keywords: Body fluid, Blood stain, Forensic identification, Haemagglutination, Saliva analysis

INTRODUCTION

Identification in the field of forensic science has evolved over a period of time to currently into an art of science which involves various specialties. Role of medical health care provider as forensic pathologist/odontologist goes all together with the police investigators in establishing the identity of an individual in conditions like mass disasters [1]. The purpose of human identification is one of the major fields of study and research in forensic science as it deals with the human body remains. The basic unit of cell, DNA was established in 1953 by Watson and Crick (1953), who discovered the double-helix structure of DNA, (responsible for the genetic inheritance of human race) this later led to important evolution in every field of science. This discovery was the base of the development of methods that showed characteristics of each person like their individuality or identification specifically based on the DNA sequence [2].

While DNA profiling has become the principal technique for individualization of biological evidences, ABO blood grouping is still a useful test method in the initial stages of crime investigation [3].

The term blood group is applied to inherited antigens detected on red cell surface by specific antibodies. Once the human body establishes the blood group it remains same throughout the life and this formed the basis of the use of blood group substance in medico-legal examination [4]. The ABO system consists of antigens found on the outer surface of red cells and corresponding antibodies in serum. A significant proportion of individuals are secretors which means that the antigens which are present in their blood will also be found in other body fluids such as saliva. Two options prevail in the molecular basis of the secretor system. An individual can be a Secretor (Se) or a Non-secretor (se) which is completely independent of whether the individual is of blood type A, B, AB, or O, suggesting that someone can be an A secretor or an A non-secretor, a B secretor or a B non-secretor [5].

In simple terms, a person is said to be a secretor if he or she secretes their blood type antigens into their body fluids like the saliva, the mucus, whereas on the other hand, a Non-secretor does not put or if so at all very little of their blood type antigens into these fluids [5].

Although anti A and anti B haem-agglutinins in saliva were analysed in 1928, it was however not utilized as evidence in the medico legal cases due to insufficient techniques available during those days. Later, a lot of modifications were done in the techniques to determine 100% accuracy in determining blood group antigens in body fluids. The two tests used to type blood and body fluids for ABO and other blood group systems are Absorption Inhibition and Absorption Elution methods. Absorption inhibition method was developed in 1923 in Italy by Vitorio Sieacusa [6]. Besides blood these antigens are secreted in various other body secretions from which blood group can be determined. This method works by reducing the strength of antigens present in the stains [5]. In the present study an attempt has been made to determine the blood group antigens in saliva and to check the efficacy of absorption inhibition method to do the same.

MATERIALS AND METHODS

A cross-sectional study was carried out in the year 2013 (May to July) among the randomly selected 80 systemically healthy individuals, comprising 20 each of blood group A, B, AB and O reporting to the Haematology Department of the Pacific Dental College And Hospital, Udaipur, aged between 17-70-Years-Old, who volunteered to participate in this study. There were no criteria set for this study as patients chosen were selected on random basis with volunteering enforcement by patient themselves. The study protocol was reviewed and approved by the Institutional Ethical Committee. The nature of the study was explained and a written consent obtained from the subjects.

Study group consisted of 20 subjects in each blood group (A, B, AB and O), whose venous blood was drawn from the cephalic

vein of right arm, centrifuged for five minutes to collect pure form of indicator erythrocytes and at the same time 4 -5 ml of whole unstimulated saliva was collected in a test tube by bending their heads for five minutes and directly collecting the saliva into the container. Blood grouping was done for the collected blood by ABO blood typing and estimation of salivary blood group antigens was done by standard Absorption Inhibition Method.

Procedure for Absorption Inhibition Method

Test tubes with saliva were placed in boiling water bath for 10 minutes and allowed to cool. Cooled test tubes were centrifuged for 10 minutes at 3000 rpm. After discarding the supernatant, clear saliva was collected using pipette. For control test tube we added one drop of saline in each.

Four test tubes were taken with 2 each labeled as TEST and CONTROL. Control tubes were taken to ensure that the antisera were not diluted beyond its capacity to agglutination. Stock agglutinating reagent was carefully adjusted to 1:8 titers and one drop of diluted antisera was added to each tube respectively. To every TEST tube, one drop of clear saliva and to CONTROL tube one drop of saline were added, later mixed, and finally incubated at room temperature for minimum of 10 minutes. Then one drop of appropriate indicator erythrocytes was added to each tube, mixed and incubated at room temperature for 10 minutes. For saline reaction in the CONTROL, tubes were centrifuged for 10 minutes [6-9].

Reaction for agglutination was recorded. Negative reaction test were re-evaluated using same procedure, and if negative again it was considered as negative.

All control samples showed clumping as there was no antigen present. The test group was considered as positive if agglutination was not seen, indicating that antigen-antibody reaction had taken place between saliva and antisera, and there was no antibody left for RBCs to react, indicating the presence of blood group and vice versa was applied for negative test group samples.

RESULTS

Out of 80 subjects 59 were males and 21 females [Table/Fig-1]. A slightly higher percentage of secretor status was observed in females (100%) than in males (90%). Groups A and O revealed 100% secretor status for both males and females, while groups B and AB revealed 95% secretor status [Table/Fig-2].

DISCUSSION

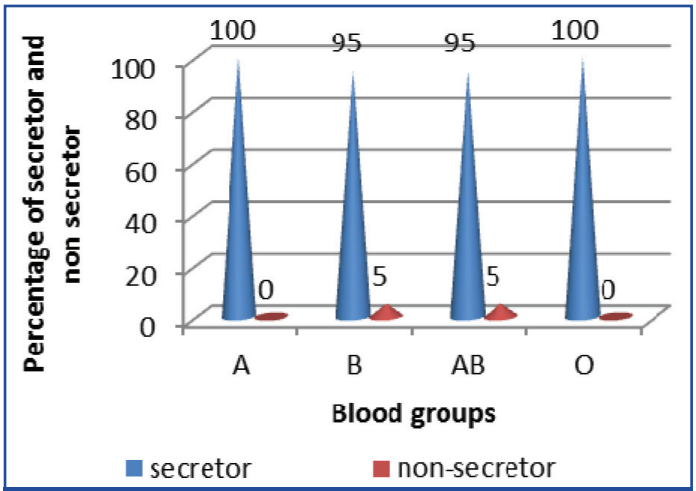
After the advent of blood group system by Karl Landsteiner in 1900, the ABO blood group and Rh system distributions have shown marked variation around the world and also some variation in different areas within the same country. It has been reported by various studies that the O blood type being common in American, Canadian and Saudi population, the B type in Chinese and Indian individuals, and the A type in Eskimos [8,10,11].

The source of blood group antigens is the water soluble substances that are present in most body fluids and organs of a secretor. Yamakami discovered the presence of A and B antigens in saliva [8]. This gave way to note in 1930 by Lehrs and Putko that secretors or non secretors existed for these antigens as two distinct groups [8]. With the concept once a blood group is established in an individual it remains unchanged throughout his life, the use of blood group substances is based in medico legal examination. Blood group would be determined with 100% accuracy from the saliva of those subjects who were secretors of antigens in their saliva [4].

Finding of 99% secretor in this study is higher than the studies conducted by Motghare P et al., who conducted a study in Maharashtra population to determine blood group from saliva and to know the secretary status [9]. Here they studied 200 subjects (101 males and 99 females), 100 randomly selected and 100 taken

Blood groups	Total number of study subjects	Males	Females
A	20	15	5
B	20	15	5
AB	20	14	6
O	20	15	5
Total	80	59	21

[Table/Fig-1]: Gender distribution of study subjects among each blood Group.



[Table/Fig-2]: Percentage distribution of the secretors and non secretors among different blood groups.

Author name	Sample used	Method used	Result
Pawan motghare et al., (2011) [9]	saliva	Absorption inhibition method	Out of 200 samples 83% were secretors. Among females 83.8% showed secretor antigen and 81% in males.
Guriender kaur et al., (1988) [12]	saliva and sweat stain	Inhibition and Elusion method.	87.5% were secretors in saliva. 21.94% in sweat with inhibition and 95.8% with elution method.
M.H Graves (1978) [13]	vaginal sample	Inhibition method and Elusion method	Out of 25 samples 12 were of O group with 9 (75%) secretors and 13 were of A group with 8 (61.5%) secretors.
Robert Thaler et al (1976) [8]	saliva	Haemagglutination Inhibition tests	out of 40 samples 31 were (77.5%) were secretors
Saboor M et al., (2014) [14]	saliva	Haemagglutination Inhibition tests	64.4% were secretors while 35.6% were non-secretors. Blood group B has the highest secretor (79.5%) frequency while Blood group AB has the lowest (45.5%)

[Table/Fig-3]: Studies on secretary status of blood group antigens In various body fluids using different methods.

from 25 families. The saliva was tested using absorption-inhibition method. They found 83% secretors in their study subjects of which 83.8% were female secretors and 81% were male secretors [Table/Fig-3].

Kaur G et al., conducted a study on both saliva and sweat stains and observed that 87.5% were secretor in saliva out of 72 samples tested [12] [Table/Fig-3]. In 1978 MH Graves studied 25 vaginal samples among which 17 (68%) were secretors of blood group antigens [13] [Table/Fig-3]. Out of which 75% were of group O and 61.5% in group A [Table/Fig-3]. These two studies compared Absorption-Inhibition and Absorption-Elution Methods in the detection of ABO (H) antigens in vaginal samples, submitted in sexual offense cases and stated that the Absorption-inhibition is a

well-established method for the identification of ABO (H) antigens [14].

Thaler R et al., studied saliva samples to determine if salivary blood group substances are related to periodontal disease [8]. Forty patients were studied, among which 31 (77.5%) were secretors for blood group antigens and in their study no correlation of the periodontal indices to blood group secretor status was established [Table/Fig-3].

In the most recent study done in 2014 by Saboor M et al., to evaluate the ABH blood group among 101 healthy adult students (76 male, 25 female ranging in age from 15 to 40 years), to know the secretors and non-secretors in people of Karachi, Pakistan using haemagglutination inhibition method of saliva [14]. They concluded that frequency of ABH secretor is high (64.4%). Blood group B has the highest secretor (79.5%) frequency while blood group AB has the lowest (45.5%). This result was correlated with ours.

The use of saliva in forensic science is based on the presence of ABH blood group substances in the saliva of secretors in fairly high concentration. Since saliva may be found on various objects at the scene of a crime, care must be taken that this evidence must not be neglected or mishandled. The findings are always used in a negative way i.e., they can disprove that a blood stain or secretion at a scene of the crime comes from an accused individual. When found at crime scene, the blood substances in secretion and tissues are more complex to identify as compared to blood itself. Even in the fresh state the only antigens that can be identified are A, B, H and these require more complicated techniques. If the samples are deteriorated and present in small amount, the reliability of the tests is still further limited. However, the examination of secretions and tissues may be useful in corresponding findings from blood samples and may be particularly valuable in the absence of blood [4].

However, not much information is available on the detection of ABO blood group antigens from the sweat and saliva stains. But still an association has previously been detected between ABO blood groups, secretor status, and serum and mucosal concentrations of small intestinal alkaline phosphates which show that knowing the secretory status of various population can tell us the systemic condition for the same [10].

The present study revealed higher percentage of secretors than the other studies, where authors used different methods for determination of secretors. From the findings of the study, we conclude that absorption–inhibition method is a more accurate method for identification and determination of secretors [11]. As a dentist though we cannot match the existing evidence to dental records of same person, we can provide important clues to identity which may help the investigators. For instance, age, socioeconomic class, and history can be formulated based on mere examination of the teeth. At last correlating these evidences with those from forensic investigators the identity possibilities can be narrowed down.

CONCLUSION

In the present study 100% secretor status was observed with blood groups A and O for both males and females. The identification of blood group antigens in various body fluids like saliva chiefly, would be a useful tool in forensic examination. Although recent techniques have increased the reliability of the determination of blood group substances, adaption of the more sensitive assays may increase the utilization of these samples. Of particular value would be the development of simpler methods for blood group substances in saliva since the antigens present in the blood are also found in other body fluids as saliva.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Oct 07, 2014**
Date of Peer Review: **Jan 01, 2015**
Date of Acceptance: **Sep 21, 2015**
Date of Publishing: **Feb 01, 2016**