



## EFFICACY OF SALIVA IN DETERMINATION OF ABO BLOOD GROUPS IN HUMANS

### Forensic Sciences

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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.

**Methods:** A total of 200 subjects (100 males and 100 females) were studied. All of them were selected at random visiting to the department of oral medicine and radiology. The blood group of each subject was determined from saliva by absorption-inhibition method.

**Results:** The present study showed that 77% subjects are secretors of antigen in saliva.

**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

Forensic odontology is that branch of dentistry which deals with the law. The last half-century has seen forensic dentistry make tremendous progress globally, both in terms of research as well as application in routine casework.<sup>1</sup>

Forensic odontology primarily involves identification. Identity refers to the characteristics by which a person may be recognized and identification is the establishment of a person's individuality. Accurate identification of the dead is required both for legal and humanitarian reasons.<sup>1</sup>

Among the many biological evidences in the medicolegal cases, blood is one of the most important evidence established because once the blood group is established it remains unchanged throughout the life. The term blood group is applied to inherited antigens detected on red cell surface by specific antibodies.<sup>2</sup>

At the beginning of the 20<sup>th</sup> century an Austrian scientist, Karl Landsteiner, noted that the RBCs of some individuals were agglutinated by the serum from other individuals. He made a note of the patterns of agglutination and showed that blood could be divided into groups. This marked the discovery of the first blood group system, ABO, and earned Landsteiner a Nobel Prize.<sup>3</sup>

Forensic science utilizes the ABO blood groups for suspect exclusion in the analysis of crime scene evidence, such as blood, saliva, seminal fluid, and even hair.<sup>4</sup>

There are certain diseases which show evidence of association with non-secretor status. ABH non-secretors are reported to have a tendency toward higher factor aVIII and vWF with a greater risk for future thrombotic and heart disease. These groups are found to have a significantly higher rate of duodenal ulcer, recurrent urinary tract infection and persistent candida infection. ABH non-secretors are also have an higher prevalence of different varieties of auto-immune diseases including ankylosing spondylitis, reactive arthritis, psoriatic arthropathy, Sjogren's syndrome, multiple sclerosis and Grave's disease. Thus it is evident that the determination of secretor and non-secretor status of ABH substances has the clinical importance.<sup>5</sup>

In some medico-legal cases such as rape, robbery and hanging blood stains may not be found but saliva may be found in some form i.e. in wet form or dry form from bite marks, cigarette ends, envelop flaps,

toothpicks or articles contaminated with saliva which can be used for determination of blood group of the victim or the culprit.<sup>6</sup>

Determination of blood group from saliva may be useful in resolving claims of parentage in estates or immigration or kidnapped children, disputed paternity and for identification of mass disaster.<sup>6</sup>

Saliva was first analyzed for anti-A and anti-B haemagglutinins in 1928. However it was not used in criminal investigation because of insufficient techniques. In past few years, many modified techniques have been introduced for detecting blood group from saliva.

Two commonly used method for detecting ABO blood group is.<sup>8</sup>

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

Hence the present study is taken up to determine the ABO blood group from saliva in humans using absorption inhibition method and to determine its feasibility in medicolegal cases.

### Materials and methods

A total of 200 subjects (100 males and 100 females) were included in the study. All the subjects were selected at random from patients coming to the Department of Oral Medicine and Radiology, Divya Jyoti College of Dental Sciences and Research after signing the informed consent and after clearance by the ethical committee of the college.

Processing of blood for blood grouping was carried out in the Lalpath Labs. Processing of saliva sample was done in collaboration with the Blood Bank, Divya Jyoti College of Medical Sciences And Research, Modinagar (Uttar Pradesh.)

The patients was asked to wash their mouths with tap water and to spit two or three times, after which they were told to spit the saliva (unstimulated) pooled in their mouths in a sterile container by asking the patient to expectorate into it in clean and dry test tubes gradually over a period of 5-10 minutes. Quantity of this saliva on an average of about 0.5 ml was collected. Then 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.

Under aseptic conditions using a sterile disposable 25 gauge needle, intravenous blood, 2ml was collected from the median vein in EDTA containing test tube ( especially provided by Lal Path Lab) for estimation of blood group.

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.

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 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 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.

**Results**

The experimental group consisted of 200 subjects (100 males and 100 females) with an age range of 18-60 years. On compiling the overall results of the study, the subjects were divided into four blood groups namely A group, B group, AB group and O group. Blood group from blood was successfully determined for all the 200 subjects but the results from saliva varied.

Based on the analysis of the Blood Group from blood, 39 subjects (19.5%) of the subjects having Blood Group A, 92(46%) were having Blood Group B, 18 (9.0%) were having Blood Group AB and rest 51 (25.5%) were having Blood Group O.

Based on Salivary Analysis, in the Blood Group A Subjects (n=39), only 34 subjects could be diagnosed with Blood Group A with percentage of 88.0%. In the subjects with Blood Group B (n=92), the salivary diagnosis was made for the 61 (66.0%) of the subjects. In the subjects with Blood Group AB (n=18), the salivary diagnosis was confirmed for the 83% (n=15) of the subjects. The salivary diagnosis of the Blood Group O could be made for 44 (86%) of the subjects out of the total of 51 subjects Thus from total of 200 subjects, blood group from saliva was successfully determined for 154 subjects which accounted for 77% as secretors of antigen in saliva. (Table 1A, Table 1B, Bar Diagram 1)

The difference between the groups for the different Blood Groups when analyzed using the Chi Square test was found to be statistically significant.

Out of 200 subjects, slightly higher percentages of antigen secretors in saliva were observed in females (78%) than in males (76%). On statistical analysis using chi square test the value came out to be 0.113 and P value was 0.867 which was non significant which means antigen secretor status has no effect on sex. (Table 2, 3, and 4 and Bar Diagram 2)

**Discussion**

The field of forensic dentistry or the more professional term, forensic odontology, is the application of dentistry to the law. Forensic dentistry now has been an integral part of the American judicial system for well over three decades. Overall, forensic dentistry includes multiple areas of scientific study, where the legal system and dentistry coincide. This specialized area of dentistry includes the gathering and interpretation of dental and related evidence within the overall field of criminalistics.

The discovery of ABO blood grouping by Land Steiner 1900 opened a new complex field of study with many practical applications. Over the past three quarter of century, information from studies on blood grouping has been applied in medico legal examinations. The use of blood group substances in medico legal examinations is based on the

fact that once a group is established in an individual it remains unchanged throughout his life.<sup>2</sup>

Over the last decade or so there has been a growing interest in saliva and its use as a diagnostic tool as an alternative to blood or urine. Whole saliva is a mixture of secretions released from salivary glands, gingival crevicular fluid and contains exfoliated oral epithelial cells and microorganisms.<sup>10</sup>

The term 'secretor' is used for an individual who secretes ABO blood group antigens in body fluids such as saliva, sweat, tears, gastric juice, semen, urine, etc. The basis for classification was antigenic character present on RBC membrane. Blood group antigens are not only present on RBC membrane, but also secreted in various body fluids like saliva, gastric juice, semen, amniotic fluid, sweat, urine, tears, etc.<sup>11</sup>

The agglutinogens of the ABO system present in the body tissues appear in lipoidal and water soluble forms. In about 80 percent of the people they appear in water soluble form and can be demonstrated in all the body fluids except the cerebrospinal fluid. They are not found in nerve tissues, epithelium, skin appendages, bone and cartilages. A person who possesses only the lipoidal form are known as 'non secretors', while those who possesses a water soluble form are known as 'secretors'.<sup>11</sup>

In Caucasians approximately 80% are secretor and 20% are non-secretor, and this differs from present study. It may be due to racial variation. But in Negroes 60% are secretor and 40% are non-secretor which simulates that in present study. The frequencies of ABO blood grouping reveal that group 'O', 'A', 'B' and 'AB' are 86%, 86%, 66% and 83% respectively.<sup>6</sup>

The results of our study were in accordance with the study of Sonia Guinsberg Saldhna,<sup>12</sup> Kimura A,<sup>13</sup> Emeribe AO,<sup>14</sup> Kim W,<sup>15</sup> Jaff MS,<sup>16</sup> Motgare P,<sup>6</sup> Jelinkova P,<sup>17</sup> S Akhter,<sup>5</sup> Sikandar Khan Sherwani,<sup>18</sup> A. Sylvia Devi.<sup>19</sup>

**Conclusion:**

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.

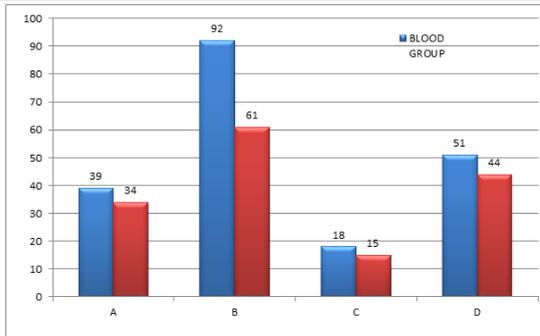
**Table 1A:** Number of subjects of different groups as determined from saliva and blood in 200 subjects

BLOOD GROUP	BLOOD GROUP DETERMINED		PERCENTAGE %
	FROM BLOOD	FROM SALIVA	
A	39	34	88
B	92	61	66
AB	18	15	83
O	51	44	86
TOTAL	200	154	77

**Table 1B- ASSOCIATION BETWEEN BLOOD GROUPS AND SALIVASECRETOR STATUS IN STUDY SUBJECTS**

BLOOD GROUP	Saliva		Chi Square	P value	Significance
	Secretors	Non -secretors			
Blood Group A (39)	34 (87.17%)	5 (12.83%)	11.109	0.011	Significant
Blood Group B (92)	61 (66.30%)	31 (33.70%)			
Blood Group AB (18)	15 (83.33%)	3 (16.67)			
Blood Group O (51)	44 (86.27%)	7 (13.73)			

**BAR DIAGRAM 1 -ASSOCIATION BETWEEN BLOOD GROUPS AND SALIVA SECRETOR STATUS IN STUDY SUBJECTS**



**Table 2: SECRETOR STATUS OF 100 MALE SAMPLES AT RANDOM**

BLOOD GROUP	SECRETORS	NON SECRETORS	TOATAL
A	17	04	21
B	35	16	51
AB	04	00	04
O	20	04	24
TOTAL	76	24	100

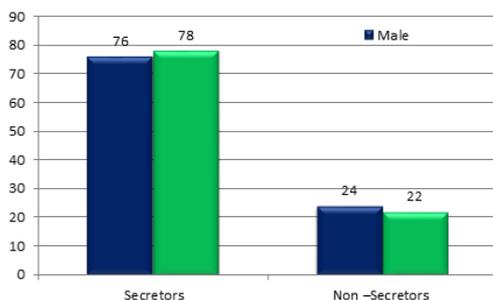
**Table 3: SECRETOR STATUS OF 100 FEMALE SAMPLES AT RANDOM**

BLOOD GROUP	SECRETORS	NON SECRETORS	TOATAL
A	17	01	18
B	26	15	41
AB	11	03	14
O	24	03	27
TOTAL	78	22	100

**Table 4: COMPARISON BETWEEN MALES AND FEMALES FOR THE SALIVARY SECRETOR STATUS**

Salivary status	Males	Females	Chi Square	P value	Significance
Secretors	76	78	0.113	0.867	Non - Significant
Non -Secretors	24	22			

**BAR DIAGRAM 2: COMPARISON BETWEEN MALES AND FEMALES FOR THE SALIVARY SECRETOR STATUS**



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