ORIGINAL RESEARCH

Diagnostic Accuracy of Absorption Elution Assay to Detect Specific Blood Group Antigens in Saliva of Children

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Abstract

Context: Invasive blood typing during a research process induces fear and anxiety in children. Specific blood group antigens, being the exclusive domain of the erythrocyte were also reported in bodily secretions like sweat, semen, and even saliva.

Aims: To determine the sensitivity, specificity, and diagnostic accuracy of absorption elution assay to identify blood group antigens in the saliva of children and to validate its usage in blood grouping and Rhesus (Rh) typing.

Materials and methods: Unstimulated saliva was collected from 60 schoolchildren who have already undergone blood investigations. The samples were subjected to absorption elution assay to screen for the presence of Rh factor and A and B antigens. The data obtained were statistically analyzed using Pearson's Chi-square test.

Results: The sensitivity, specificity, and diagnostic accuracy of absorption elution assay to detect Rh factor in saliva were 85.71, 75, and 84.96%, respectively, for screening blood group antigen A were 80, 97.78, and 93.33%, respectively, and for screening blood group antigen B were 80.95, 97.44, and 91.67%, respectively. About 80% of the findings from the absorption elution assay matched with the medical records with a statistically significant difference (p-value < 0.001).

Inference: Sensitivity, specificity, and diagnostic accuracy values obtained indicate that absorption elution assay could be a valid diagnostic method to screen blood group antigens in saliva.

Keywords: Absorption elution method, Blood typing, Saliva, Specific blood group antigens.

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Key Message

Blood group antigen determination could become one of the important diagnostic criteria to predetermine the susceptibility of chronic diseases because of their codominant genetic inheritance. Blood typing through saliva might substitute the current invasive procedures that have a drastic impact on a child's behavior. Absorption elution assay could be a reliable diagnostic method for blood tying in saliva.

Introduction

The blood grouping system has been found with a significant undeniable association with the occurrence of diseases like hemolytic disease of the newborn, blood transfusion reactions, graft rejection, and spontaneous abortion.[1] Anstee has postulated the biological role of blood group antigens in the pathogenesis of infectious diseases[2] and a certain type of blood group granted a selective resistance against infectious disease.[3]

In dentistry, experimental and clinical research has found a significant association between ABO blood groups and various oral diseases like dental caries,[4] periodontal diseases,[5] salivary gland tumors,[6] oral cancer,[7] etc. Codominant inheritance of either A and B antigens or both on the ninth chromosome as genetic components made beneficial to correlate the genetic predisposition of these oral diseases including malocclusion with an individual's blood group.[8] Thus, blood group antigen determination could become one of the important diagnostic criteria to predetermine the susceptibility of chronic oral diseases in humans.

Blood grouping requires an invasive procedure like a needle prick or venipuncture with an inbuilt risk of causing a physical disturbance, discomfort or pain, or psychological disturbance to the children or their parents. The proposed National Ethical Guidelines for Biomedical Research Involving Children imply that invasive procedure like blood sampling has a minor increase in the risk level over minimal.[9]

Hence, a nontherapeutic study involving an invasive procedure requires a strong justification to initiate the research process as no ethical committee would countenance any study. A substitute for the current invasive procedure is required to alleviate the complications of traditional methods that have a drastic impact on a child's behavior.

Specific blood group antigens (A and B) are variably expressed through body fluids (except cerebrospinal fluid), such as saliva, tears, semen, urine, gastric juice, and breast milk in addition to their presence on blood cells and platelets.[1,10,11]

Salivary diagnostics is an emerging field in pediatric dentistry because of its ease of availability and presence of a wide spectrum of

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biomarkers for the assessment of oral and systemic health.[12] Hence the objective of this study was to evaluate the presence of specific blood group antigens in saliva using a noninvasive absorption elution method. A null hypothesis was that there is no difference in ABO blood grouping between salivary samples and medical reports obtained using blood samples.

Materials and Methods

The study was conducted between November 2019 and January 2020. Sixty children between 4 and 9 years of age who have attended the Department of Pedodontics and Preventive Dentistry were randomly evaluated. The eligibility criteria were clearly defined that included (1) Cooperative children with high parent compliance, (2) Children having authenticated medical records enclosed with fundamental blood investigations, (3) Systemically healthy children who are devoid of debilitating diseases and salivary disorders, and (4) Children who are not under any medication. Ethical clearance was obtained from Institutional Review Board for the study protocol. Informed consent was taken from the parents/guardians of selected children and a short case history was recorded.

Saliva Collection

Each child was seated in a relaxed Coachman's position,[13] head tilted down, and mouth open to allow the pooled saliva to drip passively from the lower lip into the dry sterile test tube. Two milliliter of saliva was collected by draining method without any stimulation as stated by Yamuna Priya and Muthu Prathibha[14] and kept in boiling hot water bath to denature salivary as well as the bacterial enzymes as mentioned by Sen et al.[15]

Index Test

All the salivary samples were subjected to the absorption elution method[15] in the following manner. The collected saliva was evenly distributed in three test tubes and labeled as A, B, and D to which three drops of antibodies A, B, and D were added, respectively. The test tubes were shaken thoroughly which aids for the instant antigen-antibody reaction and incubated at 37°C for 5 hours. After incubation, the excess antibodies were removed by repeated saline wash. Then the test tubes were heated in a hot water bath maintained at 56°C to debond the antigen-antibody complex. A drop of freshly prepared 0.5% red cell suspension of known blood group A or B was put into respective test tubes and agitated well (Fig. 1). The test tubes were then incubated at 37°C for 15 minutes to enhance agglutination and then centrifuged at 2000 rpm for 1 minute. The presence of agglutination in a macroscopic view was considered to be a positive observation and its presence was further confirmed microscopically at 40× magnification (Fig. 2). The subject is considered a secretor of agglutinin A if agglutination occurred with the Anti-A test serum, agglutinin B secretor if agglutination occurred with the Anti-B test serum, and secretor of both if agglutination occurred with both test serums. If agglutination occurred with Anti-D sera, the subject was considered to be Rh positive. The outcome was reviewed by a trained observer who was blinded to all patient information including the blood group.

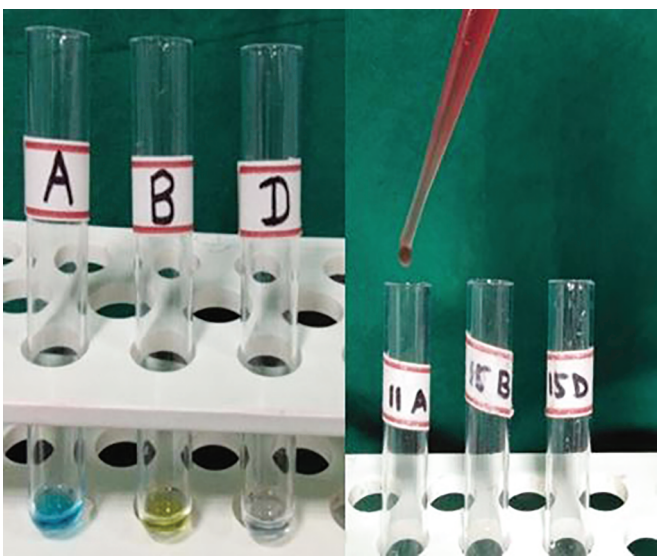

Fig. 1: Addition of RBC to antisera

Reference Standard

The blood groups of selected children were collected retrospectively from authenticated past medical blood reports.

Statistical Analysis

Sensitivity, specificity, predictive value, and diagnostic accuracy were calculated by using MediCalc easy-to-use statistical software. Data were analyzed using Chi-square test.

Demographic data: Among the 60 samples considered in this study, 48 were male, 12 were female, and the mean age was 6.93 years (Table 1 and Flowchart 1).

Results

The screening by absorption elution assay revealed that 93% tested positive for Rh factor, 25% tested positive for agglutinin A, and 35% tested positive for agglutinin B in saliva. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy for absorption elution assay to detect Rh factor in saliva were 85.71, 75, 97.85, 93.27, and 84.96%, respectively. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for screening blood group antigen A were 80, 97.78, 92.31, 93.62, and 93.33%, respectively, whereas, for screening B antigen, the absorption elution method showed 91.67% accuracy with 80.95% sensitivity, 97.44% specificity, PPV of 94.44%, and NPV of 90.48% (Tables 2 to 4).

When blood grouping from the saliva matched with the blood group of the child which was identified from the reference, the

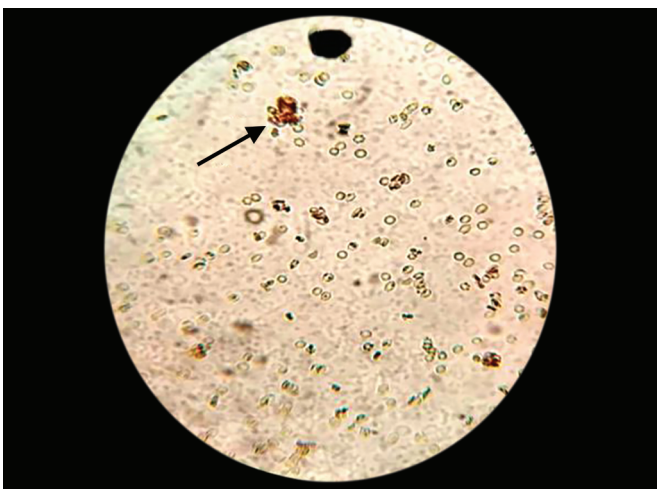

Fig. 2: Microscopic view of agglutination

test result was recorded as "positive" and if they did not match, then it was recorded as "negative." The Chi-square test showed that 80% of the findings from the absorption elution assay matched with the medical records with a statistically significant difference with Pearson's Chi-square value of 200.144 and *p*-value –0.000 ($p < 0.001$) (Fig. 3).

Discussion

The term blood group is applied to the presence of inherited antigens on the red cell surface by specific antibodies. Blood typing advent has revolutionized blood replacement therapy. Apart from forensic,[16] it opens up a new vista for the future study in anthropometric correlation[17] and genetic inheritance[8] of infectious,[5] serological, and immunological disorders[18] in dentistry. Several studies through invasive methods have been carried out to assess the association between blood grouping and the occurrence of oral diseases and found that nonsecretors of ABH antigens are more prone to oral diseases.[4,19]

But drawing blood by finger prick or venipuncture is one of the most fearful, painful, and distressing invasive procedures in children. Smith during his study involving blood sampling procedures in children of 6–8 years age group for nontherapeutic reasons has concluded that the "risk" of invasive procedure for blood typing is minimal in normal children but questionable in unusually anxious or unwilling children.[20] Children with painful experiences in early childhood can often exaggerate negative memories of pain and anxiety at subsequent procedures. Hence, there is a need for a noninvasive substitute for blood grouping either for chair-side therapeutic investigation or nontherapeutic research purposes, thus preventing both physical and psychological distress in children.

Human saliva is easily available, constituting abundant secretion of proteins, diagnostic biomarkers, and agglutinins.[12,13] By 1926, it was evident that the presence of A and B antigens were not confined to red cells, but also present in seminal fluid and saliva in soluble form. In secretors, antigens are detected in the secretions of the goblet cells and mucous glands of the gastrointestinal tract such as saliva, gastric juice, bile, meconium, etc., genitourinary tract secretions such as spermatic fluid, vaginal secretions, ovarian cyst fluid, urine, etc., and respiratory tract secretions as well as in milk, sweat, tears, and amniotic fluid. Secreted antigens are mostly carried on glycoproteins of high molecular weight called mucins, but are also present in milk and urine as free oligosaccharides. The secretion of these antigens into the body fluids has widened the scope of their prefatory risk assessment.[21] Chauhan and Gera confirmed the potential role of anti-sera A and B in indirect ABO blood group analysis in the saliva of an individual using the conventional tile method.[22] Many advanced techniques with greater accuracy to determine blood group antigens in body fluids were available of which absorption elution assay was considered to be the most sensitive[23,15] and literature has revealed that a wide scope of absorption elution assay exists in forensic dentistry.[24,25]

Kimura et al. have specifically detected ABO blood groups of secretory saliva when mixed with other body fluids (e.g., semen, vaginal secretion, urine, sweat, and serum) using the absorption elution method having a strong hemagglutination[26] and no studies have been reported in children regarding the applicability of absorption elution method to identify blood group specific antigens A and B in saliva for replacing invasive blood typing in pediatric dentistry.

In this study, the absorption elusion technique was used with certain modifications for blood grouping from saliva which include incubation, absorption, elution, and agglutination reaction. The distribution of ABO grouping from saliva with absorption elution

Table 1: Demographic profile of study population

Variable	*Percentage of children (n = 60)*
Gender	
Male	(48) 80%
female	(12) 30%
Age-group (years)	
4–6	(28) 47%
6–9	(32) 53%
ABO group distribution	
A group	(13) 21.67%
B group	(19) 31.67%
AB group	(2) 3.33%
O group	(26) 43.33%
Rh group distribution	
Rh D positive	(56) 93.33%
Rh D negative	(4) 6.67%
Blood group distribution	
A–	(1) 1.67%
A+	(12) 20%
B–	(1) 1.67%
B+	(18) 30%
AB+	(2) 3.33%
O–	(2) 3.33%
O+	(24) 40%

Table 2: Performance of absorption elution method for screening Rh factor agglutinins in saliva

Contingency table (2 × 2)				*Accuracy measure*	*Value (95% CI)*
Index test	*Reference standard*			*Sensitivity*	*85.71% (73.78–93.62)*
	Positive	*Negative*		Specificity	75% (19.41–99.37)
Positive	48	1	49	Prevalence (*)	93%
Negative	8	3	11	Positive predictive value (*)	97.85% (89.26–99.6)
Total	56	4	60	Negative predictive value (*)	27.27% (14.38–48.17)
				Diagnostic accuracy (*)	84.96% (73.38–92.88)

*These values are dependent on disease prevalence; CI, confidence interval

Flowchart 1: STARD flow diagram

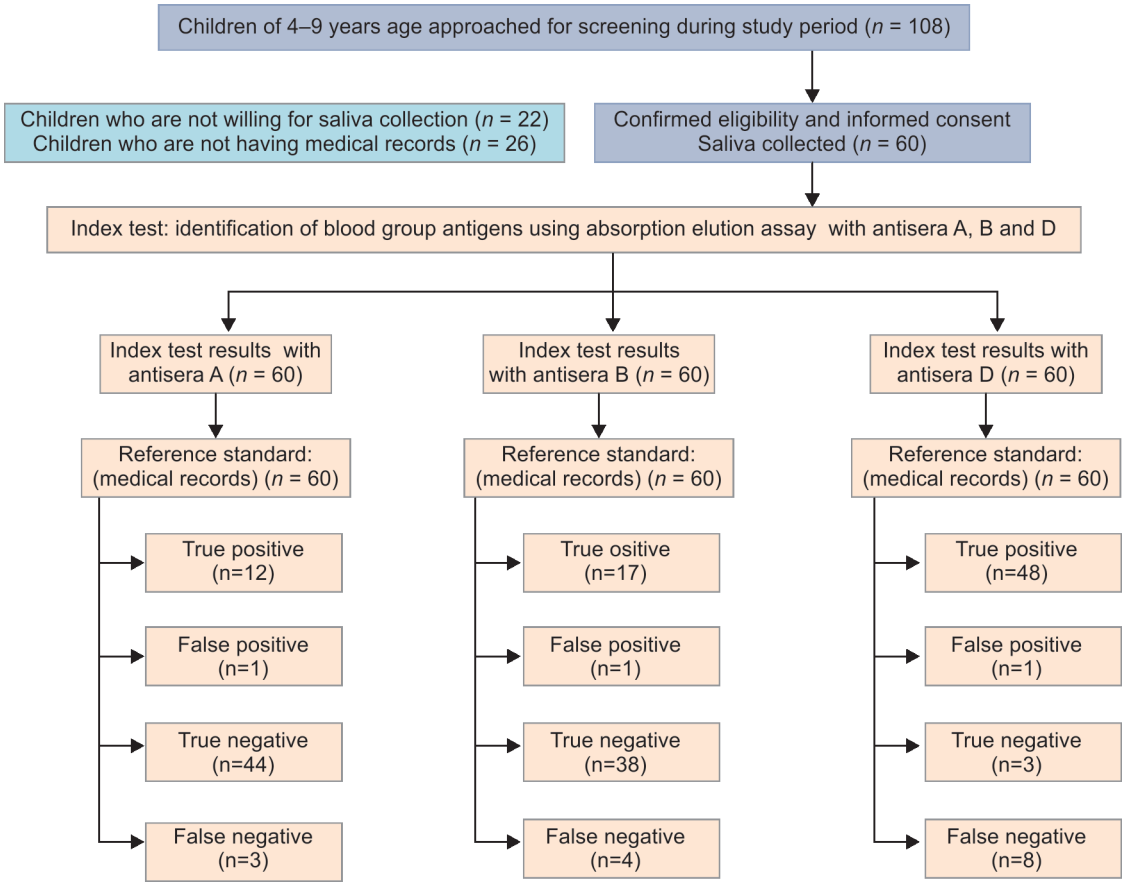

Table 3: Performance of absorption elution method for screening agglutinin A in saliva

Contingency table (2 × 2)				*Accuracy measure*	*Value (95% CI)*
Index test	*Reference standard*			*Sensitivity*	*80% (51.91–95.67)*
	Positive	*Negative*	*Total*	Specificity	97.78% (88.23–99.94)
Positive	12	1	13	Prevalence (*)	25%
Negative	3	44	47	Positive predictive value (*)	92.31% (62.96–98.83)
Total	15	45	60	Negative predictive value (*)	93.62% (84.19–97.58)
				Diagnostic accuracy (*)	93.33% (83.80–98.15)

*These values are dependent on disease prevalence; CI, confidence interval

Table 4: Performance of absorption elution method for screening agglutinin B in saliva

Contingency table (2 × 2)				*Accuracy measure*	*Value (95% CI)*
Index test	*Reference standard*			*Sensitivity*	*80.95% (58.09–94.55)*
	Positive	*Negative*	*Total*	Specificity	97.44% (86.52–99.94)
Positive	17	1	18	Prevalence (*)	35%
Negative	4	38	42	Positive predictive value (*)	94.44% (70.84–99.17)
Total	21	39	60	Negative predictive value (*)	90.48% (79.71–95.83)
				Diagnostic accuracy (*)	91.67% (81.61–97.24)

*These values are dependent on disease prevalence; CI, confidence interval

method was A– (0%), A+ (16.67%), B– (1.67%), B+ (23.33%), AB+ (5%), O– (16.67%), and O+ (36.67%). Of the 60 samples, 48 (80%) correctly matched with actual blood type.

An individual could be either secretor or nonsecretor pertaining to their genetic determination to secrete ABH blood group antigens in body fluids.[21] Parekh et al. showed the presence of blood group

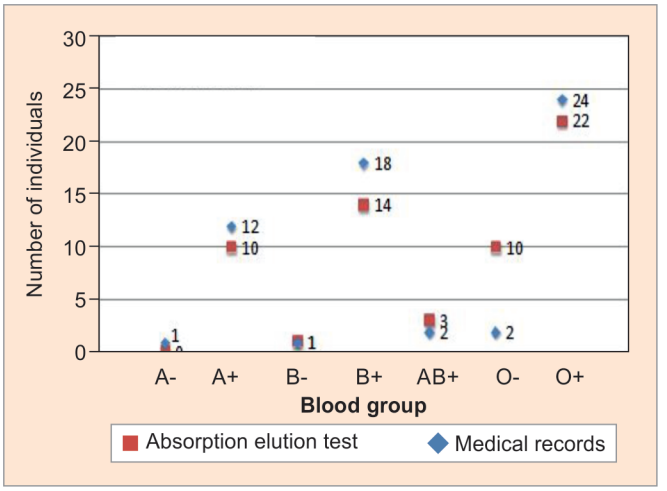

Fig. 3: Comparison of absorption elution ABO blood grouping with medical record data

antigen in dental pulp (93 cases) and also in saliva (79 cases) in adults using absorption elution assay and found that 79% of subjects were secretors.[27] Motghare et al. determined blood group from saliva using absorption inhibition method in subjects of 10–60 years and showed about 83% of subjects are secretors of antigen in saliva.[28] Sen et al.[15] proved the ability of absorption elution assay to provide better sensitivity over other techniques. Kimura et al. used the sandwich method with monoclonal antibodies and determined blood groups from mixed body fluids with 71% secretory status.[26] Compared to previous studies,[24–28] the sensitivity difference of absorption elution test arisen in the present study could be due to the difference in sampling, methodology, geographic diversity of the population, racial variation, and particularly age. Present results with high sensitivity and specificity indicate that absorption elution assay can be a valid diagnostic method to screen blood group antigens in saliva.

In A+ blood group samples in this study, three salivary samples did not show any agglutination, hence misinterpreted as O–. Four salivary samples of B+ blood group children did not show A or B blood group antigens of which two samples did not show Rh factor, hence misinterpreted as O– or O+. Similar findings were reported with O+ where three samples did not show any Rh factor and were misinterpreted as O– blood group. This is due to the nonsecretory status of concerned children. One salivary sample of A– blood group was misinterpreted as A+ and also one O– sample was misinterpreted as AB+. These could be due to contamination or technical error.

Even though salivary hemagglutinins came to light in 1928, their isolation was not in use due to inadequate procedures. Recently, Tejasvi et al. compared the slide agglutination method with that of the absorption inhibition method from saliva samples in adults. They observed a secretory status of about 86.66% and concluded absorption inhibition method was a better method in the determination of secretor status from saliva.[29] But, Sen et al.[15] stated that the absorption elution method detects more true positive cases than the absorption inhibition method, thus justifying its application in the current study. Nihal Açikgöz found the absorption elution method to be reliable on alone for blood typing when the amount of sample was minimal and insufficient compared to the absorption inhibition assay.[30] Hence, absorption elution assay was chosen in the current study, and according to the information available to date, this is the first study carried out to evaluate the presence of specific blood group antigens, especially in children's saliva using the noninvasive absorption elution method.

ABH secretors secrete ABH substances according to their blood groups. "O" blood group secretes only H substance, A blood group secretes A and H substances while B blood group secretes B and H substances in the fluids. The present study did not screen for H antigen, due to the unavailability of anti-H sera which is considered to be one of the limitations apart from the small sample size. The other major limitation of this method is that the blood type identification can only be performed on individuals who have secretor genes, whereas individuals devoid of secretor genes cannot be used as samples. Insufficient content, low antigen concentration, and degradation of substances in the saliva by temperature, humidity, etc. can influence the blood typing through saliva.

Inference

Absorption elution assay showed better diagnostic accuracy to screen A, B, and Rh agglutinins with the highest sensitivity of 80, 80.95, and 85.71%, respectively, and the highest specificity of 97.78, 97.44, and 75%.

Conclusion

The estimation of blood grouping using saliva with absorption elution assay has been found to be a reliable screening method to detect blood group antigens in saliva with reasonably high sensitivity, specificity, and diagnostic accuracy. Hence, this method could be a valid alternative to invasive blood grouping procedures, especially in anxious children with needle phobia. It also provides a broad scope in the field of forensic investigations for blood typing through saliva. However, a more technique-sensitive approach is needed for the detection of the H antigen. Further research with larger sample size and evaluation with anti-H Sera is warranted to evaluate its credibility.

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