Research Article

Detection of ABO IgA immunoglobulin, saliva from a healthy Sudanese individual at Khartoum state in 2023

Razan Bashir Osman AbdAlhadi[1], Albaraa Abdulfatah Mohammed[1], Ghanem Mohammed Mahjaf[2], Tibyan Abd Almajed Altaher[3], Mayada Mansoor Seliman Abdalla[4], Mosab Nouraldein Mohammed Hamad[5*]

[1]Department of Haematology and Immunohematology, Faculty of Medical Laboratory Sciences, National University, Sudan.
[2]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan.
[3]Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Shendi University, Sudan.
[4]Department of Haematology and Immunohematology, Faculty of Medical Laboratory Sciences, Shendi University, Sudan.
[5]Assistant professor, Microbiology department, Faculty of Medicine, Elsheikh Abdallah Elbadri University, Sudan.

Corresponding author: *Mosab Nouraldein Mohammed Hamad*
Assistant professor, Microbiology department, Faculty of Medicine, Elsheikh Abdallah Elbadri University, Sudan.
Corresponding Email: musab.noor13@gmail.com

Abstract:

Background: Individuals develop naturally occurring ABO antibodies during their first month of life. However, the method of antigen presentation is unclear yet. The ABO antigen may have been presented via mucosal cavities; to know the way of antigen presentation, we need further studies to confirm it. ***Objectives***: This study was aimed at detecting specific ABO IgA immunoglobulin, anti-A, and anti-B antibodies in saliva samples from healthy individuals in Khartoum State in 2023. ***Methods:*** This was a descriptive cross-sectional study conducted at Khartoum-Sudan State and included 60 samples from healthy individuals with blood groups A, B, and O. The samples were tested by the immunological method (sandwich ELISA), and the data were gathered and analyzed by the statistical package for social science 20. ***Result***s: The known blood group saliva sample, which was collected from Sudanese health individuals, was tested by using sandwich ELISA (secretory IgA) to confirm the result. The majority of the samples were positive for anti-B and 15% were non-reacted. The known blood group B was tested by using sandwich ELISA (secretory IgA) to confirm the result. All the samples were positive for anti-A and 5% were non-reacted. The known blood group O saliva samples were tested by sandwich ELISA (secretory IgA) to confirm the result. All the samples had anti-A and anti-B antibodies. ***Conclusion***: The study concluded that the majority of the samples were positive for anti-A and anti-B antibodies; there was no significant change between age and gender with IgA antibodies. ABO natural-occurring antibodies result from obvious antigen presentation, and the presence of ABO IgA in saliva proves this antigen presentation.

Keywords: *ABO, Detection, CBC, IgA immunoglobulin, saliva, Sudanese healthy individual.*

Introduction:

Karl Landsteiner discovered the first blood group system, ABO, in the early 20th century. He identified blood groups based on the presence of antigens on RBCs and antibodies in serum. The system was divided into blood groups A and B, with a third group called "O" and a fourth group called "AB". There is only one genetic locus that codes for the three different versions of the ABO blood group antigens. This system leads to six possible genotypes and four possible blood types **[1].** Antigens are three-dimensional substances on cell surfaces that elicit antibody formation. In transfusion medicine, they are found on red, white, or platelets. Antigen expression is inherited, and individuals can be homozygous or heterozygous. Blood group A (AB) individuals inherit both A and B genes, with transferases competing to convert H antigen into their respective products **[2].** Group O individuals lack the A and B genes, resulting in more H antigens in their red blood cells (provided they inherited the H (FUT1) gene, which 99.9% of people do inherit **[3].** About 80% of people have the secreter gene (Se). These people secrete water-soluble antigens in their saliva and other body fluids. Show if any individual variations in secretory ABO group antigens are present in saliva. First, determine if a person is a secretor or a non-secretor. Agglutination inhibition is the principle that is used in this study, and then the degree of agglutination is determined microscopically to show individual variations in secretory. This study revealed that there are great variations among individuals in the amount of secretory ABO group antigen that is present in saliva and body fluid **[4].** ABO antibodies were originally discovered by Immunoglobulin (Ig) M, IgG, and IgA classes are currently understood to have been identified by Karl Landsteiner in the early 1900s. Since IVIG is nearly exclusively produced as IgG, the iso-hemagglutinins found in IVIG belong to this immunoglobulin class. Blood bank cross-matching is done to make sure that recipients receive blood of the correct type to prevent a large mismatch or substantial incompatibility, which can result in significant morbidity and frequently death. This is done because ABO antibodies are extremely clinically significant **[4].** Sera of normal and immunized persons genotypes OO and A1O were fractionated to separate the three main immunoglobulin components, IgG, IgM, and IgA, from each other. Every fraction and each individual's serum had its anti-B activity tested. The results indicate that the natural anti-B antibodies are of the IgM molecular type, contrasting with the immune anti-B antibodies, which are IgM, IgG, and eventually IgA. The restriction to the IgM class and other data previously reported are discussed about the origin of the natural antibodies in the human ABO blood group system **[5].** Oral keratinocytes and dendritic cells distinguish between

commensal and pathogenic microorganisms, generating protective immunoinflammatory responses or promoting immune tolerance. Secretory immunoglobulin A (sIgA) antibodies at oral mucosal sites contribute to oral immunity by limiting microorganism colonization and invasion. Ig isotype class switching to IgA is dependent on or independent of T helper cells and facilitated by cytokines secreted by dendritic cells and monocytes. Studies in humans have shown that at least 96% of salivary IgA is synthesized at local sites, indicating that at least 96% of salivary IgA is synthesized at local sites **[6].** The study examined the clearance of IgA into saliva relative to albumin, aiming to determine selective IgA transport. The results showed a clearance ratio of 0.78 to 0.98, indicating that less IgA than albumin entered the saliva from the serum. The study supports the view that small amounts of IgA pass from serum to saliva, accounting for only a small percentage of the total IgA in saliva. There is no significant specific preferential transport of IgA from serum to saliva **[7].** Secretory and serum IgA are immunoglobulin classes with a total daily production exceeding other classes. They have mutual independence, with molecules with different physiochemical and immunochemical properties and antibody activities. They are produced by cells with different organ distributions and have different effector functions. Secretory IgA interacts with environmental antigens to prevent their penetration through various mechanisms. The primary function of serum IgA remains unknown, but it is proposed that in humans, monomeric serum IgA may protect endogenous antigens by preventing their interaction with humoral and cellular immune mechanisms, potentially leading to tissue damage **[8,9].** IgA is an immunoglobulin, meaning that its two heavy chains and two light chains are the same. The aforementioned chains are divided into two Fab regions that bind the antigens and an Fc region that mediates the effects by a flexible hinge region. IgA in humans is divided into two subgroups, known as IgA1 and IgA2. IgA1's hinge region has a longer 13-amino acid extension with three to six variable O-glycan substitutions; IgA2 does not have this. While both IgA1 and IgA2 include N-linked glycosylation sites at every heavy chain, IgA2 may be more resistant to the bacteria's proteolytic activity in secretions due to its two extra N-linked oligosaccharides. IgA2 is preferred in dimeric IgA (dIgA), which is composed of two monomeric IgAs joined by the J (joining) chain in the final Cys residues of their Fc regions. J chain is a small polypeptide that forms pentameric IgM and dimeric IgA, but little is known about the function of J chain due to the technical limitations. When one dIgA is bound to the polymeric immunoglobulin receptor (pIgR) at the basolateral side of the epithelium and thereby transported to the luminal side, the

dIgA-binding portion of the pIgR is cleaved to form the molecule sIgA. The pIgR fragment of sIgA is called the secretory component (SC) to support the stability of sIgA. Although both IgA1 and IgA2 can form sIgA, a variety of subclass proportions will happen in different tissues. For example, there is 80 to 90% IgA1 in nasal and male genital secretions, 60% IgA1 in saliva, and 60% IgA2 in colonic and female genital secretions **[10].**

Materials and methods:

Study design:

This study is a cross-sectional.

Study Area:

The study was carried out in different areas of Khartoum State.

Study population:

Healthy individuals in different areas of Khartoum State from January to May 2023.

Inclusion criteria:

Healthy individual

Exclusion criteria:

an individual who has a current COVID-19 infection, a patient with a respiratory infection, an immunocompromised patient, a patient with hypogammaglobulinemia, a patient with a viral infection, and a multiple myeloma patient.

Data Collection:

Data was collected using a standard data questionnaire consisting of basic demographic data concerning each participant obtained from the registry date base office. The laboratory data includes the hematological result.

Sample collection and processing:

A random saliva sample was obtained from Sudanese healthy individuals of non-AB blood groups (A, B, and O) in Khartoum state. All samples were tested for ABO-IgA antibodies by sandwich ELISA. This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to human sIgA. Samples (or standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human sIgA and Avidin-Horseradish Peroxidase (HRP) conjugate is added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human sIgA, biotinylated detection antibody, and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a stop solution, and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of human sIgA **[11].** Sample preparation went through a modification technique that aimed to isolate the ABO SIgA antibodies from total SIgA, and it was checked by using negative and positive controls under the supervision of the

immunology technologist team. Samples were centrifuged for 20 min at 1000×g at 2–8 °C. The supernatant was collected to carry out the assay. An equal volume of blood group A saliva samples was mixed with B cell suspension, blood group B saliva samples were mixed with A cell suspension, and blood group O saliva samples were mixed with A and B cell suspension. All samples were then incubated at 37 °C for 30 min and then washed with normal saline for 2 hours. Wells was determined for the diluted standard, blank, and sample. 100 µL was added to each dilution of the standard, blank, and sample into the appropriate wells it is recommended that all samples and standards be assayed in duplicate. It was recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations. The plate was covered with the sealer provided in the kit. Was incubated for 90 minutes at 37°C.The liquid was decanted from each well. Immediately, 100 µL of Biotinylated Detection Ab working solution was added to each well. The plate was covered with a new sealer. Incubated for 1 hour at 37 °C. The solution was drawn from each well. 350 µL of wash buffer was added to each well. Soak for 1 minute, aspirate or decant the solution from each well, and pat it dry against clean absorbent paper. The wash step was repeated three times. 100 µL of HRP conjugate working solution was added to each well. The plate is covered with a new sealer. Incubated for 30 min at 37°C, the wash process was repeated five times as conducted in step 90, and 90 µL of substrate reagent was added to each well. Covered the plate with a new sealer. Was incubated for about 15 minutes at 37 °C. The plate was protected from light. The microplate reader was preheated for about 15 minutes before OD measurement. 50 µL of Stop Solution was added to each well, and the optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm (cut-off value of 0.2, more than 0.2 possible).

Ethical consideration:

Ethical approval was obtained from the research ethics committee of the National University of Sudan, Faculty of Medical Laboratory Sciences. All individuals were provided with written informed consent.

Data Analysis:

The data obtained through the study were entered and analyzed by computer using Microsoft Office Excel and the SPSS statistical program to calculate the mean, standard deviation, and *P-value* by t-test. $P<0.05$ was considered statistically significant.

Results:

The gender of all participants in this study was 56% male and 43% female **(Table 1).** The variation in the age group of the participants was highest from 11 to 20 (40%) years, 21 to 30 (21%) years, and 31 to 40 (10%) years **(Table 2).** In the blood group among the participants, an equal percentage

of participants (33.3%) of blood groups A, B, and O were obtained **(Table 3).** The known blood group A saliva samples were tested by using ELISA (secretory IgA) to confirm the result; the majority of the samples were positive for anti-B and 15% were non-reacted; the known blood group B samples were tested by using ELISA (secretory IgA) to confirm the result; all the samples were positive for anti-A and 5% were non-reacted. The known blood group O saliva samples were tested by ELISA (secretory IgA) to confirm the result. All the samples were positive with ELISA for anti-A **(Table 4).**

Table 1. Frequency of patients according to gender

Gender	Frequency	Percent (%)
Male	34	56.7
Female	26	43.3
Total	**60**	**100.0**

Table 2. Frequency of patients according to age groups

Age group	Frequency	Percent (%)
0-10	4	6.7
11-20	24	40.0
21-30	13	21.7
31-40	6	10.0
41-50	3	5.0
51-60	6	10.0
61-70	2	3.3
71-80	1	1.7
81-90	1	1.7
Total	**60**	**100.0**

Table 3. Frequency of blood groups

Blood Group	Frequency	Percent (%)
Blood group A	20	33.3
Blood group B	20	33.3
Blood group O	20	33.3
Total	**60**	**100.0**

Table 4. Frequency of secretory IgA among blood groups

Blood group		Frequency	Percent (%)
A	Positive	17	85
	Negative	3	15
	Total	20	100.0
B	Positive	19	95
	Negative	1	5
	Total	20	100.0
O	Positive	20	100
	Negative	0	0
	Total	20	100.0

Table 5. The correlation between Gender, Age group, and Anti-A and Anti-B ELISA (secretory IGA) test.

Correlation	Variables	*P-value*
Pair 1	Sex & anti-A	0.630
Pair 2	Sex & anti-B	0.778
Pair 3	age & anti-A	0.379
Pair 4	age & anti-B	0.406

Discussion:

Healthy individuals develop naturally occurring ABO antibodies in their serum and body fluids like milk, tears, and saliva during their first month of life (after 6 months from birth). However, the method of antigen presentation is still unclear. The ABO antigens may have been presented via mucosal cavities. In the present study, we aimed to detect the secretary ABO IgA antibodies in saliva from individuals who lack their corresponding antigen. In this current study, the results showed that all blood group O individuals have anti-A and anti-B sIgA in their saliva samples; blood group B individuals were positive for anti-A sIgA except 1 sample, which could be ABO discrepancies; and blood group A individuals were positive for anti-B sIgA except 3 samples, which could be ABO discrepancies. This study agreed with the Bell 1971 *et al.* study in Austria, which confirmed in saliva, IgA was the most abundant ABO antibody class of all non-AB blood types study there were no significant sex and age-group differences in ABO antibodies present in saliva; this finding agreed with Schonbacher *et al.,* Australia, in 2022 **[12].** A statement

that "high titer iso-agglutinins against ABH antigens were detected in saliva as well as serum. Following antigen presentation by DCs, T cells and B cells were activated, and mucosal B cells were the site of IgA class switch recombination (CSR), which replaced the immunoglobulin heavy chain C regions (C) with the downstream C gene **[13].** By extending their dendrites or by transcytosis of microfold cells (M cells), DCs were able to collect mucosal antigens **[14,15].** Evidence suggests that the small intestine goblet cells (GCs) and follicle-associated epithelium (FAE) were both engaged in antigen uptake **[16,17].** This happens in the mucosal-related lymphoid tissue, particularly in the ileum's Peyer's patches, which were covered in FAE and contained M cells. The subepithelial dome (SED) covers the B follicles and the DCs beneath the SED **[18].** Epithelial export of locally produced immunoglobulin A (IgA) polymers, primarily dimers, discharged to the lumen as secretory IgA antibodies **[19],** is a hallmark of the mucosal immune system. It is reliant upon a coordinated network of immune cells moving from inductive mucosa-associated lymphoid tissue to exocrine effector locations, including the gut lamina propria, airways, and salivary glands. The gut-associated lymphoid tissue (GALT), which is dominated by Peyer's patches in the distal ileum, is one regional mucosa-associated lymphoid tissue structure where mucosal B cells are activated. After class switching to IgA expression in GALT, the memory/effector cells migrate to draining lymph nodes like the mesenteric lymph nodes and then on to peripheral blood **[11].** Depending on mucosal immune system antigen presentation physiology, the presence of anti-A and anti-B secretory IgA indicates that antigen presentation occurred in the first months of life and ended with the production of ABO antibodies from memory cells into the peripheral blood. And now we can say the ABO naturally occurring antibodies result from obvious antigen presentation (those antigens A and B present in the body fluid of all individuals who have SE genes) via mucosal cavities of individuals with different blood groups contacting each other.

Conclusion:

The study concluded that the majority of the samples had anti-A and anti-B secretory IgA; there was no significant change between age and gender with IgA antibodies. ABO natural-occurring antibodies result from obvious antigen presentation, and the presence of ABO IgA in saliva proves this antigen presentation.

Acknowledgments

The authors gratefully acknowledge all the people who work and help us in this study.

Sources of Funding:

There was no specific grant for this research from any funding organization in the public, private, or nonprofit sectors.

Conflict of Interest:

The author has affirmed that there are no conflicting interests.

References:

1. Stussi G, Huggel K, Lutz HU, Schanz U, Rieben R, Seebach JD. Isotype-specific detection of ABO blood group antibodies using a novel flow cytometric method. Br J Haematol. 2005;130(6):954–63.
2. Dean L. Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 5, The ABO blood group.
3. Bakhtiari S, Yadegari Z, Kaviyani M, Namazi Z, Bakhshi M, secreter status of ABO Antigens in the saliva of a Defined Group of Iranian Patients with Pemphigus Vulgaris: Case-Control Study Scientifica (Cairo) 2020 Jul 28;2020:2950856.
4. Branch DR. Anti-A and anti-B: what are they and where do they come from? Transfusion. 2015 Jul;55 Suppl 2:S74-9.
5. Filitti-Wurmser S. Natural antibodies and immune antibodies of human ABO blood group system. Biochimie. 1976;58(11-12):1345-53.
6. Feller L, Altini M, Khammissa RA, Chandran R, Bouckaert M, Lemmer J. Oral mucosal immunity. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Nov;116(5):576-83.
7. Woof, J.M.; Ken ,M.A. The function of immunoglobulin A in immunity, J. Pathol 2006,208, 270-282.
8. Strugnell RA, Wijburg OL, The role of secretory antibodies in infection immunity. Nat Rev microbiol .2010 Sep;(9):657-67.
9. Pietrzak B, Tomela K, Olejnik-Schmidt A, Mackiewicz A, Schmidt M, Secretory IgA in intestinal Mucosal Secretions as an Adaptive Barrier against the microbial cell.International Journal of Molecular Sciences .2020;21(23):9254.
10. Yue Li, Liang Jin, Tongxin Chen, "The Effects of Secretory IgA in the Mucosal Immune System", BioMed Research International, vol. 2020, Article ID 2032057, 6 pages, 2020. https://doi.org/10.1155/2020/2032057.
11. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. The immune geography of IgA induction and function. Mucosal immunology. 2008 Jan 1;1(1):11-22.
12. Schonbacher M, Banfi C, Berghold A, Matzhold E, M, Wagner T, Mayr W, R, Kormoczi G, F Immunoglobulin class profile pf ABO Antibodies in saliva and serum of healthy individual transfus MedHemother 2022.doi:10.1159/000527233.
13. A. Reboldi, T. I. Arnon, L. B. Rodda, A. Atakilit, D. Sheppard, and J. G. Cyster, “IgA production requires B cell interaction with subepithelial dendritic cells in Peyer's patches,” Science, vol. 352, no. 6287, p. aaf4822, 2016.

14. J. Farache, I. Koren, I. Milo, et al., "Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation," Immunity, vol. 38, no. 3, pp. 581–595, 2013.

15. N. A. Mabbott, D. S. Donaldson, H. Ohno, I. R. Williams, and A. Mahajan, "Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium," Mucosal Immunology, vol. 6, no. 4, pp. 666–677, 2013.

16. P. Kujala, C. R. Raymond, M. Romeijn et al., "Prion uptake in the gut: identification of the first uptake and replication sites," PLoS Pathogens, vol. 7, no. 12, Article ID e1002449, 2011.

17. J. R. McDole, L. W. Wheeler, K. G. McDonald, et al., "Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine," Nature, vol. 483, no. 7389, pp. 345–349, 2012.

18. P. Brandtzaeg, H. Kiyono, R. Pabst, and M. W. Russell, "Terminology: nomenclature of mucosa-associated lymphoid tissue," Mucosal Immunology, vol. 1, no. 1, pp. 31–37, 2008.

19. Brandtzaeg P. Mucosal immunity: induction, dissemination, and effector functions. Scandinavian journal of immunology. 2009 Dec;70(6):505-15.