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The association between ABO blood groups and norovirus infections among patients suffering from diarrheal disease in Northwest Ethiopia

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Abstract

Background: *Norovirus (NoV) infection is a significant cause of diarrhea worldwide. However, all individuals are not equally affected due to environmental, viral, and host factors, particularly ABO blood groups. Indeed, data that describes the association between NoV infection and the ABO blood group is limited in Ethiopia, and this needs to be investigated.*

Objective: *This study aimed to assess the relationship between ABO blood groups and NoV infection in Northwest Ethiopia.*

Method: *A health-facility-based cross-sectional study was conducted from May 2021 to November 2021 by enrolling 550 participants with diarrhea. Fecal samples were collected and analyzed by reverse transcription PCR to identify NoVs. To further genotype the positive samples, a viral protein-1-coding gene was sequenced. In addition, blood samples were collected and tested to identify blood groupsby using the tube hemagglutination technique. The data were analyzed using SPSS version 23. Logistic regression analysis was done to assess the association between NoV infection and the independent variables.*

Result: *Among the 550 enrolled participants, 519 (94% response rate) provided the required clinical samples and epidemiological data. The majority of the study participants (249/519; 48%) had O blood group.Among the NoV-positive study subjects, the majority (34/46, 74%) were in blood group O, followed by blood group A individuals (9/46, 19.6%).The risk of NoV infection was higher for patients with blood group O than for blood group B (AOR = 1.5, 95%CI = 2–15, P= 0.01), but there was no association for other blood groups.At least one NoV-GII was identified in each of the blood groups, while NoV-GI affected individuals with blood groups O and A. Besides, GII.3 and GII.21 genotypes were common among blood group O individuals, while most (75%) blood group A individuals were susceptible to GII.17 infections.*

Conclusion: *The positivity rate of NoV infection was considerably high among individuals with blood group O. Norovirus-GII can infect all blood groups, while NoV-GI selectively affects blood groups A and O. Further large-scale studies are warranted to assess the relevance of this observation and other genetic factors.*

Introduction

Diarrheal diseases are among the most common causes of morbidity and mortality, responsible for more than 1.5 million deaths globally(1). In developing countries, particularly, diarrhea is the second most common cause of death (2), with the highest burden resting in sub-Saharan Africa and south Asia (3). As a sub-Saharan African country, diarrheal disease is also an important public health concern in Ethiopia (4). More than 25% of under-5 children are affected by diarrhea(5, 6).Viruses are the leading etiological agents of diarrhea in both high-and low-income countries(7). Norovirus(NoV) infections pose a significant burden of diarrheal diseases among all age groups globally, with variable distributions across different settings(8, 9). These viruses are responsible for about 20% of all episodes of diarrheal disease globally (1).The estimated burden is 699 million cases and 219,000 deaths annually, where 97% of mortality is attributed to lower and middle-income countries (1, 10).

Human NoVs are genetically diverse groups of viruses under the family *Caliciviridae*. The virion contains a non-enveloped single-stranded (+sense) RNA genome (11). Based on the partial viral protein 1 (VP1) sequence analysis, they are classified into 10 genogroups (GI to GII) and 49 genotypes. Of these genogroups, GI,GII, and GIV are known to infect humans. These NoV genogroups are further divided into multiple genotypes (9 GI, 27 GII, and 2 GIV) (12). Norovirus GII is by far the predominant genogroup (13), and NoV-GII, genotype-4 (GII.4), is reported as the predominant genotype globally(9, 14). However, a predominance of non-GII.4 genotypes (GII.3, GII.17, and GII.2) over GII.4 was reported in the past five years (15, 16). This might be related to their rapid evolution and a change in their binding profiles (17).

Noroviruses are highly contagious, with an estimated infectious dose of as few as 18 viral particles and prolonged shedding over an average of 8–60 days (18, 19). The main route of transmission is direct contact with an infected person, and it can also be transmitted via contaminated food and water (20, 21). Despite the high infectivity and rapid transmission, not all individuals are affected and develop symptomatic disease (22).

The association between ABO blood groups and NoV infection was reported previously, but with a conflicting result (23, 24). One previous study demonstrated that NoV recombinant virus-like particles strongly agglutinated red blood cells from group O, A, and AB donors but were less likely to do so from group B individuals (25). Another study also reported that among NoV-positive individuals, all symptomatic infections were among blood group O and group A individuals (26). In another study, genogroup-specific susceptibility was reported with NoV-GI, which mainly targets individuals with blood groups O and A (27), while NoV-GII infected all individuals irrespective of their ABO blood group status (28). In contrast, a partial or total absence of correlation between different blood group (A, B, AB, and O) antigens and NoV infection was reported (29-31).

The distribution of ABO blood groups and other host genetic factors is strongly dependent on ethnicity (32, 33). Besides, the molecular epidemiology of NoV differs between different areas or countries due to different environmental, viral (34-36), and host factors (22, 37, 38). However, studies addressing the association of host factors, especially ABO blood groups, with NoV infection are limited in Africa and have not yet been conducted in Ethiopia. Therefore, this study aimed to assess the relationship between ABO blood groups and NoV infection in Northwest Ethiopia.

Method

Study design, study period, and setting: From May 2021 to November 2021, a health-facility-based cross-sectional study was conducted to collect clinical and epidemiological data. Four data collection sites (Debre Markos, Bahir Dar, Gondar, and Debre Tabor), all of which are found in Northwest Ethiopia, were considered. In each of the study sites, one comprehensive specialized hospital and two health centers were included. The health institutions included in Debre Tabor were Debre Tabor Comprehensive Specialized Hospital, Liul Alemayehu Health Center, and Debre Tabor Health Center. In the second study site, Bahir Dar, Felege Hiwot Comprehensive Specialized Hospital, Shimbit Health Center, and Abay Health Center were included. Similarly, Gondar consists of the University of Gondar Comprehensive Specialized Hospital, Azezo Health Center, and Gondar Health Center. Lastly, in Debre Markos, Debre Markos Comprehensive Specialized Hospital, Debre Markos Health Center, and Gozamen Health Center were considered.

Sample size determination and sampling technique: A single population proportion formula was used to calculate the sample size (n = Z α/22*P (1-P)/d2). By considering the following assumptions: Zα/2 is taken as 1.96 at a 95% confidence interval (CI); P is the proportion taken from the previous study (13.3%)(39); d is the desired level of precision (3%). Finally, a 10% non-response rate was added, and the total sample size was calculated at 550. Based on the previous year's diarrheal disease case flow, the total sample size was proportionally allocated as 100, 120, 152, and 178 for each of the Debre Tabor, Debre Markos, Gondar, and Bahir Dar sites, respectively. A systematic random sampling technique was used to select the study participants from each health facility.

Data collection: Socio-demographic data were collected by trained healthcare professionals working in each of the health facilities. Once a clinical diagnosis was made based on the inclusion criteria, a pre-tested, semi-structured questionnaire was administered to each participant.

Fecal sample collection, processing, and molecular characterization: Five mL diarrheic fecal samples were collected using sterile containers from individuals of all age groups with diarrhea and self-reported to the health facilities during the study period. These fecal samples were stored at -20 °C or lower in each of the health facilities until transported to the Amhara Public Health Institute (APHI) for molecular investigation. Besides, peripheral blood samples were also collected to assess the blood group status of study participants. Once the fecal samples were taken to the APHI, they were stored at -70 °C until processed. There, ten percent (weight/volume) of fecal suspensions were prepared with nuclease-free water or 1% phosphate-buffered saline as available, vortexed, and centrifuged. Viral ribonucleic acid (RNA) was extracted from 300 uL fecal suspensions using MagaBio Plus Virus RNA Purification Kit II (Hangzhou, China). The details for the detection and sequencing or genotyping protocols, list of primers and probes that used for screening and genotyping as well as all PCR conditions are available in our recent publication (40).

Blood sample processing and ABO blood grouping: The assigned and trained health care worker in each ward of the health facilities informed the patient that he had received permission to collect epidemiological data and sent him with a request paper to a laboratory room for blood group analysis. The trained laboratory professional, in the laboratory room, instructs the client and collects 5 mL of blood. Then blood typing was done on-site at each of the study sites with a tube hemagglutination technique as previously done(41). Briefly, a forward grouping protocol was applied to assess the presence or absence of A, B, and D antigens in the red blood cells using commercially prepared antisera (Cypress Diagnostics, Langdorp, Belgium). Blood cells were placed in the three test tubes to prepare a 5% suspension of red blood cells to be tested in isotonic saline. Then one drop of each RBC suspension in each tube was mixed with a drop of anti-A, anti-B, and anti -D. These tubes were subjected to centrifugation for 3 minutes to ensure enhanced chemical interactions, particularly for weaker antibodies to react and agglutinate. The resultant matrix was gently shaken and examined macroscopically for agglutination. The tubes were categorized according to the extent of blood clumping as A+, A-, B+, B-, AB+, AB-, O+, and O-.

Statistical analysis: Data were entered and analyzed using SPSS version 23 software. Descriptive statistics were used for frequencies and percentages. The association between the outcome variables and factors was assessed using a logistic regression analysis. Those variables with *P*values < 0.05 and an adjusted odds ratio (AOR) in a 95% CI were considered statistically significant. The assumption is that the AOR within the 95% CI should not include 1. The result was interpreted and presented in a summary or displayed by using tables and figures.

Result

Socio-demographic characteristics: Among the total 550 study participants enrolled, 519 (with a 94.4% response rate) provided the required clinical samples and socio-demographic information. The age of the study participants ranged from 3 months to 85 years. More than half (51.3%) of the participants were female. From the four study sites, one-third (178/519; 34.3%) of the study participants were recruited from Bahir Dar, followed by Gondar (142/519; 27.4%). Three-fourths of the study participants were came from urban areas. In addition to this, about two-thirds (330/519; 63.6%) of our study participants and/or their parents were married. Besides, the majority (407/519; 78.4%) of the study participants were literate (they were at least able to read and write).

ABO blood groups and Rh types: We identified four blood groups (A, B, AB, and O) and assessed the Rh factor status. Nearly half (249/519; 48%) of the study participants had the O blood group, followed by blood groups A (143/519; 27.5%) and B (103/519; 19.8%). More specifically, the majority (216/519; 41.6%) were identified as blood group O with an Rh factor (O+), which was followed by A+ (119/519; 22.9%) and B+ (88/519; 17%) (**Fig. 1).**

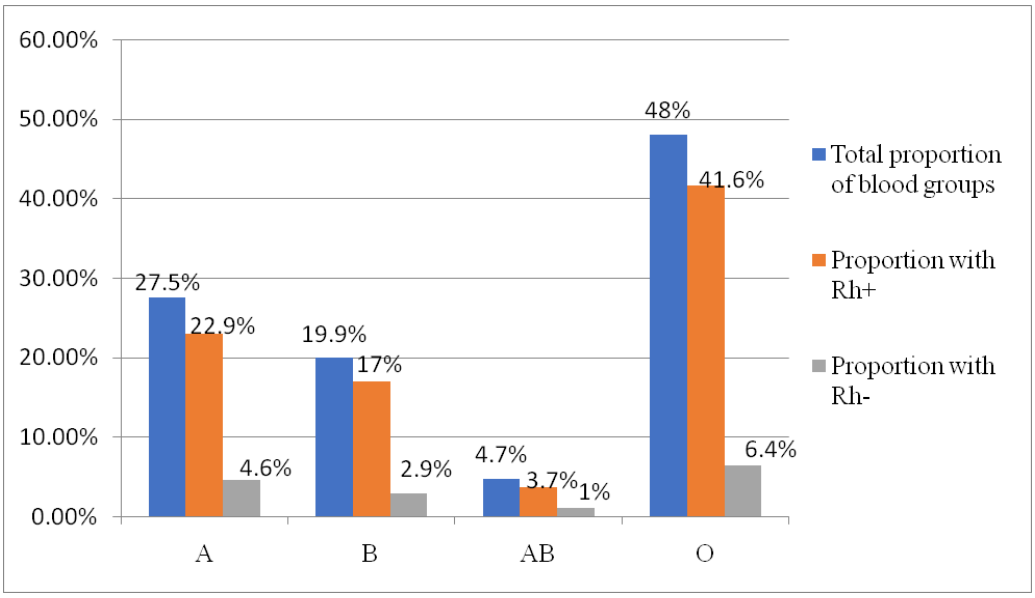

Figure 1: *The proportion of ABO blood groups and Rh status among patients with diarrhea in Northwest Ethiopia; May 2021 to November 2021*

Association between ABO blood groups and norovirus infection: Norovirus was identified in 8.9% (46/519) of the fecal samples of individuals with diarrhea. Both NoV-GI and GII were identified, with GII being predominant (38/46; 82.6%). The identified NoV genotypes were GI.3, GI.5, GII.3, GII.6, GII.10, GII.17, and GII.21. Of all the 29 successfully genotyped NoVs, genotype GII.3 was the predominant (13/29; 44.8%), followed by GII.21 (6/29; 20.7%) and GII.17 (4/29; 13.8%). In addition to this, GII.21 was identified for the first time in Ethiopia. Study participants with blood group O had the highest proportion of NoV infection (34/249; 13.7%), followed by blood group A (9/143; 6.3%), AB (1/24; 4.2%), and B 1/46 (2/103; 1.9%)(**Table 1**).

Table 1: The distribution of noroviruses across ABO blood groups of study participants in Northwest Ethiopia; from May 2021 to November 2021

NoV positivity, genogroups, and genotypes		**ABO blood groups (%)**			
		O	**A**	**B**	**AB**
Status of NoV infection	Positive	34 (13.7)	9 (6.3)	2 (1.9)	1 (4.2)
	Negative	215 (86.3)	134 (93.7)	101 (98)	23(95.8)
Genogroups	GI	6 (75)	2 (25)	0	0
	GII	28 (73.7)	7 (18.4)	2 (5.3)	1 (2.6)
Genotypes	GI.3	0	1	0	0
	GI.5	3	0	0	0
	GII.3	9	2	1	1
	GII.6	1	0	0	0
	GII.10	1	0	0	0
	GII.17	1	3	0	0
	GII.21	4	1	1	0

Among the NoV-positive study subjects, the majority (34/46, 74%) were blood group O, followed by blood group A individuals (9/46, 19.6%). Norovirus-GII was detected across participants with O, A, B, and AB blood groups, with the highest proportion (28/38; 73.7%) among blood group O participants. Based on the multiple logistic regressions analysis, the probability of NoV infection was increased amongunder-5 children (AOR = 1.4, 95% CI: 2.7–18, P = 0.02), the elderly (AOR = 5, 95% CI: 1.7–16, P = 0.015), individuals living in the Bahir Dar area (AOR = 1.5, 95% CI: 1.6–22, P = 0.014), and Debre Tabor (AOR = 2.5, 95% CI: 1.8–23, P = 0.001).Besides, the odds of NoV infection among blood group O individuals were 1.5 times higher than B blood groups (AOR: 1.5, 95%CI: 2–15, *P* = 0.001). Norovirus-GI was detected only in participants with blood groups O and A. Except for GI.3, all the other NoV genotypes were detected in participants with blood group O. The GII.3 genotype was detected across the four blood groups. Similarly, GII.21 was identified in participants with blood groups A, B, and O. In addition to this, three-fourths (3/4; 75%) of the GII.17 genotypes were detected in participants with blood group A. However, the difference was not statistically significant (*P*value > 0.05)(**Table 2**).

Table 2: The association of ABO blood groups and other variables with norovirus infection among patients with diarrhea in Northwest Ethiopia; May 2021 to November 2021

Variable	Categories	Norovirus status		COR (95% CI)	*P*value	AOR (95% CI)	*P*value
		Positive	Negative				
		N (%)	N (%)				
Sex	Male	25 (10)	228 (90)	1.3 (0.7-2.3)	0.4		
	Female	21(8)	245 (92)	1			
Age group in years	<5	20 (12.5)	160 (87.5)	5.6 (1.9-16.7)	0.002	1.4 (2.7-18)	***0.02**
	5 to 17	9 (8.2)	110 (91.8)	3.5 (1.3-9)	0.011	1.1 (0.2-17)	0.11
	18 - 64	9 (4)	225 (96)	1	1		
	> 64	8 (33.3)	24 (66.7)	12 (4-35)	0.000	5 (1.7-16)	***0.015**
Blood groups	O	34 (13.7)	215 (86.3)	2.7 (3.6-12)	0.02	1.5 (2-15)	***0.01**
	A	9 (6.3)	134 (93.7)	0.9 (0.08-5.3)	0.6	0.7 (0.5-8)	0.25
	AB	1 (4.2)	23 (95.8)	0.8 (0.2-25)	0.8	0.4 (0.2-14)	0.7
	B	2 (1.9)	101 (98)	1		1	
Study area	Debre Tabor	17 (17.2)	82 (82.8)	5 (1.6-15.4)	0.005	2.5 (1.8-23)	***0.001**
	Bahir Dar	15 (8.4)	163 (31.4)	2.7 (1.2-6.25)	0.017	1.5 (1.6-22)	***0.014**
	Gondar	10 (7)	132 (25.4)	2.25 (1.01-4.7)	0.032	2.8 (0.9-20)	0.13
	Debre Markos	4 (4)	96 (18.5)	1		1	

***AOR**, Adjusted odds ratio; **COR**, Crude odds ratio; *statistically significant*

Discussion

In the present study, we tried to demonstrate the association between the ABO blood group and NoV infections by analyzing blood and fecal samples collected from diarrheal patients recruited from the four major and proximate cities located in Northwest Ethiopia. In this study, NoV was predominantly identified in the participants with blood group O (13.7%) followed by blood group A (6.3%). Despite a significant number of participants, with blood group B involved, only about 2% of them were positive for NoV infection.

Three-fourths of the study participants who were positive for NoV infection had O blood group, while blood groups B and AB were less likely to be infected with NoV. Our findings are in agreement with different studies (24, 31, 42). Besides, in the present study, at least one NoV-GII was identified in each of the four blood groups, while infection with NoV-GI was observed among individuals with blood groups O and A. This is in agreement with a few previous studies (27, 31). Protection of individuals with blood groups B and AB for both genogroups was also reported previously, which supports our finding(22, 24).

Our findings also showed that at least one genotype of NoV-

GII was found among individuals with O blood groups. Besides, the predominant genotype (GII.3) was identified in all of the blood groups, more commonly among blood group O and blood group A individuals. This finding is in agreement with a study conducted in China(43).Although it was difficult to compare as it was a systematic review and meta-analysis, significant susceptibility patterns of O blood groups were also reported in China, which supported our finding (24).An absence of correlation among all blood groups (A, B, AB, and O) with NoV infection was also reported (29-31). This might be due to the existence of different factors other than the ABO blood group system that play a great role in the susceptibility of NoVs (11, 44).

In the present study, GII.21 was identified among all blood groups except AB blood groups. However, this justification requires further investigation. In addition to this, GII.17 genotypes were identified among individuals with blood groups A and O. In contrast to this, one previous study reported that one GII.17 infection was found in an individual with blood group O (31).The difference might be explained by host factors other than the ABO blood group, including the secretory antigen status of the individuals. Most individuals, termed secretors (having an active fucosyltransferase (FUT2) gene), express the histoblood group antigens in different body fluids that commonly act as receptors for NoV. Hence, they are commonly sensitive to some NoV infections, while others with inactivated or mutated FUT2 genes, termed non-secretors, are resistant to NoV(11). It might also be due to the host microbiota (like Enterobacter cloacae and Escherichia coli) that can mediated NoV attachment to the host via HBGA-like carbohydrates expressed on the surface of these bacteria(44). Moreover, the previous studies, the presence of Lewis^{b} antigen among secretors(45), and Lewis^{a} antigen among non-secretors (46)might play a role in NoV attachment.

In our study, there was a significant difference in the positivity rates of NoV across the different age groups, with the extreme age groups being significantly affected. Our finding is in agreement with some studies (47, 48). The possible explanation for the increased susceptibility of these age groups might be either due to their increased environmental exposure (49)or their weakened immune system (50). Similarly, our findings varied with study sites, where a significant increase was reported at the Debre Tabor and Bahir Dar sites. This might be explained either due to the increased diversity of the population and contact-mediated infection in Bahir Dar sites and/or due to the variability of weather conditions (relatively lower temperature, increased humidity, and increased rainfall) in the case of Debre Tabor sites that favor this virus replication (51, 52).

This is the first study conducted to assess the association between ABO blood groups and NoV infection in Ethiopia by considering multiple sites across all age groups. However, other host genetic factors, including secretory antigens and Lewis antigens, that might have a relationship are not assessed.

Conclusion and recommendation

The positivity rate of NoV was significantly higher among individuals with O blood groups compared to others. Norovirus-GII infected individuals of all blood groups, while NoV-GI affected blood groups A and O. This study might provide an input for viral-host interaction studies and vaccine design programs for NoV. Further large-scale studies are warranted to assess the relevance of this observation and identify other genetic factors that might affect the susceptibility of individuals to the different NoV genotypes.

Abbreviations:

AOR— Adjusted odds ratio
APHI— Amhara Public Health Institute
FUT2— Fucosyltransferase 2
NoVs— Noroviruses
NoV-GII— Norovirus-genogroup II
NoV-GI— Norovirus-genogroup I
ORF— Open reading frame
PCR— Polymerase chain reaction
RNA— Ribonucleic acid
RT-PCR— Real-time reverse transcription polymerase chain reaction

Declarations:

Ethical approval and consent to participate: The project was approved by the University of Gondar institutional review board with reference number V/P/RCS/05//765/2021. Written informed consent/assent was obtained either directly from the participants and/or their parents for children. The study was conducted according to the rules and regulations of the Helsinki Declarations. To maintain confidentiality, the data was recorded with a special code. The study participants were free not to participate or withdraw at any time.

Consent for publication: All authors agree to publish this manuscript in this journal

Data availability: The datasets supporting the conclusions are included within the manuscript. However, upon a reasonable quest, the data are available from the corresponding author [DT].

Competing interests: The authors declare that they have no conflict of interest.

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