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Original Research Article

Distribution of influenza A and B antibodies and correlation with ABO/Rh blood grouping

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ABSTRACT

Background: Influenza is a clinically-significant infection with significant number of globally reported annual deaths. The aim of this study was to study the distribution of influenza A and B antibodies in Najran, the Southwest region of Saudi Arabia, and to investigate the correlation between demographic characteristics and influenza virus antibody levels.
Methods: Enzyme linked immunosorbent assay was used to detect antibody level of influenza A and B. The correlation with ABO/Rh blood groupings was also examined. The total number of participants was 252. Only twenty-four subjects received the flu vaccine.
Results: It was found that 33.7% and 24.1% of unvaccinated subjects were IgG-positive for influenza A and B, respectively. Interestingly, the antibody levels of the unvaccinated participants were higher than the vaccinated group. A significant difference was found between unvaccinated participants with O+ and influenza A and B antibody levels (**p=0.0045). The antibody level was inversely correlated with age in influenza B IgG subjects but not influenza A IgG (r=-0.1379; R squared=0.01900; p=0.0375). Forty-three subjects (17%) were positive for antibodies of both influenza A and B.
Conclusions: IgG antibody positivity is greater in cases of influenza type A compared to influenza B. A significant correlation was found in the unvaccinated group between influenza B IgG antibody levels and age, but not influenza A (*p=0.0375). More research is needed to investigate the role of O+ blood group in influenza infections.

Keywords: Anti-influenza antibodies, Blood groups, Flu vaccine

INTRODUCTION

Influenza virus is a seasonal human pathogen that causes severe symptoms globally. It is also an enveloped orthomyxovirus that possess segmented negative stranded RNA genome.[1] Influenza is a contagious respiratory condition occurring mostly in the winter season and is associated with fever, cough, sore throat, fatigue, and runny and/or stuffy nose. Some infected people develop complications, such as pneumonia, which can be life-threatening and result in death if not adequately treated.[2] Influenza A virus strains including H1N1 and H3N2 are responsible for most of the severe cases and deaths worldwide.[3] It is estimated that seasonal influenza infects up to 15% of the global population and causes nearly half a million deaths.[4]

The virus contains two glycoprotein spikes called hemagglutinin and neuraminidase which undergo constant mutations.[5] This global pathogen can agglutinate erythrocytes of different species via binding to sialic acid receptors, which are linked to galactose via an α 2.6 linkage.[6] It is worth investigating ABO blood groups and influenza disease mechanism. The ABO blood groups were the first human blood typing system to be discovered, which were classified into A, B, AB, and O subtypes according to the presence or absence of erythrocyte antigens identified as A, B, and H.[7] Subsequent discovery has led to identification of another blood group called the Rhesus (Rh) system, which divides blood into either positive or negative based on presence or lack of Rh antigen, importantly the D antigen, on erythrocytes.[8] Beyond the clinical significance of blood groups in immunohematology, numerous publications have reported accumulating evidence that certain ABO blood groups also a play role in various human diseases such as coronary heart disease, disorders of haemolytic transfusion reactions, infectious diseases, malignancies, and neurological disorders.[9]

The relationship between the ABO blood group and human susceptibility to influenza infection has been an interest to researchers since the 1960's.[10] Various observational and serological studies have reported that individuals with O, A, and B blood groups have high tendency of severe influenza infection. However, Horby et al stated that "the data are inconsistent" because of the lack of coordinated efforts related to cohort studies, and this inconsistently necessitates further accumulation of evidence.[2,11]

The objective of this study was to determine seropositivity of influenza A and B in the Southwest, Kingdom of Saudi Arabia (KSA). The second objective was to investigate whether (or not) a correlation exists between ABO/Rh blood groups, age, and the level of influenza antibodies. Third, the difference between vaccinated and unvaccinated individuals was assessed, and whether the antibody level differs between the two groups.

METHODS

Subjects and sample collection

This study was conducted between December 13, 2020 and February 1, 2021 using a random sampling design (Figure 1). All participants (n=269) were fit, healthy adults (>18 years old), recruited among blood donors at King Khalid hospital and maternity children hospital, Najran city, KSA. All blood donors lived in Najran and were not reactive to human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell lymphotropic virus (HTLV), *Treponema pallidum* (syphilis), and *Plasmodium* species (malaria). Out of 269 participants, 228 were not vaccinated, 24 were vaccinated, and 17 had incomplete data (vaccination status was unknown) and were excluded from the study. Demographic data including age, gender, nationality, and influenza vaccination status were obtained using a structured questionnaire.

Five milliliters of venous blood were collected in plain tubes, allowed to clot at room temperature for 20 to 30 min, and centrifuged at 4,000×g for 5 min. The resulting serum was collected, aliquoted into 1.5 ml tubes and frozen at -20 °C until used for antibody testing. The sample size was calculated using OpenEpi open source epidemiologic statistics for public health version 3.01. A total population of 869 participants who have donated blood at the period of sample collection were included. The calculation was performed at a 95% confidence level and 5% marginal error. The sample size, based on these parameters, should have been 267 participants. After implementing the exclusion criteria, 252 participants were included in the study.

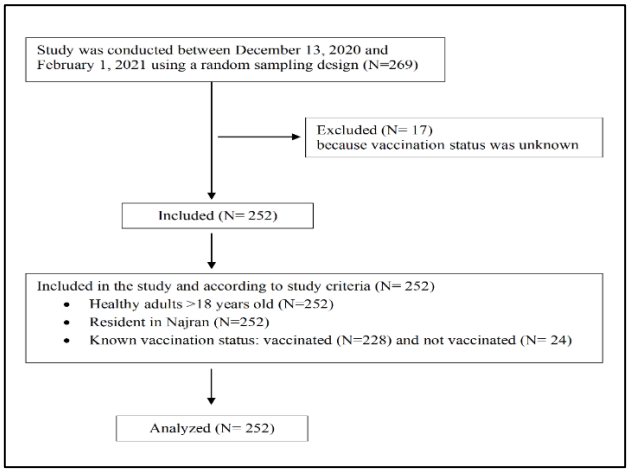


Figure 1: The study design with inclusion and exclusion criteria.

Blood grouping

ABO and Rh blood group tests were conducted using IH-500 fully automated blood typing system (Bio-Rad Laboratories Inc., USA), which provides in vitro serological analysis for blood grouping and antibody detection of blood specimens based on results from gel card images.

Detection of influenza IgG

Enzyme-linked immune-sorbent assay (ELISA) was used to measure influenza A IgG and influenza B IgG levels in the samples according to the manufacturer's instructions (Vircell, Granada, Spain, catalogue number G/M1007-EN-02 and G/M1008-EN-02 respectively). Briefly, 100 µl of the serum diluent were added to all wells, and then 5 µl of the controls and serum samples were added. After reagents addition, washings and incubation, ELISA plates were quantified using SPECTROstar nano absorbance reader (BMG Labteck, Germany) at 450/620 nm. The

level of IgG in each sample was represented as antibody index and calculated using the formula: (sample optical density [OD] / cut off serum mean OD) X 10. According to the kit instructions, the subject is considered negative when the IgG index is <9, positive when the index is above 11, and equivocal if the antibody index lies between 9 and 11.

Statistical analysis

The analyses were conducted using GraphPad Prism software, USA, to statistically determine whether an individual's blood group, age, and vaccination status would impact the level of influenza IgG.

Ethical approval

The study was approved by Najran health ethics committee of general directorate of health affairs at Najran city, KSA (IRB No. 2020-24E). Written informed consent was obtained from all subjects after they received an explanation of the study.

RESULTS

Demographic characteristics

Demographic data include unvaccinated and vaccinated subjects ranging from 18 years to 65 years (Table 1). Table 2 shows descriptive statistics of IgG antibodies against influenza A and B results among the subjects. Averages of IgG levels among the unvaccinated subjects regarding ABO/Rh (Table 3) and nationalities (Table 4) were included. Although there is no statistical significance, it is worth noting the higher values. High influenza A antibody level was noticed with B blood group. The sample size of most nationalities is low apart from Yemeni and Saudi Arabian subjects.

Table 1: Demographic characteristics of participants as respondents of the study.

Demographics characteristics	Frequency (n)		Percentage (%)
	Unvaccinated	Vaccinated	
Age (Years)			
≤ 25	50	4	21.42
26-36	106	9	45.63
37-47	53	7	23.81
48-58	19	3	8.73
>58	0	1	0.39
Gender			
Male	228	24	100
Female	0	0	0
Nationality			
Saudi	142	20	64.28
Non-Saudi	86	4	35.71
Total	228	24	100

Table 2: Descriptive statistics of influenza A IgG and B IgG antibodies results among unvaccinated subjects.

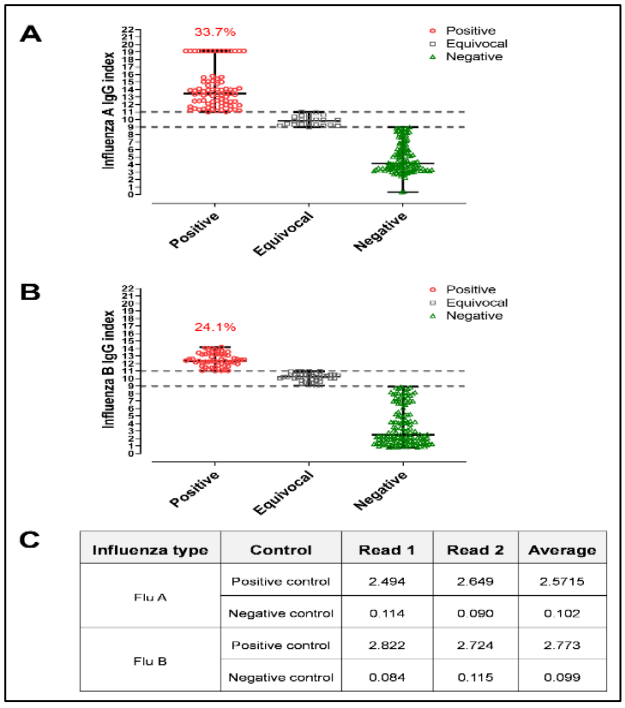
Variables	Influenza A IgG			Influenza B IgG		
	Positive	Equivocal	Negative	Positive	Equivocal	Negative
N	77	22	129	55	36	137
Minimum	11.002	9.003	0.328	11.007	9.059	0.789
Median	13.458	9.815	4.151	12.345	10.219	2.503
Maximum	19.136	10.973	8.980	14.167	10.970	8.893
Mean	14.134	9.840	4.872	12.484	10.175	3.633
SD	2.668	0.629	1.987	0.889	0.499	2.544

Table 3: Averages of IgG antibody levels of unvaccinated subjects with respective ABO/Rh blood groupings.

Variables	N	Influenza A	Influenza B
		Average IgG index	Average IgG index
AB+	8	8.65	7.74
A+	51	9.21	6.88
B+	20	10.36	8.07
O+	126	7.92	6.55
A-	9	8.55	6.54
B-	4	9.06	8.63
O-	10	7.51	5.73

Table 4: Averages of IgG antibody levels of unvaccinated subjects with respective nationality. The nationality of one subject was unknown.

Country	N	Influenza A	Influenza B
		Average IgG index	Average IgG index
Unknown	1	13.16	12.26
Nigeria	1	11.32	10.22
India	2	11.57	8.99
Bangladesh	3	8.97	8.12
Pakistan	5	11.81	9.52
Sudan	10	7.34	6.21
Egypt	11	8.01	5.27
Yemen	54	7.77	5.75
Saudi Arabia	141	8.64	7.14



Influenza type	Control	Read 1	Read 2	Average
Flu A	Positive control	2.494	2.649	2.5715
	Negative control	0.114	0.090	0.102
Flu B	Positive control	2.822	2.724	2.773
	Negative control	0.084	0.115	0.099

Figure 2 (A-C): Overall levels of IgG antibodies against influenza A and influenza B virus among unvaccinated subjects. The figure shows that 33.7% of the overall subjects have influenza A IgG antibodies while 24.1% are positive for influenza B IgG. Controls readings within range as suggested by manufacturer.

Correlation between ABO/Rh blood groups and positive unvaccinated subjects

First, the overall levels of IgG against influenza A and influenza B of the unvaccinated were determined. Negative, equivocal, and positive samples were as shown in Figure 2. It was noticed that 33.7% from all subjects were positive for influenza A.

However, only 24.1% of subjects developed antibodies against influenza B. Very high readings were shown in 14 samples (Figure 2A). According to the kit instructions, 5µl of the serum sample should be added to the concerned well containing 100µl of sample diluent. No further dilution was done for all samples even for samples with very high antibody results. With regards to ABO blood groupings, the anti-influenza A IgG was higher than anti-influenza B. No statistical difference was found between antibody level from different blood groups (Figure 3). However, a statistical difference was found between influenza A and B antibodies from the O+ group (Figure 4).

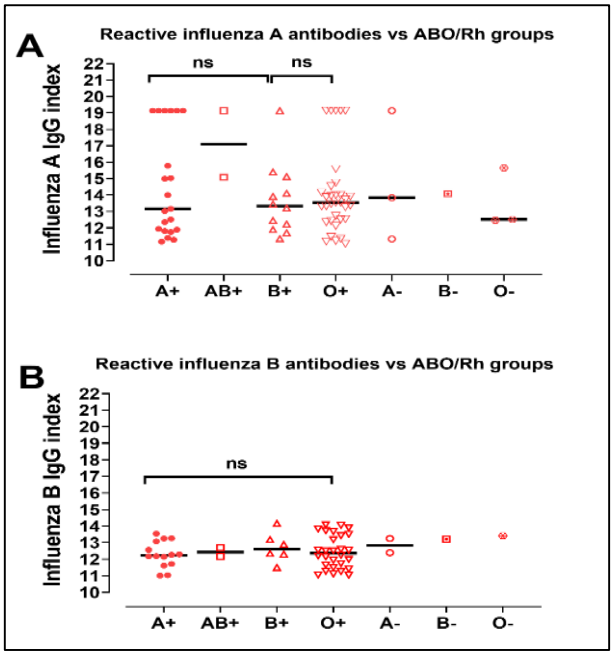


Figure 3 (A and B): All blood groups vs. anti-influenza IgG antibody levels from positive unvaccinated subjects of influenza A and B. ABO blood groups with low sample size were not compared. NS=not significant.

Influenza A IgG and B IgG antibodies versus age and vaccination status

Although 21.4% of the unvaccinated and vaccinated subjects aged between 18 years to 25 years, and 23.8% were between 37 and 47 years, the highest levels of antibodies belonged to the group who aged 26 to 36 years that represented 45.6% of the overall study population,

but the correlation was not statistically significant. Figure 5 indicates that age correlated with anti-influenza antibody levels. An inverse correlation noticed between age and influenza B IgG levels of the unvaccinated group but not influenza A (p=0.0375). One of the objectives of the current study was to examine the vaccinated subjects. No statistical difference was observed between the vaccinated and unvaccinated subjects (Figure 6).

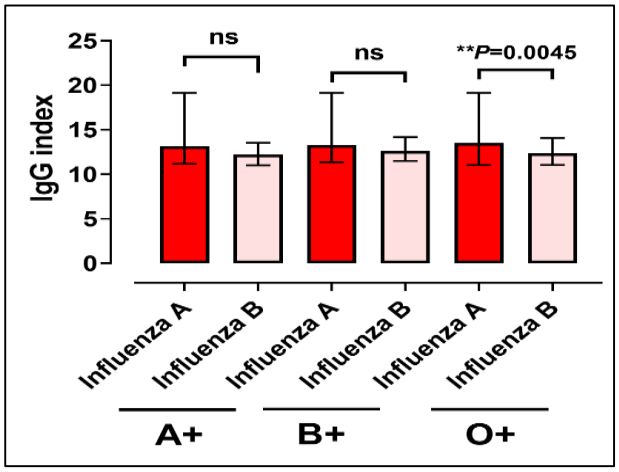


Figure 4: Influenza A and B antibody indices of the positive "unvaccinated" group versus three blood typing groups A+, B+, and O+. Statistical analysis was done using Mann-Whitney U test.

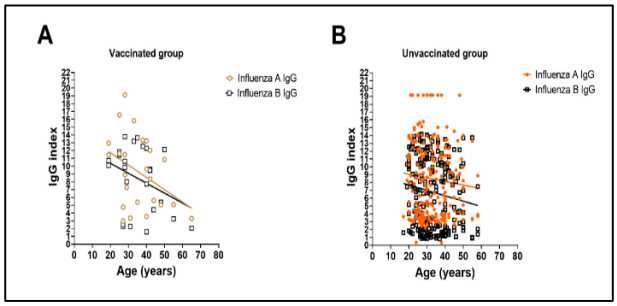


Figure 5 (A and B): Age versus IgG index of vaccinated group. No significance found in vaccinated group of both influenza types. A significant correlation was found between age and influenza B IgG antibodies of unvaccinated group, (r=-0.1379; R^2=0.01900; p=0.0375) but not influenza A (r=-0.08713; R^2=0.007592; p=0.1899). For vaccinated group, statistical data are as follows: (r=-0.3870; R^2=0.1498; p=0.0617) for influenza A; and (r=-0.3426; R^2=0.1174; p=0.1012) for influenza B.

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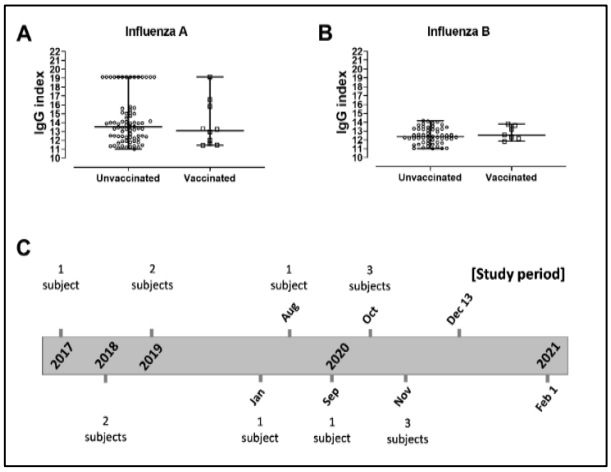

Figure 6 (A-C): Represents statistical analyses of vaccinated and unvaccinated group no statistical difference was found using Mann-Whitney test. Study timeline and vaccination periods. Out of 24 vaccinated subjects, 18 answered question about vaccine time. There are 4 subjects who did not know exact vaccine administration time.

DISCUSSION

This study worked on the following objectives: (1) influenza virus seropositivity among blood donors in Saudi Arabia according to demographic features of the population sample. (2) Correlation between ABO/Rh blood grouping and antibody positivity. (3) Differences between vaccinated and unvaccinated participants in antibody level.

Influenza virus continues to cause seasonal infections and deaths worldwide.[4] A study similar to ours is by Mahallawi et al interpreted their data as an antibody index. They studied the correlation between age and influenza A and B antibodies in 360 male participants. Interestingly, a significant correlation between age and influenza B antibody levels but not with influenza A was found in our study, unlike Mahallawi et al study in which they observed the significance with influenza A but not B.[12]

We found that for influenza A, the antibody level was highest compared to influenza B. Fourteen participants (5.5%) presented the highest antibody level the test can detect. Samples' dilution was according to the manufacturer's instructions in which 5 µl of the serum sample were added in 100 µl serum diluent, and no

further dilutions done to all samples. Our study included ABO/Rh blood typing as a possible variable. However, no statistical difference was found between influenza A and B in all blood groups with the exception of O+ (Figure 4). The number of O+ subjects was higher than in all other blood types, hence the difference. In Figure 3, statistical analyses were done for groups with higher number of samples. In a review of 32 publications about the distribution of ABO and Rh blood groups in Saudi Arabia, the results were in line with ours. This comprehensive review found that 51%±8% were O, 27%±4% A, 18%±8% B, and 4%±2% AB, and majority of blood donors (92%±2%) were Rh positive.[13] In our study, 55.3% were O+, 22.4% A+, 8.8% B+, and 3.5% were AB+. These percentages are in line with these studies.

It is worth reviewing related publications. In a narrative review by Dalbhi et al geographical locations determined the responses to influenza H1N1 treatment measures, and acute kidney injury developed in 33% of patients who were admitted to the hospital.[14] A retrospective study in Saudi Arabia concerning influenza A from 2010 to 2016, 54.2% (9262 cases) were confirmed, mostly in the Riyadh Region. The case fatality rate was 3.6%.[15] Influenza is a clinically significant infection that can cause severe symptoms. Chronic rhinosinusitis is common in the Southwest, KSA, at different age groups including children.[16] In Abha city, Southwest of Saudi Arabia, 312 primary health care workers including nurses were surveyed to assess the coverage of seasonal influenza vaccine. The coverage rate was 45.5%. Surprisingly, 40% feared the side effects and 23% lacked knowledge about the vaccine. Misconceptions about the vaccine was a barrier to increasing the coverage rate.[17] While our study included vaccinated subjects, the low number of vaccinated participants (24 out of 252) could be indication that most people had some concerns about the vaccine. In Western Saudi Arabia, a cross-sectional study found that confirmed cases of influenza virus between 2015 and 2019 were reported using polymerase chain reaction (PCR), 27.3% with influenza A and 30.7% with influenza B (n=1928). October was the peak month of infections, and no co-infection with subtypes was reported.[18] However, in our study, the reverse was true in which 33.7% were influenza A positive, 24.1% were influenza B positive, and 17% were infected with both types. It can be concluded that around one-third of the population had influenza.

In our study, only male subjects were included which might be considered as a limitation. Usually, blood donors were male, probably because they experience less vasovagal reaction when compared to females.[19] We attempted to collect female samples, however; there were challenges to do that. Females are seldom donating blood. While the overall number of samples were 252, most of them were unvaccinated. Therefore, most statistical analyses were done with the unvaccinated group variables. Only 24 out of 252 subjects have taken the vaccine, which suggests that the awareness about flu vaccine should be heightened. Out of the 24 vaccinated subjects, 18 knew the period in which flu vaccine has been administered. The vaccine has been taken since 2017 through 2020. While the results were confirmed with laboratory tests, no statistical difference was found between the two groups (Figure 6). It is difficult to suggest that the level of antibody in vaccinated subjects was less than the unvaccinated group (Figure 6), since re-exposure to influenza virus might have increased the antibody level with time.

It can be concluded that about one-third of the population had influenza infection. No significant correlation was found between ABO/Rh blood groups with influenza virus among unvaccinated subjects, apart from O+ group versus antibody levels of influenza A and B. Although the level of antibody was higher in the unvaccinated subjects, flu vaccines are highly recommended to reduce the global burden of this clinically-significant virus. All in all, the number of vaccinated subjects was small, and awareness programs should be implemented about flu vaccine to reduce the disease burden.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Najran Health Ethics Committee of General Directorate of Health Affairs at Najran city, KSA (IRB No. 2020-24E).

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