

Natural immunity to influenza A and B among Saudi blood donors in Al Madinah Al Munawarah, Saudi Arabia

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ABSTRACT

الأهداف: معرفة مدى انتشار الأجسام المضادة في مصلى المتبرعين بالدم السعوديون وبقاء الأجسام المضادة من النوع الذي يدوم طويلاً وربط ذلك بعدة عوامل مثل مؤشر كتلة الجسم والعمر.

المنهجية: جميع المشاركين هم سعوديون كانت لديهم الرغبة في المشاركة وتم قبولهم للتبرع. أجريت هذه الدراسة خلال الفترة من أبريل 2019م ويوليو 2019م. وكان المشاركون بالغين أصحاء سعوديون كانت لديهم الرغبة في المشاركة وتم قبولهم للتبرع ببنك الدم المركزي، المدينة المنورة، المملكة العربية السعودية. تم استخدام تحليل الكشف عن الأجسام المضادة بطريقة الأليزا مستويات الجلوبيولين المناعي (IgG) ضد الأنفلونزا A و B في عينات المصل.

النتائج: أظهرت النتائج أن الإنفلونزا من النوع أ كانت انتشارها بين المتبرعين 29.2% بينما النوع ب كانت 38.6% كما أن العلاقة بين انتشار الأجسام المضادة للفيروسين كانت قوية وإيجابية ($r=0.708$, $p>0.001$). كذلك كان هناك تناسب طردي قوي بين تركيز الأجسام المضادة للفيروس من النوع أ وزيادة العمر ($p>0.01$). لم تظهر الدراسة علاقة بين تركيز الأجسام المضادة للفيروس أ و ب وزيادة مؤشر كتلة الجسم ($p<0.05$). أخيراً، أظهر تحليل الانحدار الخطي أنه يمكن التنبؤ بمستوى الجسم المضاد للأنفلونزا أ حسب العمر ($p>0.05$) ومؤشر كتلة الجسم ($p>0.06$).

الخلاصة: أظهرت النتائج أن قرابة ثلث المتبرعين بالدم السعوديين لديهم أجسام مضادة للفيروس تم اكتسابها جراء الإصابة بالفيروس حيث تم استبعاد من تم تطعيمهم ضد الفيروس.

Objectives: To investigate the seroprevalence of influenza viruses (A and B) among blood donors in Kingdom of Saudi Arabia.

Methods: The present investigation was conducted between April 2019 and July 2019. Participants were healthy adults recruited from the central blood bank Al Madinah Al Munawarah, Kingdom of Saudi Arabia. Immunoglobulin G (IgG) levels against influenza A and B were measured in serum samples using ELISA.

Results: The results showed that 29.2% of the sample had significant concentrations of influenza A IgG antibody, whereas 38.6% had significant concentrations of influenza B IgG antibody. A strong correlation was found between the levels of influenza A and influenza B antibodies ($r=0.708$, $p<0.001$). The number of individuals identified as negative for influenza A IgG antibody increased with age ($p<0.01$). In addition, no correlations were identified between influenza A IgG and influenza B IgG and body mass index (BMI), ($p>0.05$). Finally, linear regression analysis showed that the level of influenza A antibody can be predicted by age ($p<0.05$) and body mass index (BMI) ($p<0.05$).

Conclusion: Approximately one-third of Saudi Arabian adults presented significant levels of influenza A and B antibodies in our study. Demographic factors, including age and BMI, might contribute to influenza A antibody levels.

Keywords: influenza A, influenza B, seroprevalence, IgG, Al Madinah Al Munawarah

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Influenza is a highly transmittable, acute viral infection of the respiratory tract.¹ Influenza belongs to the family *Orthomyxoviridae*, and 4 types of influenza have been identified, including influenza A and B, which are enveloped ribonucleic viruses.^{2,3} The global burden of influenza is high.^{4,5} Approximately, 100,000 individuals die each year from direct influenza virus infections of the lower respiratory tract.⁴ In addition, more than one-quarter million individuals die from influenza-related complications globally.^{6,7} A recent review highlighted the extra-pulmonary complications (damage to organs other than the lungs) associated with influenza infections that can increase the healthcare burden associated with influenza infections.⁸ Individuals with underlying illnesses, such as respiratory and cardiovascular diseases, reduced immunity, and asthma, are at high risk of developing severe influenza symptoms, which may result in hospital admission. The implementation of influenza vaccine programs might limit the spread of the disease to susceptible individuals and decrease the overall burden of the disease.⁹ The usefulness of vaccination programs depends largely on the selection of appropriate vaccines that target those influenza strains that are common to the country in question. Therefore, determining the seroprevalence of the population is important for guiding the selection of influenza vaccine strains.

The Ministry of Health (MOH), Kingdom of Saudi Arabia (KSA) has established a free influenza vaccination that is available at all primary health care facilities. However, limited studies were performed to investigate the effectiveness of this vaccine in this country. For example, in a small sample-size study conducted in Al Madinah Al Munawarah, KSA, the influenza vaccine was shown to be effective for initiating humoral immune responses in recipient subjects.¹⁰ Additional national studies remain necessary to examine the effectiveness and persistence of anti-influenza antibodies among vaccinated individuals. In general, natural influenza virus infections produce wide-ranging and long-lasting immunity. In contrast, the immune response that develops in response to vaccination is narrow and short-lived.^{11,12}

In the current study, healthy blood donors were examined to investigate the current seroprevalence of influenza A and B Immunoglobulin G (IgG)

antibodies in KSA. Blood donors have also been used in similar previous studies performed in Australia and in Germany.^{13,14}

Other factors, such as age and obesity, have been shown to affect the humoral immune response.^{15,16} For example, a recent study showed that age was a valid predictor of the humoral immune response to pathogens and vaccines. Reduced and delayed immune responses to influenza viruses have also been reported in obese individuals.^{15,16} In addition, a study conducted on a trivalent influenza vaccine showed a greater decline in the antibody titer in obese individuals compared with that in normal-weight individuals when examined 12 months after vaccination.¹⁷

The study aim to investigate the seroprevalence of influenza A and B IgG antibodies in Al Madinah Al Munawarah, KSA. In addition, the results were compared based on various demographic parameters, including age and body mass index (BMI).

Methods. This study was conducted using a cross-sectional design and was conducted between April 2019 and July 2019. All participants (N=360) were healthy adults (>18 years old), recruited among blood donors at the central blood bank (CBB), Al Madinah Al Munawarah, KSA. On average, approximately 90 individuals donate blood to the CBB each day. During the recruitment period, every 5th subject from the list of donors was invited to participate in the study. Subjects with human immunodeficiency virus (HIV), hepatitis B virus (HBV), human T-cell lymphotropic virus (HTLV), hepatitis C virus (HCV), syphilis, and malaria were excluded from the study. Any subject who has ever received a flu vaccine was also excluded (n=13). The Human Research Ethics Committee of the College of Applied Medical Sciences at Taibah University, KSA, approved the study procedures (IRB No: SREC/AMS 2019/62/CLD). Written informed consent was obtained from all subjects after a full explanation of the study objectives was provided. The sample size was calculated using G power, version 3, and at least 240 participants were estimated to be necessary to achieve a power of 0.8, an alpha of 0.05, and moderate effects.

Blood samples were obtained in plain tubes. After coagulation was completed, the samples were immediately centrifuged at 4,000×g for 5 minutes. The serum was collected, aliquoted into 1.5 ml tubes, and immediately stored at -20°C until used for enzyme-linked immunosorbent assay (ELISA) testing.

Demographic data, including age, gender, nationality, and influenza vaccination status, were obtained using a structured questionnaire. Weight was

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obtained using a digital body weight scale, whereas height was measured using a wall-based body height chart. Body mass index was calculated from weight and height, as previously described.¹⁸

Indirect ELISA was used to measure influenza A and influenza B IgG antibody levels in the samples (Vircell, Granada, Spain, catalog number L-G/M1007-EN-02 and L-G/M1008-EN-02), according to the manufacturer's instructions. The ELISA plates were quantified using an EL x 808 Absorbance Reader (BioTek, USA) at 450/620 nm. When the level of IgG was below the cut-off value, the subject was considered negative. However, when the IgG level was above the cut-off value, the subject was considered positive. Equivocal results were also interpreted, according to the kit instructions.¹⁹

Statistical analysis. The Statistical Package for Social Sciences, version 22 (IBM Corp, Armonk, NY, USA) was used to perform data analysis. Pearson's correlation coefficient and linear regression analyses were conducted to analyze the relationships between IgG data and demographic variables. A p -value ≤ 0.05 was considered significant.

Results. A total of 360 individuals volunteered to participate in this study from the Al Madinah Al Munawarah region, KSA. The response rate was 62%. The demographic characteristics of the sample population are presented in **Table 1**. The mean age was 32.5 ± 10.2 years, and the mean BMI was 28.67 ± 5.9 (range 14.9-49.2). Among all participants, approximately 96% ($n=345$) were nonsmokers. All participants were healthy blood donors and did not suffer from any chronic or inherited diseases.

The ELISA assay was used to measure influenza A and B IgG antibody concentrations for all 360 participants. **Figure 1** shows the distribution of participants according to the measured levels. Among the sample population, 29.2% of individuals ($n=105$) had significant concentrations of influenza A antibody, whereas 61.4% participants ($n=221$) were identified as negative and 9.4% ($n=34$) were identified as equivocal. Additionally, 38.6% of participants ($n=139$) had significant concentrations of influenza B antibody, whereas 56.1% ($n=202$) participants were found to be negative and 10.6% ($n=39$) of participants were found to be equivocal.

Among the sample population, only 25% ($n=90$) presented significant levels of both influenza A and B IgG antibodies simultaneously. Furthermore, a strong correlation was found between the levels of influenza A and influenza B IgG antibodies ($r=0.708$, $p<0.001$),

Table 1 - Demographic data of participants.

Parameter	n (%)
Mean age (years)	32.5±10.2
Age range	18-66
Mean BMI	28.67±5.9
BMI range	14.9-49.2
BMI categories	
<18.5	5 (1.3)
18.5-24.9	101 (28.1)
25-29.9	125 (34.7)
30-39.9	112 (31.1)
>40	17 (4.7)
Gender	
Male	360 (100)
Female	0 (0)
Tobacco use	
Yes	15 (4.2)
No	345 (95.8)

BMI: body mass index

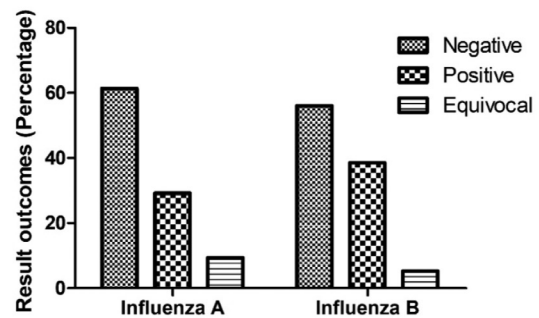


Figure 1 - Distribution of participants according to influenza A and influenza B antibodies. Influenza A and influenza B antibodies were measured using enzyme-linked immunosorbent assay (ELISA) of serum samples. Values less than 9 IU/ μ L were considered negative. Values between 9-11 IU/ μ L were considered equivocal. Values more than 11 IU/ μ L were considered positive.

using Pearson's correlation coefficient analysis (**Figure 2**).

To examine the impacts of age and BMI on influenza A and B antibody levels, participants were stratified into various subgroups (**Figures 3 & 4**). A significant negative correlation was found between influenza A antibody levels and participant age ($r=-0.138$, 95% confidence interval (CI): [-0.238 - -0.035], $p=0.009$). No significant correlation was detected between age and influenza B antibody levels ($r= -0.028$, 95% CI: [-0.1316-0.07498], $p=0.588$), (**Figure 3**).

Figure 4 shows the relationships between BMI and antibody levels. No significant correlations were

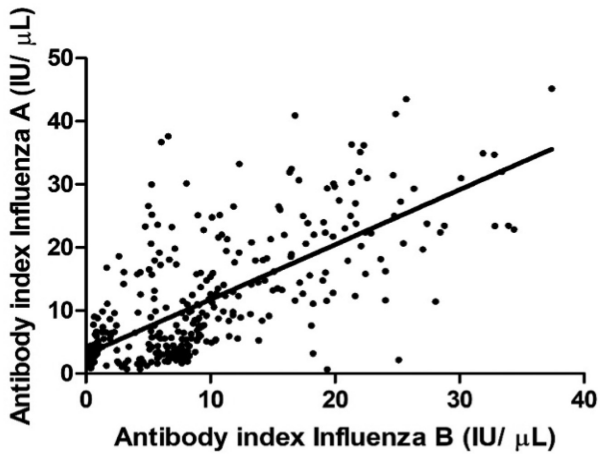


Figure 2 - Correlations between the levels of influenza A and influenza B antibodies. Values are expressed in IU/μL. A strong correlation was found between the level of influenza A antibody and the level of influenza B antibody ($r^2=0.503$, $p<0.001$, $N=360$).

detected between BMI and either influenza A antibody levels ($r=-0.055$, 95% CI: [-0.1584-0.04777], $p=0.290$), or influenza B antibody levels ($r=0.0186$, 95%CI: [-0.1393-0.2004], $p=0.724$), (Figure 4).

Linear regression analysis ($R=0.1763$, $R^2=0.0314$, adjusted $R^2=0.026$) showed that the level of influenza A antibody can be predicted by age ($B=-0.112$, $p=0.018$, 95% CI: [-0.205 to -0.020]) and BMI ($B=-0.104$, $p=0.046$, 95% CI: [-0.206 to -0.002]). However, linear regression analysis showed no association between influenza B antibody levels and either age ($B=-0.040$, $p=0.43$, 95% CI: [-0.141-0.060]) or BMI ($B=-0.046$, $p=0.41$, 95% CI: [-0.157-0.064]).

Discussion. In this study, the seroprevalences of influenza A and B IgG antibodies were examined in Al Madinah Al Munawarah, KSA. The results showed that 29.2% of the studied population had significant

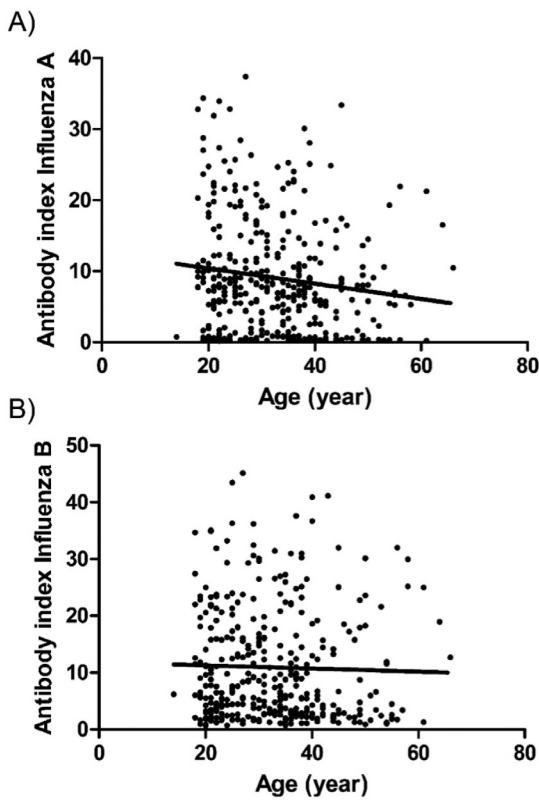


Figure 3 - Correlations between the levels of influenza A and influenza B antibody and age. A) A significant correlation between influenza A antibody levels and age was found ($r= -0.138$, $p<0.01$). B) No correlation between influenza B antibody levels and age was found ($r= -0.028$, $p>0.05$).

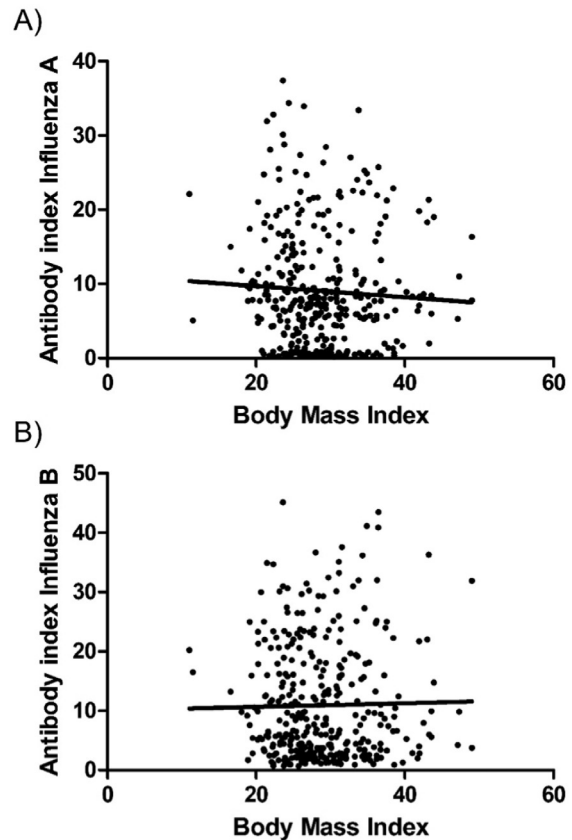


Figure 4 - Correlations between the levels of influenza A and influenza B antibody and BMI. A) No correlation between influenza A antibody levels and BMI was found ($r= -0.055$, $p>0.05$). B) No correlation between influenza B antibody levels and BMI was found ($r=0.0186$, $p>0.05$).

concentrations of influenza A IgG antibody, whereas 38.6% had significant concentrations of influenza B IgG antibody. In addition, a strong correlation was found between the levels of influenza A IgG and influenza B IgG antibodies, and a strong negative correlation was found between influenza A IgG antibody levels and age.

According to the present findings, approximately 29.2% of the sample population were positive for influenza A IgG antibody. Because the study participants were restricted to those who have never received an influenza vaccine, these significant levels of influenza A IgG antibody are likely due to humoral immune responses due to influenza infections during the individuals' life times. The reported influenza A IgG-positive frequency in this study was similar to that from another study performed in the Middle East, which reported a prevalence of 24% for Influenza A (H3N2) IgG.²⁰ In a recent study performed in KSA and based on the information obtained from the influenza A (H1N1) surveillance project, which was carried out from 2010-2016, a seasonal trend was observed, with a fatality rate of approximately 3.6%.²¹ When examining influenza B, approximately 38.6% of our sample population were positive for influenza B IgG antibody. A similar percentage was reported by a study conducted in Riyadh, KSA in 2016, with a reported prevalence of 35% for influenza B IgG-positive individuals. However, the sample size examined in Riyadh study was relatively small (n=100) and the nationality of the participants was not disclosed.²² Infection with influenza B is frequently misinterpreted as a more minor disease than infection with influenza A. However, several studies have verified that influenza B can also cause epidemics that result in a significant proportion of people requiring hospitalization, similar to those driven by influenza A.^{23,24} Typically, B-Victoria infects naive children/adolescents, whereas B-Yamagata infects both children and adults.²⁵⁻²⁷

The influenza vaccine is seasonal and is recommended to be administered in KSA from September-March, which corresponds to the seasonality of Influenza in KSA. Influenza B virus has been shown to contribute considerably to winter respiratory disease, causing seasonal epidemics every 2 to 4 years. Influenza A epidemics have been reported to peak approximately 2 to 3 weeks earlier than influenza B epidemics in most Gulf states.²⁰ In the present study, samples were collected after the winter season, slightly after exposure to the viruses would be expected. Approximately one-quarter of participants were IgG-positive for both influenza strains A and B. Many factors are associated with

influenza infections, such as exposure, transmission pressure, which is influenced by crowding or population density, seasonality, and duration. Thus, the fraction of the Saudi Arabian population that were IgG-positive for both influenza strains appears to be more susceptible to influenza virus infection compared with the rest of the sample population. In general, human immunity reproduces and is significantly prejudiced via former influenza infections or vaccinations.^{28,29} More studies remain necessary to investigate these findings.

The association between influenza A IgG levels and the age of participants agrees with the findings of other studies, which showed an age-dependent immune response.^{30,31} The acquisition of higher antibody titrations among young participants is most likely due to recurrent viral exposure viruses and the presence of a strong immunological memory response compared with older individuals. However, among children, an inverse relationship between age and IgG influenza A and B levels has been reported in the Netherlands. Therefore, more studies of influenza viruses in KSA are necessary to examine the patterns of infection and epidemiology and to uncover the mechanisms that underlie the observed association between age and antibody titration.

The present study did not find any correlation between BMI and either influenza A or B IgG levels. However, linear regression analysis indicated that influenza A IgG levels were predicted by BMI. This result agreed with those reported by previous studies, in which BMI correlated with the immune response to influenza and other bacterial respiratory infections.^{15-17,32} In addition, epidemiological data have suggested a relationship between body weight, the risk of infection, and the outcomes of infectious diseases.³²

The majority of studies conducted in KSA examining the spread of respiratory infections (including influenza) have been performed among Hajj and Umrah populations.³³⁻³⁶ The collection of comprehensive data regarding the prevalence of influenza viruses in KSA remains necessary. Thus, systematic national laboratory surveillance is strongly recommended to provide a clear picture of the spread of viral infections throughout the country. The findings presented in this study might provide feedback to the healthcare stakeholders to explore the dynamics of viral transmission throughout the country and identify prevention measures to minimize the spread of influenza and, consequently, lower the infection burden on health sectors. Moreover, implementing a national vaccination campaign would be a useful prevention method to improve the acquired immunity against influenza viruses.

Study limitations. Among the study limitations is the stratification of the sample into different age and BMI categories. Therefore, the confirmation of the present findings in a larger study sample is strongly recommended. In addition, the present study was conducted on blood donors in Al Madinah Al Munawarah, KSA, which may induce population biases. Therefore, expanding the study to include other regions in KSA, with a better representation of the overall population, is also recommended for future investigations.

In conclusion, the present study reported the seroprevalences of influenza A and B IgG antibodies in a population in Al Madinah Al Munawarah, KSA. Approximately 29.2% of the sample population had significant concentrations of influenza A IgG antibodies and 38.6% of influenza B IgG antibodies. In addition, in one-quarter of the sample, significant concentrations of both influenza A and B IgG antibodies were found. Factors such as age and BMI might contribute to Influenza A IgG levels. These results can provide feedback to healthcare providers in Al Madinah Al Munawarah, KSA, although more comprehensive studies that include other regions of the country are strongly recommended.

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