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ORIGINAL RESEARCH article

The seroprevalence of IgM and IgG antibodies production among expected COVID-19 patients: A retrospective cohort study

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Abstract: Antibody tests can identify people with a resolving or past severe acute respiratory syndrome coronavirus 2 infection and thereby help researchers and public health experts better understand the epidemiology of severe acute respiratory syndrome coronavirus 2. This study is a retrospective study that included 187 Libyan individuals, who attended Attshkhesy (the diagnostic) laboratory in Alkhoms City, Libya, between January 01, 2021, and August 28, 2021. The mean ages of males and females were 48.8 and 46.8, respectively. The study utilized the CLIA quantitative antibody test. To perform the CLIA quantitative antibody test, a high throughput assay apparatus known as the YHLO-iFlash 1800 Chemiluminescence Immunoassay Analyzer was utilized, along with assay reagents called iFlash-SARS-CoV-2 IgM/IgG (manufactured by YHLO Biotech, Shenzhen, China). In female subjects, the concentration of severe acute respiratory syndrome coronavirus 2 IgM was higher than that of IgG in all age groups. Interestingly, in male subjects, the results showed the opposite, where the concentration of severe acute respiratory syndrome coronavirus-2 IgG was much higher than that of IgM in all age groups. When male data were plotted against the female data, the concentration of severe acute respiratory syndrome coronavirus 2 IgM in females was much higher than that of IgM in males in all age groups. Merged IgM-male and IgM-female results showed that IgM concentrations were higher in females than males at all age groups, which means that the incidence of recent COVID-19 infection was higher in females than in males. On the other hand, the IgG antibody prevalence in females was always higher than in males except in age groups 41-50 years and 51-60 years, which can be used as an indicator of high acquired immunity among females due to possible reinfection of females with COVID-19 virus.

Introduction

On December 31, 2019, the pneumonia outbreak was attributed to a novel strain of coronavirus known as 2019-nCoV [1], later renamed SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). The Wuhan strain has been identified as a new strain of Beta-coronavirus from group 2B, sharing approximately 70.0% genetic similarity with SARS-CoV [2]. The inadequate diagnosis of COVID-19 has contributed to the severity of the disease due to the stress caused by false positive results and the spread of the disease due to false negative results. The main reason for misclassifying symptomatic patients as either having COVID-19

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or not was the lack of RT-PCR test sampling of respiratory specimens from the lower tract [3]. A timely diagnosis through serological testing provides a better and more comprehensible way of understanding the patterns of SARS-CoV-2 IgG/IgM seroconversion [3]. The detection of IgG/IgM antibodies is crucial in determining the duration and origin of humoral responses against SARS-CoV-2, as these antibodies can be detected a few days after the onset of the disease and may persist in the body for years after infection [3]. In the case of COVID-19, the IgM and IgG responses can be observed from the second week of the disease. However, in coronaviruses, IgM and IgG levels tend to diminish over time in humans. For instance, IgG antibodies against SARS-CoV-1 were found to decrease approximately two years after infection, rendering the individual susceptible to reinfection once the immune response has completely diminished [4]. Similarly, in MERS-CoV, IgG levels gradually declined after one year from the onset of infection. Understanding the duration of protective immunity is crucial to preventing reinfection and predicting the response to vaccination [5]. Several serologic assays, such as the chemiluminescence immunoassay (CLIA) assay, have been developed to combat this pandemic. Antibody tests, in particular, are useful in assessing the prevalence of the disease in the population, controlling the spread of infection, evaluating the efficacy of new vaccinations, and indicating the severity of COVID-19 [5, 6]. Therefore, this study aimed to examine the prevalence of IgG/IgM antibodies among suspected COVID-19 patients using the CLIA quantitative antibody test. This study specifically focuses on assessing IgM and IgG antibodies in various potential COVID-19 subjects in the city of Al-Khums, Libya, as no specific study of this nature has been conducted in this area, to the best of our knowledge. This research will aid in predicting the likelihood of reinfection among the local population and estimating the duration and effectiveness of vaccines, as well as the need for vaccine boosters.

Materials and methods

CLIA quantitative antibody test: This test was conducted on a total of 187 individuals, comprising 66 females and 121 males. The means of the age of males and females are 48.8 and 46.8 years, respectively. The goal of this test was to determine their IgG/IgM antibody statuses. The blood samples of each volunteer were collected between the dates of January 1 and August 28, 2021, at the Attshkhesy (the diagnostic) laboratory in Al-Khums City, Libya. To perform the CLIA quantitative antibody test, a high throughput assay apparatus known as the YHLO-iFlash 1800 Chemiluminescence Immunoassay Analyzer was utilized, along with assay reagents called iFlash-SARS-CoV-2 IgM/IgG (manufactured by YHLO Biotech, Shenzhen, China). The testing procedure adhered to the official guidelines [7]. A cutoff value of 10 AU/ml was established for the CLIA quantitative antibody test. The antibody test targeted the S antigen, which has the potential to stimulate the production of neutralizing antibodies, and the N antigen. Before measuring the CLIA samples, a quality check test was conducted daily. The company determined the expected value and confidential range of the calibration reagent for each lot, and only after confirming that the values fell within the predetermined range were the tests carried out on the participants.

Participant's acceptance criteria: This study included individuals who have been infected or harbor suspicions of being infected by the coronavirus, regardless of the presence of symptoms or prior administration of the Coronavirus vaccine. Ethics approval and consent to participate this study was granted by the Faculty of Pharmacy, El Mergib University, Al-Khums, Libya. This retrospective study was performed using archival data; therefore, there was no need to obtain patient consent.

Statistical analysis: The differences in IgM and IgG antibody prevalence in serological assays were expressed as mean±SEM by GraphPad Prism 6 (GraphPad, USA) software. Statistical significance was tested using two-way ANOVA, followed by Sidak's post hoc test. A p-value of 0.05 was considered significant and is indicated with asterisks with *, **, or *** according to the level of significance.

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Results

A total of 187 individuals were included in this study. The mean ages of the males and females were 48.8 and 46.8 years, respectively. Despite their ages being variable, no data was recorded from ages less than 10 years old and the proportion of the males was 64.7% (n=121). **Figure 1** shows the general pattern of distribution of individuals according to their gender, age, and SARS-CoV-2 IgG and IgM antibody titers in their serum samples.

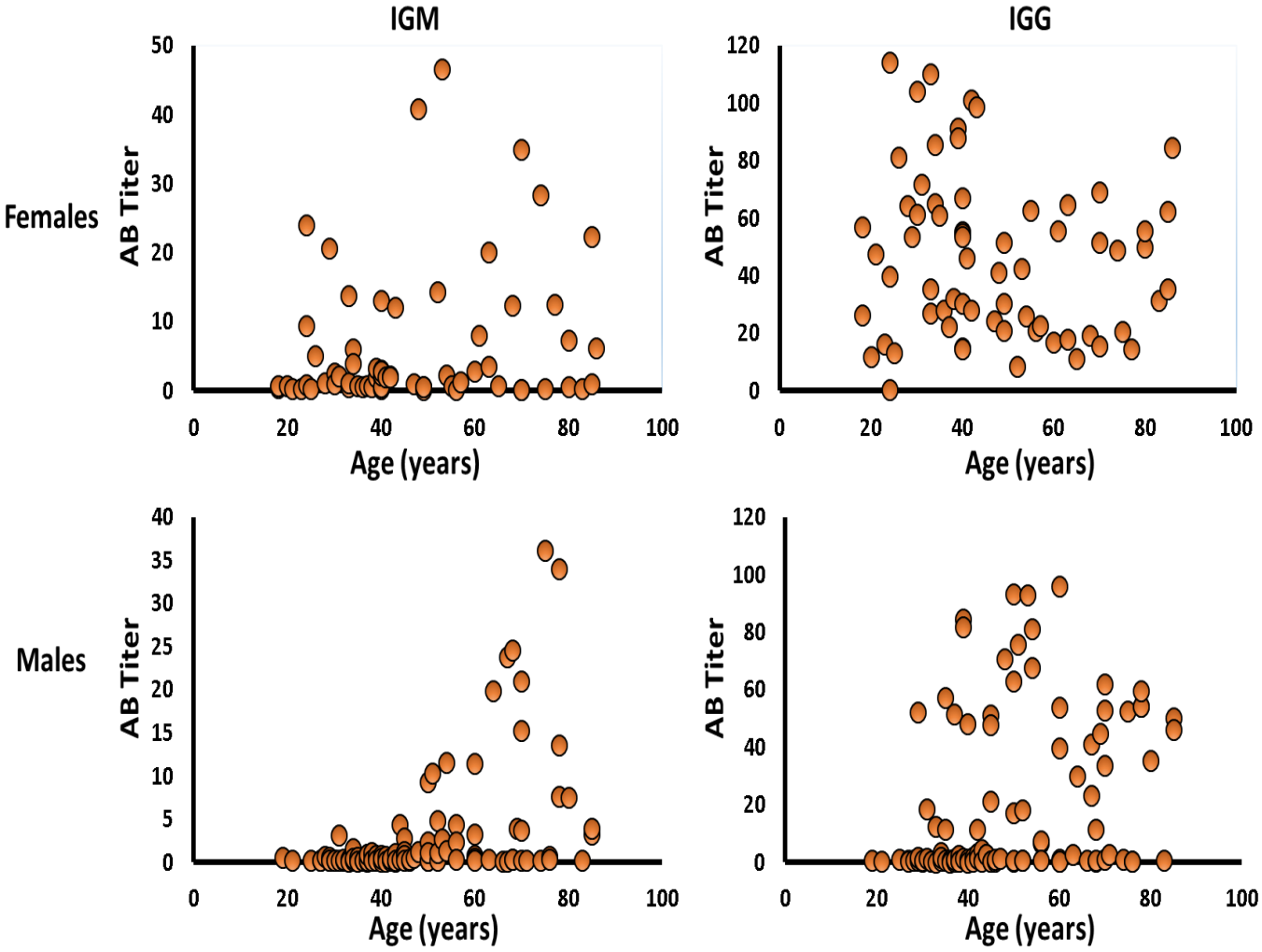

Figure 1: General pattern of distribution of the individuals according to gender, age, and SARS-CoV-2 IgG and IgM antibody titers in their serum samples

The distribution of the male subjects according to their age is presented in **Figure 2**. The highest IgG antibody concentration recorded in the age group 51-60 years was at a mean of 33.9±9.4. Data showed a general pattern of elevated IgG antibody concentrations at ages older than 50 years. The age group (21-30 years) included the lowest concentration of IgG antibody at a mean of 5.9±2.5. On the other hand, the CLIA quantitative antibody test results of males showed the highest IgM antibody concentration at the age groups 61-70 years and 71-80 years at means of 14.11±6.5 and 11.14±4.7 AU/ml, respectively. The IgG and IgM antibodies merged results showed that IgM concentrations were higher than IgG concentrations at all age groups. However, data showed a significant difference between IgG and IgM concentrations in the age groups of 51 years to 60 years and 81 years to 90 years.

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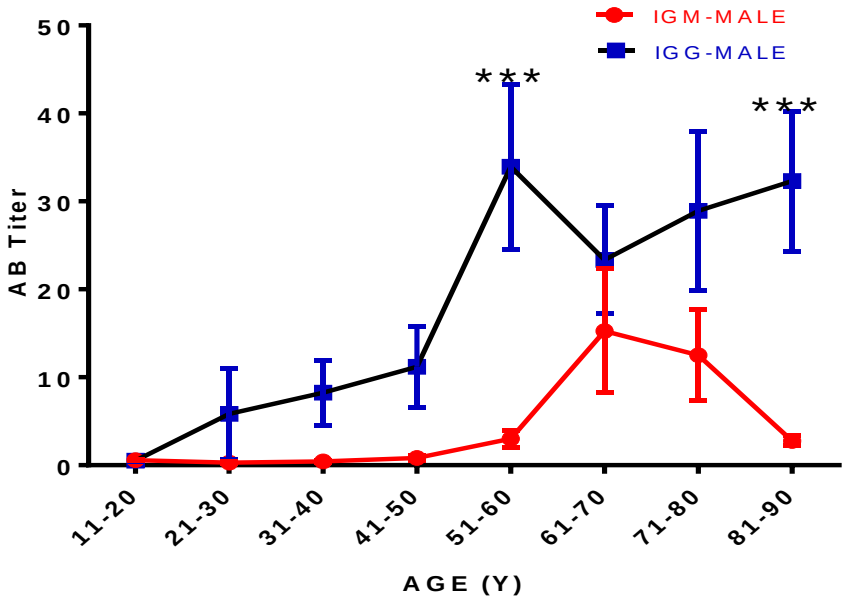

Figure 2: Trends in the SARS-CoV-2 IgG and IgM antibody titers for the age groups in males

The proportion of female individuals was 35.2% (n=66). The distribution of female individuals according to their age is presented in **Figure 3**. The highest IgG antibody concentration recorded in the age group 61-70 years was at a mean of 36.06±22.5. The IgM antibody data of female subjects showed that the highest IgM antibody concentration was recorded in the age group of 21-31 years, at the mean of 54.08±10.9 AU/ml. Merged data showed that IgM concentrations were higher than IgG concentrations at all age groups. Data also showed a significant difference ($p<0.05$) between IgG and igM concentrations only in the 21-30-year age group.

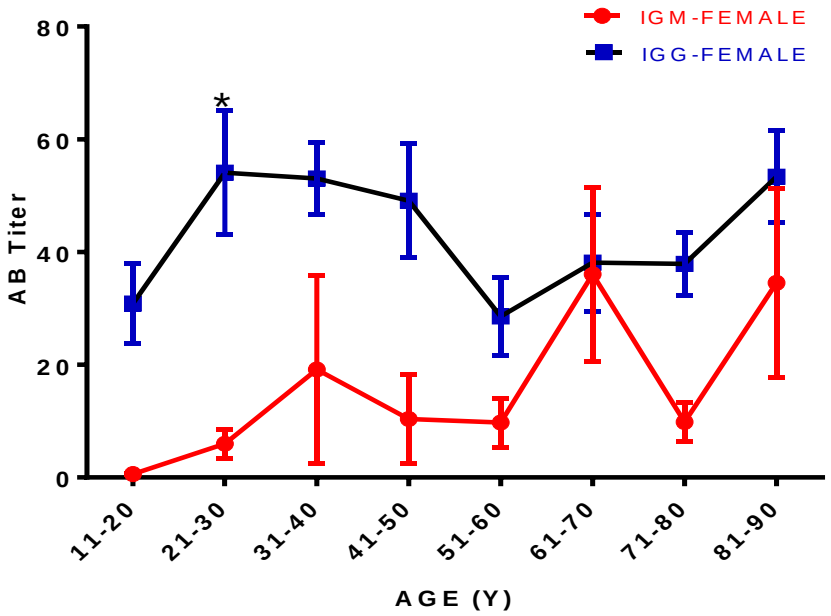

Figure 3: Trends in the SARS-CoV-2 IgG and IgM antibody titers for the age groups in females

In **Figure 4**, merged data of IgM-male subjects and IgM-female subjects showed that IgM concentrations were higher in female individuals than in male individuals at all the age groups with no significant difference ($p>0.05$).

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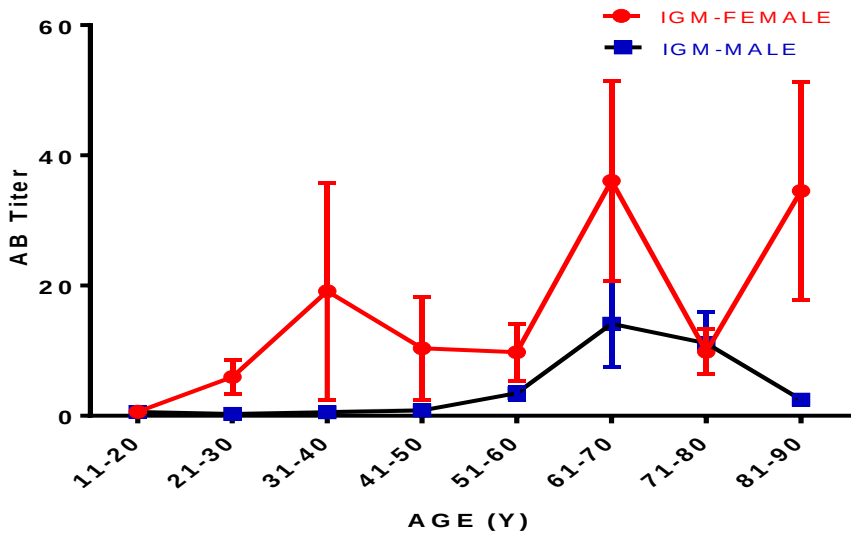

Figure 4: Trends in the SARS-CoV-2 IgM antibody titers for the age groups

The CLIA quantitative antibody test results showed that the IgG antibody prevalence in female individuals was always higher than in male individuals, except in age groups 41-50 years and 51-60 years as shown in **Figure 5**. Interestingly, data showed a significant difference ($p<0.05$) between IgG antibody titers only at younger ages (<50 years old).

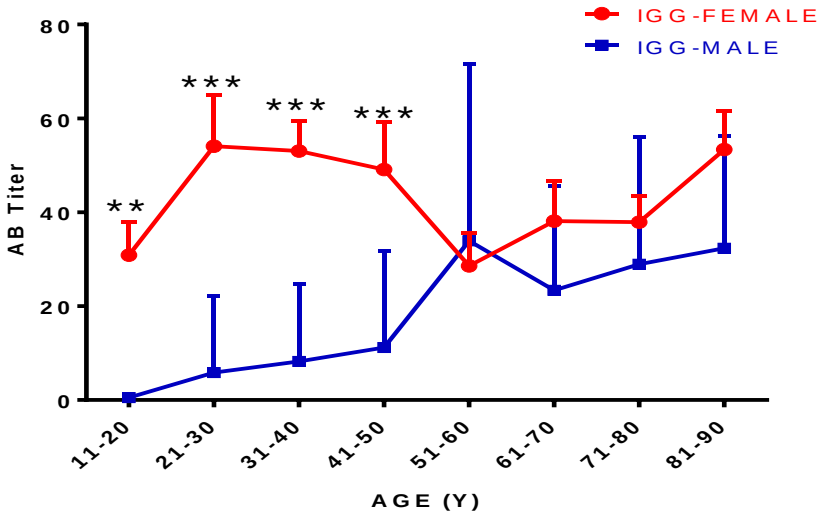

Figure 5: Trends in the SARS-CoV-2 IgG antibody titers for the age groups

Discussion

The seroprevalence of IgM and IgG antibody production among expected COVID-19 patients varies over time. In long-term follow-up studies, the seroprevalence of IgM antibodies decreases, while IgG antibodies remain higher than in six-month follow-up patients [8]. In this study, the SARS-Cov-2 IgM and IgG concentrations were investigated in 186 COVID-19 individuals from Al Khoms City, Libya. In females, the concentration of SARS-Cov-2 IgM was higher than that of IgG in all age groups. Interestingly, in males, the results showed the opposite, where the concentration of SARS-Cov-2 IgG was much higher than that of IgM in all the age groups. The SARS-Cov-2 IgM concentration reached a peak at the age group (61-70 years) and then decreased slowly. When male results were plotted against female results, the concentration of SARS-Cov-2 IgM in females was much higher than that of IgM in males in all age groups. The present data are in

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harmony with other published data, where evidence indicates that antibody development following infection likely confers some degree of immunity from subsequent infection for at least six months. Nearly, all immunocompetent persons develop an adaptive immune response following SARS-CoV-2 infection, including B and T cell-mediated immunity [9] due to antiviral humoral and cellular immune responses, respectively. Antibodies-including IgM, IgG, and IgA-against S and its subunits can be detected in serum within 1-3 weeks after infection. IgM and IgG antibodies can arise simultaneously; however, IgM (and IgA) antibodies decay more rapidly than IgG. The humoral immune response appears to remain intact even with the loss of specific antibodies over time because of the persistence of memory B-cells [10]. Studies of persons infected with the SARS-CoV-1 and Middle East Respiratory Syndrome (MERS-CoV) coronaviruses demonstrated measurable antibodies for 18-24 months following infection [11].

The kinetics of anti-SARS-CoV-2 antibodies show variations. Anti-RBD IgG antibodies remain high up to one year of follow-up, while anti-N IgG antibodies decrease over time [12]. another study found that SARS-CoV-2 IgG spike antibody titers decrease significantly over nine months after infection, with seropositivity rates declining over time [13]. A cohort study conducted over nine months found that the positivity rate of IgG antibodies remained high, with three distinct kinetics of antibody response observed [14]. Additionally, the duration of IgG antibody production is independent of COVID-19 severity [8]. There was also a correlation between IgM and IgG antibody production and COVID-19 clinical outcomes. Serum IgM levels were positively associated with survival and negatively associated with comorbidity [15]. IgG levels were associated with longer hospitalization [16]. IgG and IgM levels were higher in deceased patients compared to discharged patients [17]. Additionally, COVID-19 patients with fatal disease had decreased SARS-CoV-2 neutralizing antibody titers and lower SARS-CoV-2 spike-specific IgG levels [18]. IgG antibodies generally become detectable 10-14 days after infection and normally peak around 28 days after infection. This pattern of antibody development seen with other infection often does not apply to SARS-CoV-2, however, IgM sometimes occurs after IgG, together with IgG or does not occur at all [19]. However, median IgM detection occurs 5 days after symptom onset, whereas IgG is detected a median 14 days after symptom onset. In this study, IgG levels significantly decline after two or three months [20, 21]. Thus, these findings suggest that IgM and IgG antibody production may play a role in predicting clinical outcomes in COVID-19 patients. Moreover, the seroprevalence of IgM and IgG antibody production among expected COVID-19 patients can vary, and further research is needed to understand the factors influencing antibody production and treatment in Libya [22].

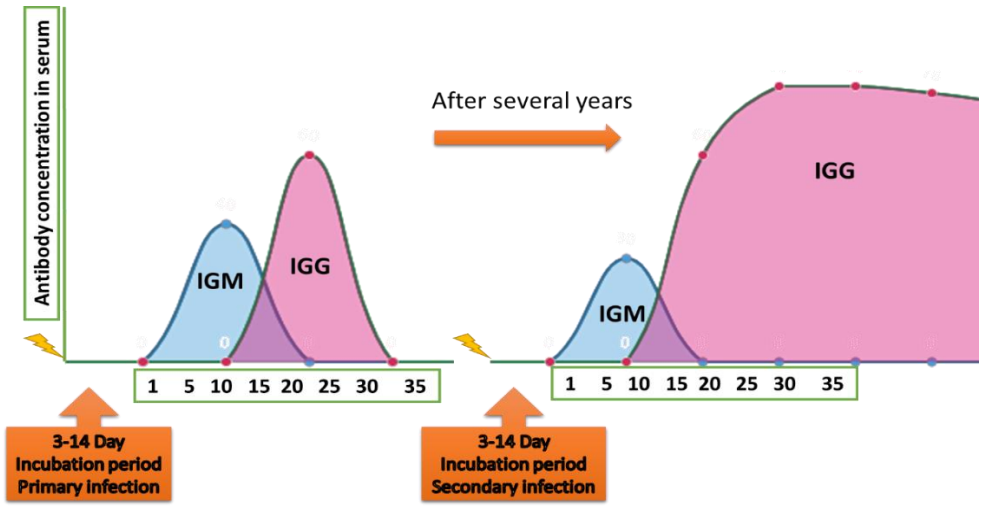

Figure 5: SARS-CoV-2 IgM and IgG antibodies levels and kinetics over time

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Conclusion: Merged IgM-male and IgM-female data showed higher IgM concentrations in females which indicates a high incidence rate of COVID-19 infection in females. The IgG antibody prevalence in females was always higher except for those 40 years old and over. This can be used as an indicator of high acquired immunity among females due to possible reinfection of females with the COVID-19 virus.

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Author contribution: IMA & MAM conceptualized the research idea and performed data analysis and interpretation**.** MSA, HMM, MSA & BOA collected data. HMA drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.