SARS-COV-2 SEROSURVEY AMONG BULGARIAN HEALTHCARE WORKERS BEFORE AND AFTER VACCINATION

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ABSTRACT

Background: In the response to the current COVID-19 pandemic caused by the novel SARS-CoV-2, one of the groups at higher risk were healthcare workers (HCWs), especially those who worked on the frontline. The presence of SARS-CoV-2 specific IgG antibodies (seropositivity) in certain populations provides better understanding of virus circulation and transmission. Our aim was to study the seroprevalence rates of anti-SARS-CoV-2 antibodies among a group of healthcare workers before and after vaccination/ COVID-19 infection.

Material and Methods: We determined the presence of SARS-CoV-2 specific IgG and IgA antibodies against S-antigen of by ELISA method. In this study, we enrolled 74 healthcare workers and three months later, 48 of the participants were followed up. At the baseline, none of the participants was vaccinated or had suffered COVID-19.

Results: SARS-Cov-2 specific IgG antibodies were found in 32.4% of the participants. Higher prevalence of class IgA antibodies – 44.6% was detected. All samples that were IgG seropositive were also positive or borderline for IgA antibodies. Overall, virus-specific antibodies were not detected in 40.6% of HCWs in the group. During the follow-up (after vaccination and/or COVID-19 infection) high rates of both IgG and IgA seroprevalence were established. SARS-C0V-2 specific IgG antibodies were detected in 95.8% of the participants. Statistically significant difference was found in the levels of IgG and IgA antibodies both before and after vaccination, p<0.0001.

Conclusions: Based on detection of anti-SARS-CoV-2 IgG antibodies, seroprevalence of 32.4% was established in an unvaccinated group of HCWs. Our survey demonstrated that asymptomatic COVID-19 infection may induce weaker humoral immune response, with production of IgA but not of IgG antibodies.

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INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) pandemic caused by the novel SARS-CoV-2 exerted immense pressure on the health and public systems around the world over the past three years (2020-2022). In response to the pandemic, one of the groups at highest risk were healthcare workers (HCWs), especially those who worked on the frontline in COVID-19 units. Some authors considered that in countries with lower vaccination coverage as Bulgaria, the risk was even higher (1). According to the official statistics, as of June 2023, 26 519 Bulgarian health workers were infected with SARS-CoV-2 (2). This number is probably underestimated.

Seroepidemiological studies might be a helpful tool to give insight into asymptomatic infections, as well as those not registered in official statistics. The presence of SARS-CoV-2 specific IgG antibodies (seropositivity) in certain populations provides a better understanding of the viral circulation and transmission. Also, large-scale population studies could predict the future development of the pandemic (3).

Since the beginning of COVID-19 pandemic, many serological studies have been carried out, both in specific groups and in the general population. The reported seroprevalence rates vary widely. A study from Poland reported 25.2% seroprevalence of anti-SARS-CoV-2 antibodies among HCWs prior vaccination availability (4). Socan at all. observed a seroprevalence rate of 20.4% among hospital staff during the second COVID-19 wave in Slovenia (5).

After the first COVID-19 wave in France, SARS-CoV-2 IgG seroprevalence rate among personnel in hospitals was 1.1 % (6). In the UK, 28.0% seropositivity was found after the first COVID-19 wave (7). A prospective cohort study from Saudi Arabia, conducted in June 2020, reported a seroprevalence of 10.8% (8). Seroprevalence of 45.3 % – twice as high in comparison with the local community, was established among HCWs from Western Switzerland after the second wave of the infection (10). Many factors, such as the timepoint, vaccination coverage, demographic, socio-economic characteristics, etc., should be taken into account when comparing data for the seroprevalence in different areas (3).

Studies have shown that higher IgG seroprevalence rates were found among medical personnel with frequent exposure to COVID-19 patients. It was estimated that the exposure together with the use of personal protective equipment are important and specific risk factors (10-12).

Our aim was to study the presence of anti-SARS-CoV-2 antibodies among healthcare workers from the Military Medical Academy, Sofia, which is one of the biggest hospitals treating COVID-19 patients in Bulgaria.

MATERIALS AND METHODS

Sample collection and testing was conducted twice – in November 2021 and in March 2022. At the start of the study we enrolled 74 healthcare workers from one hospital (Military Medical Academy, Sofia, Bulgaria). Three months later, 48 of the participants were followed up. At the time of the first sampling, none of the participants was vaccinated nor had evidence or history of previous SARS-CoV-2 infection. We determined presence rates of IgG and IgA antibodies against the S1 domain of the Spike (S) protein of SARS-CoV-2 by ELISA kits (EUROIMMUN, Germany). The samples were processed in accordance with the manufacturer`s instructions. The results are presented semi-quantitatively as the ratio of the extinction of the tested sample over the extinction of the calibrator (S/CO). The results were interpreted as follows: positive if S/CO ratio ≥1.1, borderline if S/CO ratio ≥0.8 to <1.1 and negative if S/CO ratio <0.8. Statistical analysis was performed with SPSS software using the Kruskal-Wallis independent samples test. A p value < 0.05 was considered statistically significant. Graphs were made with GraphPad Prism 9 software. The study was approved by the Institutional Review Board of NCIPD (approval number 4/17.02.2021).

RESULTS

The baseline study in November 2021 involved 74 HCWs, of whom 48 males, and 26 females, with an average age of 41.74 ±12.02 years. Positive values of SARS-CoV-2 specific IgG antibodies were found in 32.4% (24/74) of participants, and in 4.1% (3/74) borderline levels were detected. Interestingly, the prevalence of IgA antibodies was higher than the prevalence of IgG antibodies. In 44.6% (33/74) of the participants, IgA antibodies were detected and in additional 13.5% (10/74) borderline values of IgA antibodies were found **(Figure 1).** The number of HCWs with positive and borderline results for IgA antibodies (43/74) was higher than the number of HCWs with positive and borderline results for IgG antibodies (27/74) and this difference was statistically significant ($p<0.05$).

We analysed the relationship between the presence of IgG and IgA antibodies. All samples that were seropositive for IgG antibodies were also positive or borderline for IgA antibodies (32.4%). Only one sample with borderline levels of IgG was negative for IgA antibodies. Almost 23.0% of the tested serum

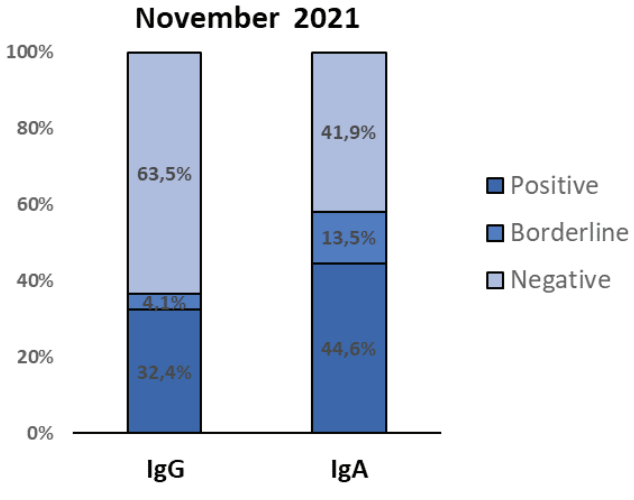

Figure 1. Seroprevalence rates of IgG and IgA SARS-CoV-2 antibodies in a group of unvaccinated healthcare workers. The results are presented as percentage (%) of the positive, borderline and negative values.

Table 1. SARS-CoV-2 IgA antibodies in healthcare workers with negative, borderline and positive IgG antibodies with 95.0% confidence interval.

Antibodies result	Number of participants	%, (95.0% CI)
IgG(neg.) IgA(neg.)	30	40.5% (95.0% CI: 29.4, 51.8)
IgG(neg.) IgA(pos.)	8	10.8% (95.0% CI: 3.7, 17.9)
IgG (neg.) IgA(bord.)	9	12.2% (95.0% CI: 4.7, 19.6)
IgG (bord.) IgA (neg.)	1	1.4% (95.0% CI:-1.3, 4.0)
IgG(bord.) IgA(pos.)	2	2.7% (95.0% CI: –1.0, 6.4)
IgG(pos.) IgA(bord.)	1	1.4% (95.0% CI:-1.3, 4.0)
IgG(pos.) IgA(pos.)	23	31.0% (95.0% CI: 20.5, 41.6)
Total:	**74**	**100%**

neg. = negative; bord. = borderline; pos. = positive; CI = Confidence interval

samples were positive or borderline only for IgA antibodies. The presence of specific antibodies at this time point may be considered indicative of previous exposure to the pathogen and/or asymptomatic infection. Overall, 40.5% of HCWs in the group were seronegative for both IgG and IgA antibodies **(Table 1).**

Three months after the initial testing, we performed a follow up of 48 HCWs from the original group. During this time, all participants had been vaccinated and/or recovered from COVID-19. As expected, high seroprevalence rates were established. Class IgG antibodies were detected in 95.8% (46/48) and only two participants were seronegative – 4.2% (2/48). With one exception, all participants were found to have class IgA antibodies, or 97.9% (47/48) of them. Of the participants, 91.7% (44/48) were positive and 6.3% (3/48) – borderline (Figure 2). Both seronegative participants were vaccinated; one of them had neither IgG nor IgA antibodies, while the other had borderline IgA values.

We compared the results from November 2021 with those from March 2022 in order to evaluate

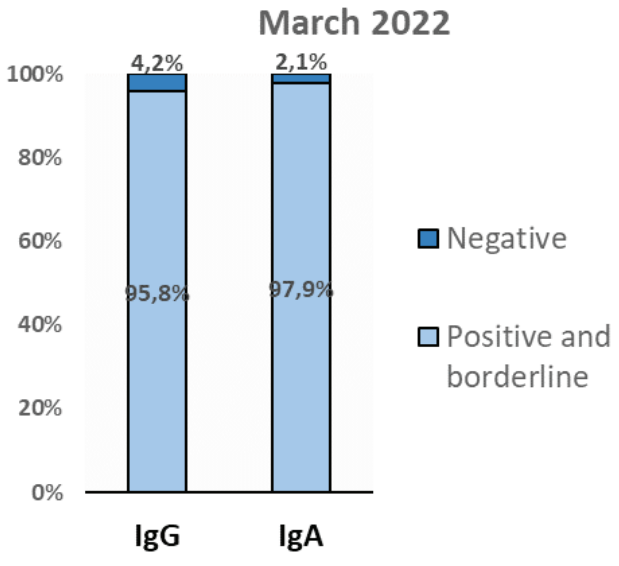

Figure 2. Seroprevalence rates of IgG and IgA SARS-CoV-2 antibodies during the follow-up. The results are presented as percentage (%) of the positive, borderline and negative values.

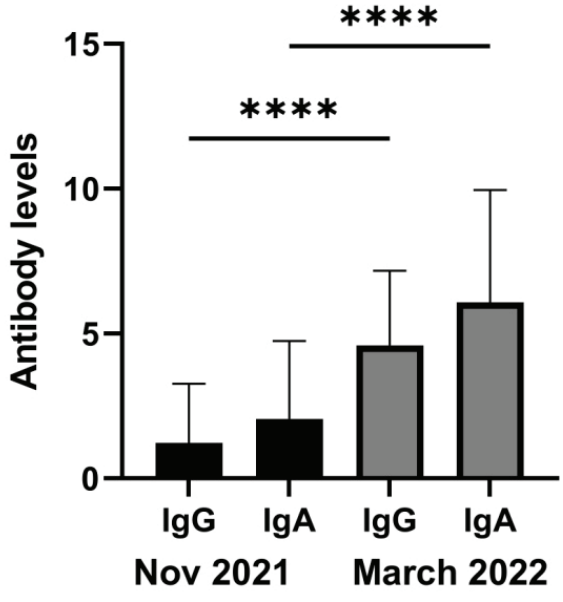

Figure 3. A comparison of mean S/CO ratio of IgG and IgA antibody levels in November 2021 and March 2022. The statistical differences were determined by the Kruskal–Wallis test (****, p<0.0001)

the alteration in mean antibody levels. We found statistically significant differences between the levels of both IgG and IgA antibodies detected during the two studies, p<0.0001 **(Figure 3)**

In this study no correlation between the seropositivity and the participants' age or sex was observed.

DISCUSSION

Based on the detection of anti-SARS-CoV-2 IgG antibodies, we observed a seroprevalence of 32.4% in an unvaccinated group of Bulgarian HCWs. During the follow up, after vaccination and/or COVID-19, we observed an increase of the seroprevalence rate to 95.8%. The relatively high baseline IgG antibody rates should be considered in the context of the COVID-19 pandemic situation in the country at the time of the study. At the same time (autumn, 2021), we had the most active circulation of Delta SARS-Co-2 variant, which led to the highest COVID-19 wave with a peak incidence rate of 928.3 $_{(100000)}$ (13).

Production of IgA antibodies without IgG antibodies was detected in about 23% of the HCWs. One possible explanation for the higher seroprevalence of IgA antibodies in the baseline study might be that asymptomatic infection induces a weaker immune response without IgG engagement. Cordova et all. report lower levels of IgG seroprevalence in asymptomatic HCWs with confirmed COVID-19 in comparison with the symptomatic ones (14). Madureira et all. reported that among previously asymptomatic HCWs 12.9% were positive for neutralizing and IgG antibodies against SARS-CoV-2 (9). Others found similar seropositivity rates in both asymptomatic and symptomatic COVID-19 patients, but established significantly higher levels of IgG in the symptomatic group in comparison to those without symptoms (15).

According to Brehm et al., individuals who had received three doses of vaccine or had a previous infection plus two doses of vaccine elicited the strongest humoral immune response to SARS-CoV-2 (16). During the follow up, we found significant differences between the mean levels of both IgG and IgA before and after vaccination and/or COVID-19 infection. Our results also indicated that almost all participants had acquired virus-specific IgG antibodies. Only two HCWs were IgG/IgA seronegative after COVID-19 infection/vaccination. This finding could be explained by a short-lived antibody immune response or other reasons such as immunodeficiency or incomplete vaccination course (17, 18). One of the main limitations of the seroprevalence studies is that they do not take into account the T-cell response, which has a leading role in anti-SARS-CoV-2 immunity (19, 20).

Numerous studies on COVID-19 indicated a wide range of seroprevalence rates, with time of conduction and study design mainly accounting for the differences in the results. A study from Belgium reported seroprevalence of 15.1% before vaccination (December 2020) and an increase to 84.2% after the first vaccination among primary healthcare providers, underlining the importance of vaccination for occupational health and protection of medical personnel (21). Recently, a study from Serbia, similar to ours, reported 93.0% overall prevalence of anti-SARS-CoV-2 antibodies among HCWs before the emergence of the Omicron variant (22).

In general, the seropositivity levels have increased since the first and second waves of COVID-19 and after the mass vaccination. This is expected because, as the pandemic progresses, the proportion of individuals with either previous COVID-19 infection and/or at least one vaccine dose is growing. On the other hand, the levels of circulating IgG and IgA antibodies normally decline over time. The duration of acquired immunity after illness and vaccination is still being discussed. With the emergence and spread of new variants of SARS-CoV-2, reinfections continue to occur and affect the seroprevalence in the general population.

One limitation of this study is the relatively small group of participants. Further investigations among larger groups, and following the dynamics of seropositivity rates are necessary.

Conclusions

Our survey demonstrates that asymptomatic COVID-19 infection may induce a weaker humoral immune response, with a predominant production of class IgA antibodies and a weaker IgG antibody response. The study also emphasizes the importance of vaccination for acquiring strong protective immunity against SARS-CoV-2.

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