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Original article

PREVALENCE OF ANTI-SARS-COV-2 IGG ANTIBODIES IN SKOPJE, NORTH MACEDONIA: TWO-TIME POINTS POPULATION-BASED CROSS-SECTIONAL STUDY

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Abstract

The aim of our study was to determine the seroprevalence of IgG anti-SARS-CoV-2 antibodies in Skopje, the capital of North Macedonia at two time points, February 2021, and February 2022 and to provide information about the dynamics of the collective immunity.

We recruited a total of 1004 in the first and 971 random individuals in the second time point who answered a questionnaire and IgG anti-SARS-CoV-2 antibodies against S and N antigen were measured.

The estimated overall seroprevalence in Skopje in February 2021 was 31.4%, with no statistical difference between different age groups or gender. Positive RT-PCR test or presence of symptoms associated with COVID-19 strongly correlated with presence of anti-SARS-CoV-2 antibodies ($p<0.001$). At the second time point the overall seroprevalence was 86.8%, somewhat higher in the group of participants vaccinated against COVID-19 with one of the six different vaccines compared to the unvaccinated group (89.9% *vs.* 75.8%, $p<0.001$). In the subgroup of individuals without prior positive RT-PCR test and unvaccinated, 69 out of 105 (65.7%) had IgG anti-SARS-CoV-2 antibodies.

Estimation of population seroprevalence is valuable information that reflect the immunization status of the population in the capitol of North Macedonia. However, the emergence of different subvariants of Omicron challenges the importance of collective immunity in prevention of new infection.

Keywords: COVID 19, seroprevalence-anti SARS-CoV-2 antibodies, population study

Introduction

Over the last two years, humankind faced significant challenges caused by the pandemic spread of a new viral infectious disease COVID-19. This highly contagious disease is caused by the new human coronavirus SARS-CoV-2 and is associated with significant morbidity and mortality. The WHO declared the global pandemics on 12th of March 2020 and so far (April, 2022) it caused more than 490 million infections and over 6.1 million deaths [1,2]. The reasons driving the rapid spread of the SARS-CoV-2 are the virus human-to-human transmissibility (particularly among asymptomatic or people with very mild symptoms), the apparent lack of cross immunity from previous coronavirus infections and somewhat delayed response to the pandemic (including lack of adherence to the anti-epidemic measures)[3-5]. With the increasing availability of vaccines and the few pandemic waves, the number of new cases worldwide in March 2022 was around 42 million, with an estimated death rate of around 0.3% on the global level.

The first cases of COVID-19 in North Macedonia (total population of about 1.8 million) were detected in March 2020, when the country implemented the preventive measures recommended by the WHO. These included social distancing, wearing masks, increased personal hygiene, curfews and lockdowns for extended periods of time [6]. Nevertheless, by March 2021 there were over 115,000 COVID-19 cases (about 1 in 15 people) and almost 3,500 COVID-19 fatalities (about 1 in 500 people or about 3% of the infected people) in the country[1,2]. Overall, until April 11, 2022 in the Republic of North Macedonia the total number of COVID-19 cases was 307,654, including 9,244 fatalities - which brings the mortality rate to approximately 0.6% of the general population and 3% of the infected population[6].

Targeted immunization for COVID-19 in the Republic of North Macedonia was initiated on February 17,2021 in healthcare workers, while mass immunization in general population started in May 2021. The percentage of people with administrated two vaccine doses is 40.1% in North Macedonia (April 2022), which is lower than the worldwide average of 58.8%[7].

IgG antibodies persist in the body after the virus has been cleared and therefore sero-epidemiological studies are more suitable for estimation of the cumulative prevalence of the COVID-19 outbreak compared to virus detection by PCR or antigen testing. These population-based sero-epidemiological studies are particularly important to gain insight into the natural history of this new, suddenly emerged infectious disease (epidemiology, contagiousness, spread rate of immunity). The WHO recommends (March, 2020) the cross-sectional type of surveys as the most appropriate one when the peak transmission has been established in a region [8-11].

Numerous studies demonstrated that the population prevalence of seropositivity for SARS-CoV-2 increased in the course of the pandemic[12], varying considerably in different populations and localities. Depending on the activity of the pandemic at the time of reporting, the seropositivity rates ranged from 4.6% in Veneto region, Italy (August 2020)[5], 8% in Arizona, USA (October 2020)[13], 12% in South-East England (October 2020)[14], 17.1% in Iran (December 2020)[15], 17.6% in Ahmedabad City, India (December 2020)[16], 20% in New York, USA (May 2020)[12], 23% in Northern Italy (August 2020)[17], and as high as 34% in Nicaragua (March 2021) and 45% in a village in Ecuador (2020)[18].

This is a population-based cross-sectional study conducted in the Republic of North Macedonia, based on estimation of the SARS-CoV-2 seropositivity in a random sample of people living in Skopje, the capital of North Macedonia, at two time points (February 2021 and February 2022). The aim of this study was to provide information about the dynamics of the collective immunity and the socio-demographic factors associated with the SARS-CoV-2 infection in North Macedonia.

Material and methods

Sampling

A cross-sectional study of randomly selected people in different locations in the city of Skopje was performed at two time points, 22 February to 8 March 2021 and February 15 to February 24, 2022. In the first time point, a total of 1,004 participants were recruited, while in the second time point there were 971 participants. Participants were selected randomly and voluntarily from people who were passing by on the six different locations in the city of Skopje (Figure 1). The same collection locations were used in both sampling time points. Staff from the Institute of Immunobiology and Human Genetics, Faculty of Medicine in Skopje carried out the fieldwork. Upon informed consent, participants were recruited, donated a blood sample for subsequent laboratory analysis and completed a questionnaire. The questionnaire included information about age, sex, blood group, preexisting chronic conditions, previous history of symptoms compatible with COVID-19 (i.e., fever, cough, sore throat, rhinitis, anosmia or ageusia, tiredness, shortness of breath, diarrhea), whether they had a positive RT-PCR test results from the start of the pandemic, were they hospitalized and/or on oxygen support for COVID-19. The second questionnaire included questions about the vaccination status. An informed consent was obtained from all participants and the study was approved by the Ethics Committee at the Faculty of Medicine in Skopje.

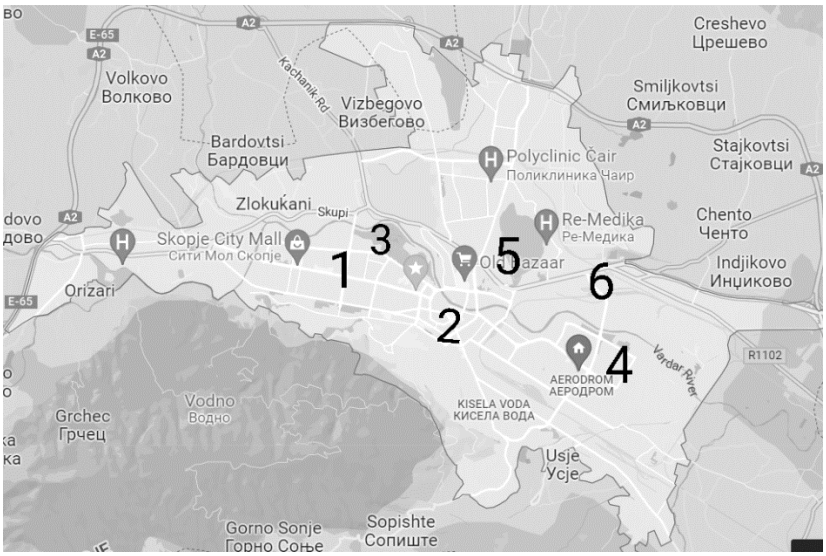

Fig. 1. Map of Skopje. Locations where the participants were selected are designated with numbers

Measurement of anti-SARS-CoV-2 IgG antibodies

Two immunoassays for detection of IgG anti-SARS-CoV-2 antibodies, both with specificity against Spike (S) and Nucleocapsid (N) antigens, were used. The samples from the first time point were analyzed with automated chemiluminescent analytical system (CLIA) (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China). According to the manufacturer's inserts (272 2019-nCoV IgG, V1.2, 2020-02), the sensitivity of the IgG test is 91.2% and the specificity 97.5%. The results were quantitative and were considered positive for presence of anti-SARS-CoV-2 antibodies for values above 1. The IgG anti-SARS-CoV-2 antibodies in the second point were tested using a manual ELISA SARS-CoV-2 IgG kit from the Institute for Application of Nuclear Energy, Belgrade, Serbia, according to the manufacturer's instructions. VICTOR2 Elisa plate reader was used to measure the optical density of the samples and an index was calculated. Values above 20 were considered positive. The reported specificity of the test is >98% with a sensitivity of >99%. We

performed a validation study on 268 samples that were tested with both methods, and we obtained concordance of 98.5%.

Statistical analysis

Descriptive analyses were performed. Data are presented as raw numbers and percentages and compared using Chi-square test or Fisher's Exact test, as applicable. p value <0.05 was considered statistically significant.

Results

Seroprevalence in 2021

At the first time point in 2021, a total of 1,004 individuals from Skopje, the capital of North Macedonia, were enrolled in the study: 630 women (62.75%) and 374 men (37.25%). The mean age of women was 43.1±16.7 years and of men 45.9±16.3 years. The demographic caracteristics of the studied population are presented in Table 1.

Table 1. Demographics of the participants and seropositivity by groups - first time point in 2021: percentige and 95% confidence intervals shown. Chi-square test p values are shown

	N	% total	Seropositive (n)	%	CI 95%
Overall	1004	100	315	31.4	28.5-34.2
Sex					
Male	374	37.25	127	34.0	29.2-38.7
Female	630	62.75	188	29.8	26.3-33.4
p value					*0.174*
Age group					
<29	240	24.10	83	34.6	28.6-40.6
30-39	196	19.68	60	30.6	24.2-37.1
40-49	190	19.07	60	31.6	25.0-38.2
50-59	151	15.16	50	33.1	25.6-40.6
≥60	219	21.99	62	28.3	22.3-34.3
Unknown	8	0.79			
p value					*0.612*
PCR test					
Positive	189	18.82	161	85.2	80.1-90.2
Negative	33	3.28	5	15.2	2.9-27.4
Untested	782	77.89	149	19.1	16.3-21.8
p value					***<0.001***
Symptoms					
Symptomatic	360	35.86	210	58.3	53.2-63.4
Asymptomatic	644	64.14	105	16.3	13.4-19.2
p value					***<0.001***
Blood group					
0+	257	29.24	82	31.9	26.2-37.6
0-	42	4.78	13	31.0	17.0-44.9
A+	317	36.06	99	36.4	26.1-36.3
A-	47	5.35	18	38.3	24.4-52.2
B+	155	17.63	44	28.4	21.3-35.5
AB+	59	6.71	15	25.4	14.3-36.5
B-	2	0.23	1	0.5	
AB-	0	0.00	0	0	
Unknown	125				
p value					*0.675*

The estimated overall seroprevalence in Skopje, in March 2021, was 31.4% (315/1004) (95% CI: 28.5-34.2). Anti-SARS-CoV-2 IgG antibodies were detected in 127 male (34%, 95% CI: 29.2-38.7) and in 188 female (29.8%, 95% CI: 26.3-33.4) participants. There was no statistical significance between sex and anti-SARS-CoV-2 IgG antibodies (χ^2=1.938, p-value=0.163).

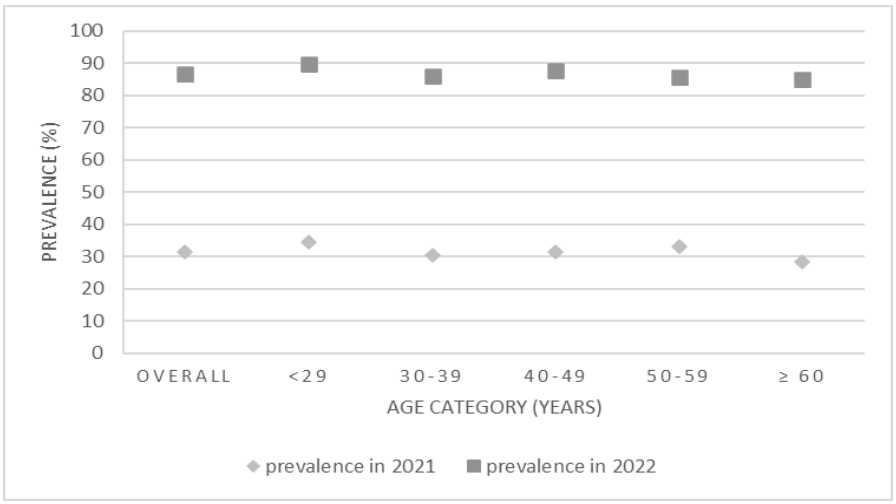

Fig. 2. Prevalence of anti-SARS-CoV2 IgG antibodies by age groups in 2021 and in 2022

We divided the participants in 5 age groups (<29, 30-39, 40-49, 50-59 and >60). The presence of anti-SARS-CoV2 IgG antibodies did not show a statistical significance between the different age groups (χ^2=2.33, p=0.675) (Figure 2).

About one fifth (222 participants) had prior SARS-CoV-2 RT-PCR test and 189 were positive. The seroprevalence of 85.2% (161/189) in the RT-PCR positive group was statistically much higher compared to 15.2% (5/33) in the RT-PCR negative group and 19.1% (149/782) untested group, as shown in Figure 3 (χ^2=73.05, p=1.26x10^{-17}).

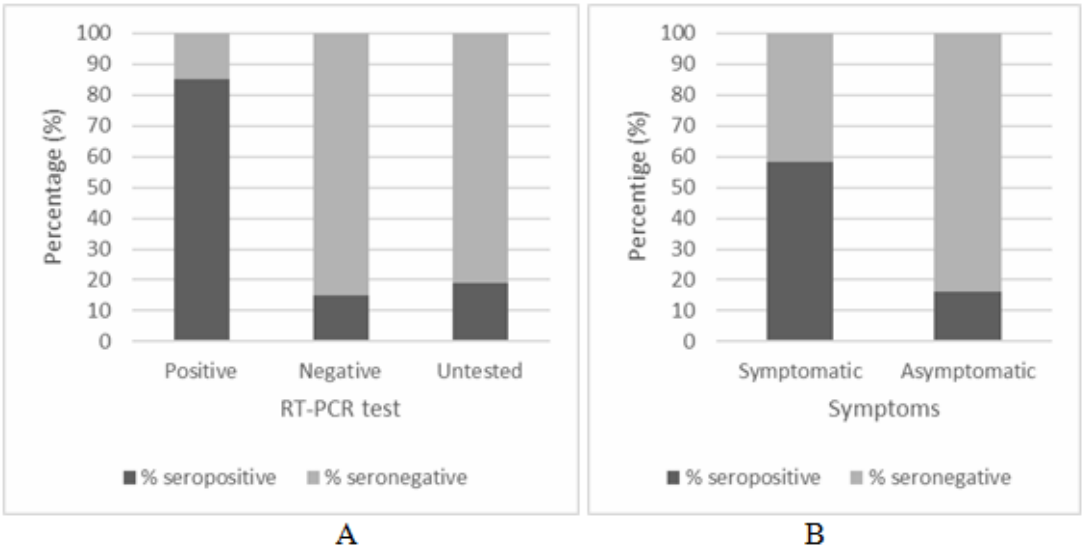

Fig. 3. Representation of seropositivity according to A. RT-PCR status (positive, negative, and untested) and B. symptoms (symptomatic *vs*. asymptomatic)

More than a third of the individuals (n=360) reported at least one symptom associated with COVID-19, and the seroprevalence in the symptomatic group was 58.33% (210/360), which was significantly higher than in the asymptomatic group 16.30% (105/644), as shown in Figure 3 (χ^2=186.48, p=1.86x10^{-42}).

Analysis of the association between the blood groups and anti-SARS-CoV2 IgG antibodies showed no statistical significance (p=0.675).

Only 52 participants (5.2%) reported at least one chronic illness (hypertension, diabetes mellitus and/or thyroid malfunction) and 20 (38.46%) of them were positive for anti-SARS-CoV-2 antibodies, like the prevalence in those who did not report a chronic illness (χ^2=1.13, p=0.287).

Five participants in our study answered that they were hospitalized for COVID-19 infection and all of them were positive to anti-SARS-CoV-2 antibodies.

Seroprevalence in 2022

At the second time point (February 2022), we recruited and analyzed 971 individuals, 640 women (65.91%) with the mean age of 46±14.7 years and 331 men (34.09%) with the mean age of 48.8±15.5 years. The demographic characteristics of the participants in the second time point are presented in Table 2.

The estimated overall seroprevalence in Skopje at this point was 86.8% (95% CI: 84.7-88.9), with 843/971 seropositive participants. We detected similar prevalence of anti-SARS-CoV-2 IgG antibodies in men (88.2%, 95% CI: 84.7-91.7) and in women (86.1%, 95% CI: 83.4-88.8).

Table 2. Demographics of participants and seropositivity by groups - second time point in 2022: percentige and 95% confidence intervals shown. Chi-square test p values are shown

	n	% Total	Seropositive (n)	%	CI 95%
Overall	971	100	843	86.8	84.7-88.9
Sex					
Male	331	34.09	292	88.2	84.7-91.7
Female	640	65.91	551	86.1	83.4-88.8
p value					*0.35379*
Age group					
<29	165	16.99	148	89.7	85.1-94.3
30-39	151	15.55	130	86.1	80.6-91.6
40-49	246	25.33	216	87.8	83.7-91.9
50-59	217	22.35	186	85.7	81.1-90.4
≥ 60	192	19.78	163	84.89	79.8-90.0
p value					*0.676302*
PCR test					
Positive	374	38.52	355	94.9	92.7-97.1
Negative	320	32.95	258	80.6	76.3-85.0
Untested	277	28.53	230	83.0	78.6-87.4
p value					***<0.001***
Symptoms					
Symptomatic	507	52.21	460	90.7	88.2-93.2
Asymptomatic	464	47.79	383	82.5	79.1-86.0
p value					***<0.001***
Vaccination status					
2 doses vaccine	760	78.27	683	89.9	87.7-92.0
Comirnaty	394	51.84	378	95.9	94.0-97.9
Vaxzervia	56	7.37	46	82.1	72.1-92.2
Sputnik V	27	3.55	23	85.2	71.8-98.6

Sinopharm	184	24.21	149	81.0	75.3-86.7
CoronaVac	98	12.89	86	87.8	81.3-94.2
Moderna	1	0.13	1	100	
Not vaccinated	211	21.73	160	75.8	70.1-81.6
p value					***<0.001***
3 doses vaccine	164	16.89	163	99.4	98.2-100.6
Comirnaty	158	96.34	157	99.4	
Sinopharm	5	3.05	5	100	
Sputnik V	1	0.61	1	100	
p value					***< 0.001***
Blood group					
0+	244	25.13	214	87.7	82.6-91.8
0-	44	4.53	40	90.9	82.4-99.4
A+	336	34.60	294	87.5	84.0-91.0
A-	43	4.43	38	82.4	72.8-92.0
B+	133	13.69	110	82.7	6.3-89.1
AB+	64	6.59	56	87.5	79.4-95.6
B-	26	2.68	23	88.5	76.2-100.7
AB-	12	1.23	9	75.0	50.5-99.5
Unknown	69				
p value					*0.711547*

The seroprevalence by age ranged from 84.7%-89.7%, with no statistical significance between the age groups. The number of participants who reported prior PCR test was 71.5% (694/971) and the seroprevalence in the subgroup that tested positive was 94.9% (355/374), higher than in the subgroup with negative PCR (80.6%, 258/320) and in the untested group (83%, 230/277). The participants who reported prior COVID-19 symptoms had seropositivity

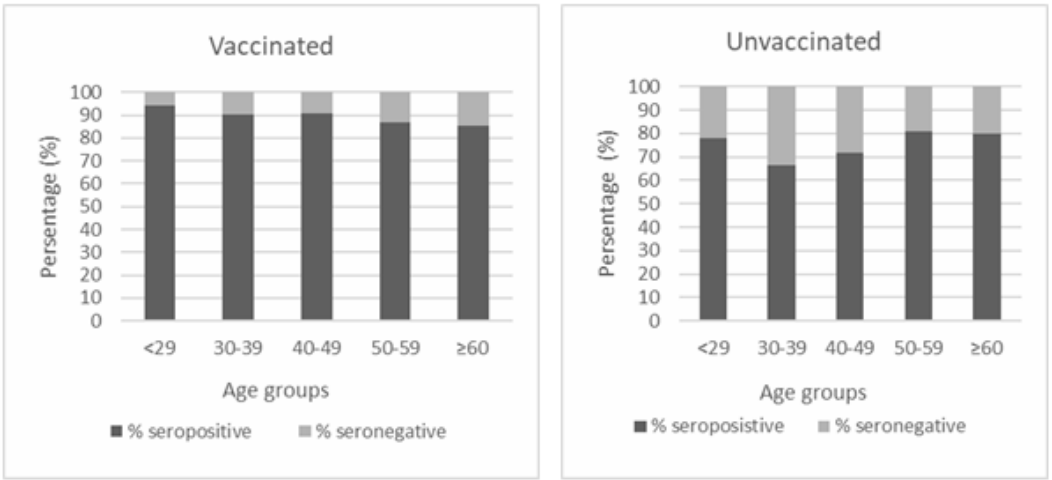

Fig. 4. Representation of seropositivity in age groups according to vaccination status A. vaccinated, B. unvaccinated

in 90.7% of the cases, significantly higher than that of the asymptomatic individuals (82.5%) ($p<0.001$).

In terms of vaccination status, 760 participants reported vaccination with two doses with one of the six available vaccines (Comirnaty, Sinopharm, CoronaVac, Sputnik V, Vaxzervria and Moderna). The percentages of seropositivity are shown in Table 2. Figure 4 presents the seroprevalence by age groups in vaccinated and unvaccinated individuals. The range of seropositivity in the group of vaccinated was between 85.5% and 94.2% ($\chi^2=7.3006$, $p=0.120829$), while in the group of unvaccinated ranged between 66.7% and 81%

(χ^2=2.4591, p=0.651979). Vaccination with three doses was reported by 164 individuals and had high seropositivity of 99.4%. At this time point, there were 105 individuals who were not vaccinated and did not have a positive PCR result or did not have prior PCR test. Two-thirds of them 69/105 (65.7%) had IgG anti-SARS-CoV-2 antibodies.

Discussion

The results of this study demonstrated a high seroprevalence (1 in 3 people) for COVID-19 one year after the onset of the pandemic in the Republic of North Macedonia (March 2020 to February 2021) and attaining collective immunity two years into the pandemic (February 2022) with nearly 9 in 10 people testing positive for anti-SARS-CoV-2 IgG antibodies in the nation's capital Skopje. According to the results from the 2021 census, Skopje is the home of about one third of the country's total population (526,502 citizens)[19]. The results from the first time point (February 2021) reflected the naturally attained humoral immunity via symptomatic and asymptomatic infection. The seroprevalence of 1 in 3 was in correlation with data from other populations, ranging between 29.9%[20] and 46.7%[21], conducted in May 2021.

At this time point, about one third of the participants reported prior COVID-19-related symptoms and about 60% of them had antibodies. Another factor strongly associated with SARS-CoV-2 seropositivity was prior positive PCR test, with the expected high seropositivity prevalence of 85.2%. In a large group of non-vaccinated Danish blood donors who had positive PCR test, presence of anti-SARS-CoV-2 antibodies was detected in 94.2% of them even after 15 months[22]. One of the reasons elaborated why not all PCR positive individuals have detectable anti-SARS-CoV-2 antibodies was asymptomatic status or if they were pregnant[23].

The reports from the Institute of Public Health of the Republic of North Macedonia[24] for the same period (beginning of the pandemic to March 2021) identified a total of 46,149 individuals with a positive PCR test - representing about 8.8% of the population in Skopje and lower than the seroprevalence of 31.4% found in this study. During this period, the anti-epidemic measures (restricted movements, quarantines, and curfews) were in force; the first case of COVID-19 in our country occurred (November 2020), while in February 2021 mass vaccination against COVID-19 started. One of the reasons for the high percentage of IgG anti-SARS-CoV-2 antibodies in comparison with the positive PCR tests can be the limited availability of PCR test for symptomatic and/or hospitalized patients, and the very limited access to publicly funded PCR testing. Asymptomatic people and the contacts were not routinely tested. Very often, the testing was performed 10 days after the onset of symptoms, missing the window of PCR positivity. This is supported by the fact that in this study 15.2% of the individuals with a previously negative PCR tests and 19.05% of the untested individuals were seropositive.

The results of seropositivity from the second time point in February 2022 indicated attainment of collective immunity - with seropositivity over the year increasing from 31.4% to 86.8%, both through new infections and immunization efforts introduced in February 2021. Similar to the first time point, the history of symptoms consistent with COVID-19, prior positive PCR test and the immunization were factors significantly associated with seropositivity. Ninety percent of symptomatic individuals, 96% of those with positive PCR test and 90% of vaccinated individuals were tested positive for anti-SARS-CoV-2 antibodies.

There was no difference in the seroprevalence of IgG anti-SARS-CoV-2 antibodies between men and women, as well as between different age groups. These findings are similar to other population-base studies[25,26]. Although there were some indices that people with certain blood groups were more prone to COVID-19, no statistical difference was observed between blood group and seroprevalence[27].

Like other viral infections, COVID-19 infection can be asymptomatic and serological tests can give insight into the infection rate on a population level [28]. The percentage of asymptomatic cases in our study group was 16.3% and this number was in the range of previously published data in other populations between 4 and 41%[29]. This percentage corresponds to around 85,800 citizens of Skopje that were asymptomatic but could be the source of spread of the disease - a fact that needs to be considered when creating public health policies, particularly in terms of enabling much more liberal access to publicly funded testing.

In February 2021, vaccination against COVID-19 started in our country, initially limited only to the healthcare personnel. During the following months, vaccines from different manufacturers became available. The vaccination uptake in North Macedonia was fairly modest with only around 40% of the population being fully vaccinated with two doses by March 6, 2022 [24]. Restrictive measures, like COVID certificates, were implemented due to the high number of cases and to promote vaccination. Following the waves of beta, delta, and omicron strains of SARS-CoV-2, as well as vaccination against COVID-19, we investigated the seropositivity rate in Skopje in February 2022. These results reflect procuracy of population and are valuable information for the Ministry of Health in planning future policies. The overall seroprevalence of IgG anti-SARS-CoV-2 antibodies was 86.8%. The procuracy of population is uniform, with no significant difference in seroprevalence between males and females, or different age groups. These findings are in concordance with other seroprevalence studies performed worldwide. Similar results were obtained in studies done in Indonesia in March 2022[30] where 99.2% of the tested individuals were seropositive, and in Great Britain with nearly 99% seropositivity[31]. A large proportion of the participants had done PCR test for different reasons (contact with COVID-19 positive, travel purposes, symptoms), but still the presence of anti-SARS-CoV-2 antibodies was detected in 94.9% of the PCR positive, 80.6% of PCR negative and in 83.0% of the untested population. Positive PCR test was strongly associated with presence of antibodies[22].

Vaccination against COVID-19 in our country was offered with one of the five available vaccines (Comirnaty, Vaxzervria, Sputnik V, Sinopharm and CoronaVac) and one participant was immunized with Moderna vaccine abroad. All vaccines elicited a strong humoral immune response and anti-SARS-CoV-2 seropositivity ranged from 80% in participants vaccinated with Sinopharm to 95.9% in participants vaccinated with Comirnaty vaccine. The higher efficacy of Comirnaty vaccine in terms of eliciting humoral immune response compared to Sinopharm in our study is consistent with the literature [32]. Studies report higher percentage of seropositivity with vaccination after infection and when testing was done one month after the vaccination[33-37]. Our slightly lower seropositivity prevalence can be explained by the fact that a large proportion of the participants in our study were vaccinated six or more months prior to antibody testing. When analyzing the presence of anti-SARS-CoV-2 antibodies after the booster dose, we detected only one participant who was negative for anti-SARS-CoV-2 antibodies, consistent with the notion that boosters prolong the protection against COVID-19[38,39].

A limitation of our study was the way in which participants were recruited. In most of the population-based studies participants were selected randomly from the registered households[25,40]. We choose to be more accessible in order to have more people willing to participate in our study. All age groups were represented in our cohort, and women more than men were willing to participate. We positioned the recruitment in six frequent places in Skopje to capture information about the different municipalities. Our sample consisted of individuals with positive or negative PCR test, as well as PCR untested population, previously symptomatic or asymptomatic and vaccinated or unvaccinated participants, and having information for these different categories is the strength of our study. We do not have

information about the seroprevalence in different ethnic groups living in Skopje because this information was not available for analysis. Our study detected only IgG antibodies against N and S antigen in order to detect both immunity from infection and from vaccination. The cellular immunity and the role it may play were not evaluated.

IgG anti-SARS-CoV-2 antibodies surveillance is a good tool in the evaluation of the immunization of the population. Although initially quarantine and various other restrictions were necessary to control the overload of the healthcare system in our country, now, two years into the pandemic it is evident that majority of the population has been immunized either through infection or vaccination or both. How long will the antibodies offer protection on individual level and the seropositivity prevalence on population level remains unclear. Our results suggest that the population in the capital of our country has acquired some degree of collective immunity, however, the emergence of subvariants of Omicron, especially BA.5 during the last few months, challenges the importance of collective immunity in prevention of new infections. We can hypothesize that the immunity acquired in the past 2 years, along with the evolutionary changes of the virus, contribute to reduction of the number of severe cases and fatalities from COVID-19. Further research is needed to objectively evaluate their individual impact.

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Conflict of interest statement. None declared.

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