Detection of anti-SARS-CoV-2 antibodies and its seroprevalence in Zavidovići municipality of Zenica-Doboј Canton, Bosnia and Herzegovina

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ABSTRACT

Objectives: Improved serological detection of specific antibodies against SARS-CoV-2 could help estimate the true number of infections.

Methods: A total of 443 serum samples provided by unvaccinated patients of all ages with unknown COVID-19 status that were originally submitted for routine screening or clinical management from outpatient laboratory during the March–April 2021 (third wave) were collected. Seroprevalence of IgM/IgG antibodies was determined by lateral flow immunoassay (Tigsun, Beijing, China).

Results: Among 443 serum samples, 186 (42.0%) were positive (incidence of 5.2/1000) with slight predominance of females, 104 (55.9%), highest seropositivity in 25–50 and 51–64 years age groups, 61 (32.8%) and 57 (30.6%), respectively (P < 0.05); rural population was more prevalent, 101 (54.3%) (P < 0.05) and active workers, 86 (41.1%). Almost equal number of patients was with or without symptoms, 48.4% and 51.6%, respectively. For the comparison, in the same period it was registered 296 (out of 855; 34.6%) PCR SARS-CoV-19 positive persons (incidence of 8.2/1000) with the higher gender (females) and the highest age prevalence in 51–64 years age group (36.8%). In the period March 2020–June 2021, it was registered 804 (out of 3323; 24.2%) (incidence of 22.3/1000) PCR SARS-CoV-19 positive persons with no significant gender and significant age difference (25–50 and 51–64 years group, respectively).

Conclusion: In the regions with high prevalence/incidence of SARS-CoV-2 in the general population (Bosnia and Herzegovina is on the World top on the number of deaths) seroprevalence measuring can help tracking the spread of disease.

Keywords: Comorbidity, coronavirus, seroprevalence, symptoms

Introduction

Coronaviruses are a complex group of viruses of the subfamily Coronavirinae in the family Coronaviridae of the order Nidovirales.[1] Human coronaviruses were first discovered in the 1960s as causative agents of self-limited upper respiratory tract infections and until 2002, they were known to cause mild infections. However, this changed with the emergence of Severe Acute Respiratory Syndrome Coronavirus (SAR-CoV).[2]

Commonly reported COVID-19 symptoms include fever, shortness of breath, cough, loss of taste or smell and muscle pain.[3] SARS-CoV-2 has an incubation period of 2–14 days and approximately 80% of those that are infected will show mild or no symptoms.[4] A sero-epidemiological study provides information on the proportion of the population exposed and, if the antibodies are a marker of total or partial immunity, the proportion of the population that remains susceptible to the virus.[5] Some serological surveys of SARS-CoV-2 have been reported. Sood et al. reported seroprevalence of SARS-CoV-2 in Los Angeles in 4.65% of cases.[6] In Milan, Italy Valenti et al. reported about seroprevalence among healthy blood donors with the percentage of 2.7%.[7] Low seroprevalence (6%) has been reported in Kenya, especially among those who do not come into contact with the intermediate hosts.[8] High seroprevalence was reported in Iran, where the IgG antibodies were detected in two studies, 22% and 34%.[9,10] Study from the Uganda showed a higher seroprevalence of antibody in general population, in 87.53% of cases.[11]
Antibody test results are important for detection of previous SARS-CoV-2 infection that triggered some immune response with or without symptoms.\(^\text{[3]}\)

Human coronaviruses are important emerging pathogens and currently the world is facing a devastating pandemic caused by SARS-2. Therefore, there is the need for continuous viral surveillance. For preventive control of the pandemic spread of coronaviruses, we need additional testing and information about seroprevalence of antibodies in the municipality.

There is no information about seroprevalence of specific antibody on SARS-CoV-19 in Bosnia and Herzegovina.

The aim of the study was to determine the prevalence of SARS-CoV-2 antibodies in Zavidovići municipality.

**Methods**

**Sample collection**

In the period of March 2021–April 2021, 443 consecutive, non-duplicate serum samples were collected from participants who were not vaccinated with inactivated SARS-CoV-2 vaccine in Health Center of Zavidovići. The population covered by this institution is 35,988 in Zavidovići municipality of Zenica-Doboj Canton, Bosnia and Herzegovina. Informed consent was obtained from each participant. Informed consent includes demographic characteristics of participants (including age, gender, place of residence, and occupation). Furthermore, study were include data on participants experience of COVID-19 symptoms during the pandemic (including fever, feverishness, dry cough, sore throat, shortness of breath, muscle pain, loss of smell and taste, diarrhea) and information of health status (diabetes mellitus-DM, hypertension-HTA, cerebrovascular insult-CVI, heart diseases, diseases of blood vessels, malignancy, and others).

About 5 ml blood samples were collected from all participants (443 samples). Immunochromatographic assay had used for the detection and qualitative of anti-SARS-CoV-2 IgG antibodies using Tigsun COVID-19 Combo IgM/IgG Rapid test (the test has high sensitivity and specificity without cross-reaction with common viruses). Detection of SARS-CoV-2 IgG antibodies: When a positive sample has been detected, the SARS-CoV-2 IgG antibodies in the sample had bounded with colloidal gold-labeled SARS-CoV-2 antigens to form an immune complex. When the complex passes the Test Line (T), it had been bounding to the anti-human IgG monoclonal antibody immobilized in the Test Line (T) of the device to form a new complex. This had generated a colored test band that indicates a positive result. When the SARS-CoV-2 antibody level in the specimen was zero or below the target cutoff, there were not been a visible colored band in the Test Line (T) of the device. This indicated a negative result (http://www.tigsun.com/Content/2020/08/14/1712513045.html).

**Statistical analysis**

Descriptive analyses were reported as mean and percentage for different variables. The Chi-square test was used to investigate the association between variables. All analyses were performed by SPSS version 15.0 software. \(P < 0.05\) was considered significant.

**Results**

Overall, 443 participants were included in the study. Among 443, 186 (42.%; cumulative inc. 5.2) were seropositive while 257 (58.0%) were seronegative on virus COVID-19. Of the 186 seropositive samples (participants), 104 (55.9%; cumulative inc. 5.7) were females and 82 (44.1%; cumulative inc. 4.6) were males [Table 1]. There was no significant difference between genders in the seroprevalence.

The most seropositive samples were detected between 25–50 and 51–64 years old (33% and 31%; out of 186; cumulative inc. 4.7 and 7.5), followed by > 65 years old (26%; cumulative inc. 13.0), respectively [Table 1].

The seropositive patients were mostly from rural residence (54%; out of 186), and among of seropositive patients, 51% were active worker, followed by house person (24%) and retired (20%) [Tables 1 and 2].

Ninety seropositive patients (48.4%; out of 186) were detected with the specific symptoms. The most frequently symptoms were fever (60%; out of 90), muscle pain (46.7%), dry cough and loss of smell (36.7%), and others [Table 2].

The most patients with the specific symptoms were detected between 25 and 50 years old (36.7%; out of 90), followed by 51–64 years old (30.0%), while patients without symptoms were most detected between 51 and 64 years old (31.2%; out of 96), followed by 25–50 and > 65 years old (29.2%) [Table 3].

Overall, 81 (43.5%) patients had at least one underlying disease. Diabetes mellitus was the most frequent underlying disease (14.8%; out of 81), followed by hypertension (11.1%) and heart disease (8.6%). More than one comorbidity were detected in eight patients (9.9%) [Table 2].

NOTE: From the beginning of pandemic March 2020 to June 2021, a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test for SARS-CoV detection was performed using a nasopharyngeal swab in Zavidovići municipality in total of 3323 cases. Among 3323 patients, 804 (24%, cumulative inc. 22.3) were positive on COVID-19. A total of positive samples, 51% were males (cumulative inc. 22.9). The most positive samples were detected in > 65 years old (33%; cumulative inc. 70.9) followed by 25–50 and 51–64 years old (31% in both age groups; cumulative inc. 18.8 and 32.5), respectively [Table 4].
Table 1: Seroprevalence of anti-SARS-CoV-2 antibodies among Zavidović municipality population

| Characteristic                  | Total No of participants | Positive No | Positive % | Negative No | Negative % | P | No of population | Cumulative incidence/1000 |
|--------------------------------|--------------------------|-------------|------------|-------------|------------|----|------------------|---------------------------|
| **Gender**                     |                          |             |            |             |            |    |                  |                           |
| Females                        | 256                      | 104         | 41.3       | 152         | 59.1       |    | 18102            | 5.7                       |
| Males                          | 187                      | 82          | 44.1       | 105         | 40.9       |    | 17886            | 4.6                       |
| Total                          | 443                      | 186         | 41.8       | 257         | 58.2       |    | 35988            | 5.2                       |
| **Age (years)**                |                          |             |            |             |            |    |                  |                           |
| 0–14                           | 29                       | 15          | 51.7       | 14          | 48.3       | <0.05 | 6075             | 2.5                       |
| 15–24                          | 29                       | 4           | 2.2        | 25          | 97.8       |    | 5422             | 0.7                       |
| 25–50                          | 147                      | 61          | 41.4       | 86          | 58.6       |    | 13115            | 4.7                       |
| 51–64                          | 131                      | 57          | 43.9       | 74          | 56.1       |    | 7611             | 7.5                       |
| ≥65                            | 107                      | 49          | 45.7       | 58          | 54.3       |    | 3765             | 13.0                      |
| Total                          | 443                      | 186         | 41.8       | 257         | 58.2       |    | 35988            | 5.2                       |
| **Place of residence**         |                          |             |            |             |            |    |                  |                           |
| Urban                          | 192                      | 85          | 44.2       | 107         | 55.8       | <0.05 | 8174             | 10.4                      |
| Rural                          | 251                      | 101         | 40.3       | 150         | 59.7       |    | 27814            | 3.6                       |
| Total                          | 443                      | 186         | 41.8       | 257         | 58.2       |    | 35988            | 5.2                       |

Discussion

This study demonstrated a prevalence of positive antibody to SARS-CoV-2 of 42% in general population. Human coronaviruses are known to have a wide distribution. They are endemic to most countries in the world but usually limited information is available on their presence and circulation, especially from small countries, such as from Bosnia and Herzegovina. Information about seroprevalence will provide a picture about the possible level of exposure and population-based immunity against human coronaviruses. 

Seroprevalence of anti-SARS-CoV-2 in our study population was 42%, respectively, which is higher than in the report from Iran, 33% and 22%. Other studies reported that the seroprevalence of anti-SARS-CoV-2 antibodies was 1% in Germany, 0.7% in Texas, 9% in Austria, 11% in Switzerland and 2.7% in Milan, Italy. Some study, for example from Italy, showed that estimated period-prevalence of COVID-19 varies from 0.35% to 13.3%, and meta-analysis study from Rostami et al. showed that results varied from 1.5% in South America to 5.3% in Northern Europe. In 2021, 2 years from the beginning of pandemic, this is expected and the reasons for this is little higher seroprevalence of anti-SARS-CoV-2.

Seroprevalence of antibodies was higher in females than in males (56% and 44%, respectively), and it is similar in the reports form Spain, Iran, and Uganda. These results were contradictory with the reports from Iran (43% in males and 37% in females) and from South Korea (11% in male and 4% in female). Seroprevalence of antibodies was significantly more prevalent in the age group of 25–50 years old and it is similar in the reports from Iran and Switzerland. The highest seroprevalence in other studies was detected in the following age groups: 0–5 years old in Uganda and more than 60 years old in South Korean population. Exposure made age groups 0–5 years old and more than 60 years old more susceptible to contracting human coronaviruses resulting in the high seropositivity. We found that young children had significantly lower seroprevalence than the other age groups. A single positive young child out of 29 in our sample suggests that infection was less prevalent in children than in adults or active workers during this epidemic. These results are consistent with a small but growing body of evidence suggesting that young children are both infected and develop severe disease less often than adults, but much uncertainty remains. We included only children who were brought for biochemical testing of blood samples at the time of the research and immunological responses to infection might be different in younger children. More studies are needed to better understand infection and antibody dynamics among young children.

Differences in the prevalence of antibodies in urban and rural locations may be due to population density, demographics, use of mass transit, standard of living, the habit of using primary health care and other factors and different prevalence of IgG antibody in different localities is not surprising.

The highest seroprevalence was detected in active workers (44%, 31 with the lower education, 27 with the secondary education, 16 with a college degree, six as administrators and only two health professionals, followed by house person and retired, and it is contradictory with the report from Uganda (42% in pre-school children and 37% in students). The
Table 2: Characteristics of seropositive participants in the study

| Characteristic     | No (%) of participants | Total No of positive participants | % of total number |
|-------------------|------------------------|-----------------------------------|-------------------|
| Occupation        |                        |                                   |                   |
| Active worker     | 82                     | 44.1                              |                   |
| Unemployed        | 2                      | 1.1                               |                   |
| Retired           | 38                     | 20.4                              |                   |
| Student           | 9                      | 4.8                               |                   |
| Kids              | 8                      | 4.3                               |                   |
| House person      | 45                     | 24.2                              |                   |
| Other             | 2                      | 1.1                               |                   |
| Total             | 186                    | 100                               |                   |
| Symptoms          |                        |                                   |                   |
| With              | 90                     | 48.4                              |                   |
| Without           | 95                     | 51.1                              |                   |
| without data      | 1                      | 0.5                               |                   |
| Total             | 186                    | 100                               |                   |
| Type of symptoms  |                        |                                   |                   |
| Fever             | 54                     | 60.0                              |                   |
| Feverishness      | 22                     | 24.4                              |                   |
| Sore throat       | 16                     | 17.8                              |                   |
| Dry cough         | 33                     | 36.7                              |                   |
| Shortness of breath | 20                  | 22.2                              |                   |
| Muscle pain       | 42                     | 46.7                              |                   |
| Loss of smell     | 33                     | 36.7                              |                   |
| Loss of taste     | 30                     | 33.3                              |                   |
| Diarrhea          | 8                      | 8.9                               |                   |
| More than two symptoms | 59              | 65.6                              |                   |
| Total participants with symptoms | 90               | 100                               |                   |
| Chronic diseases  |                        |                                   |                   |
| DM                | 12                     | 14.8                              |                   |
| HTA               | 9                      | 11.1                              |                   |
| CVI               | 2                      | 2.5                               |                   |
| Heart diseases    | 7                      | 8.6                               |                   |
| Diseases of blood vessels | 2         | 2.5                               |                   |
| Oncological       | 5                      | 6.2                               |                   |
| Other             | 44                     | 54.3                              |                   |
| More than two     | 8                      | 9.9                               |                   |

Table 3: Distribution of seropositive patients with and without symptoms

| Age (Total No of seropositive patients) | Seropositive participants | P |
|----------------------------------------|---------------------------|---|
|                                        | with symptoms | without symptoms |   |
| 0–14 (15)                              | 7 (6.3) | 8 (8.3) | >0.05 |
| 15–24 (4)                              | 2 (2.2) | 2 (2.1) |      |
| 25–50 (61)                             | 33 (36.7) | 28 (29.2) | |
| 51–64 (57)                             | 27 (30.0) | 30 (31.2) | |
| ≥65 (49)                               | 21 (23.3) | 28 (29.2) |     |
| Total (186)                             | 90 (48.4) | 96 (51.6) | |

probable cause for this finding in our study is the higher risk of exposure and transfer of the virus between workers compared to other population groups.

Number of asymptomatic patients was higher (51.6%) than number of symptomatic patients, and it is similar to the reports from China[19] and Republic of Korea.[20] In Iceland and Italy number of asymptomatic patients was detected in ≥44%, which is lower than in our study.[21,22] Our findings also indicate differences between symptomatic and asymptomatic patients in age groups, with the prevalence of asymptomatic patients slightly increased with the age for people older than 50. Possible reason for this difference could be a high-risk population in the present study and a greater involvement in community activities.[16] This report indicates that patients with COVID-19 can transmit the disease regardless of their symptomatic status, and if they are not identified in a timely manner, they could become moving sources of infection and lead to massive transmission of disease.[23] They must be identified and quarantined to eliminate the transmission of SARS-CoV-2.

Fever, followed by muscle pain and dry cough, was the most prevalent of symptoms (60%, 47% and 37%) associated with the seroprevalence of anti-SARS-CoV antibody, and it is similar with the report from Iran,[9] Jeddah,[24] and Iraq.[25] Fever and anosmia were also common findings in report from California.[6] In Germany, fever, dry cough and anosmia were also the most prevalent symptoms associated with a positive anti-SARS-CoV-2 antibody test.[26] Cause of these more prevalent symptoms such as fever, dry cough, and others were more specific for COVID-19, than other similar diseases such as cold and flu.

The most common comorbidities were diabetes mellitus (15%), followed by hypertension (11%) and findings are lower than in report from Italy,[27] but higher than in report from Jeddah.[24] In this study, there is connection between positive samples on COVID-19 tested with PCR [Table 4], and seroprevalence of antibodies between ages [Table 1] (similar prevalence). In the future, vaccines will be the most effective way to combat COVID-19 to reduce the burden on the health system. The number of confirmed SARS-CoV-19 infections is largely underestimated. Seroprevalence surveys are of the utmost importance to assess the proportion of the population that has already developed antibodies against the virus and might potentially be protected against subsequent infection.[14] As recommended by the WHO, monitoring changes of seroprevalence over time is also crucial at the beginning of an epidemic to anticipate its dynamics and plan an adequate public health response.[28]
Table 4: Results of SARS-CoV-2 PCR in the period March 2020–June 2021

| Characteristic (No of participants) | Positive | Negative | Intermediate | No data | \( P \) | No of population | Cumulative incidence/1000 |
|-------------------------------------|----------|----------|--------------|---------|--------|----------------|--------------------------|
| Gender                              |          |          |              |         | >0.05  |                |                          |
| Females (1402)                      | 394 (49.0)| 983 (39.9)| 7 (29.2)     | 18 (60.0)|        | 18102          | 21.8                     |
| Males (1921)                        | 410 (51.0)| 1482 (60.1)| 17 (70.8)    | 12 (40.0)|        | 17886          | 22.9                     |
| Total (3323)                        | 804 (24.2)| 2465 (74.2)| 24 (0.7)     | 30 (0.9) |        | 35988          | 22.3                     |
| Age (years)                         | <0.05    |          |              |         |        |                |                          |
| 0–14 (100)                          | 9 (1.1)  | 91 (3.7) | 0            | 2 (6.7) |        | 6075           | 1.5                      |
| 15–24 (223)                         | 34 (4.2) | 189 (7.7)| 1 (4.2)      | 1 (3.3) |        | 5422           | 6.3                      |
| 25–50 (1254)                        | 247 (30.7)| 1007 (40.9)| 6 (25.0)    | 8 (26.7) |        | 13115          | 18.8                     |
| 51–64 (894)                         | 247 (30.7)| 647 (26.2)| 7 (29.2)     | 5 (16.7) |        | 7611           | 32.5                     |
| ≥65 (798)                           | 267 (33.2)| 531 (21.5)| 10 (41.7)    | 14 (46.7)|        | 3765           | 70.9                     |
| Total (3323)                        | 804 (24.2)| 2465 (74.2)| 24 (0.7)     | 30 (0.9) |        | 35988          | 22.3                     |

Conclusions

In general, after 2 years from the beginning of pandemic, it is still small number of seroprevalence in this study, and one of the reasons could be: (1) Inadequate time from exposure to form IgG response to COVID-19; (2) a significant number of first responders successfully kill the virus with respiratory tract IgA defenses, so the virus never enters in the blood; (3) IgG antibodies once produced, do not persist for very long; (4) and small number of participants included in this study.

Human coronaviruses are important emerging pathogens and currently the world is facing a devastating pandemic caused by SARS-2, there is therefore need for continuous viral surveillance.

Authors Declaration Statements

Ethics approval and consent to participate
The present study has been approved by the Director of Institute for Health and Food Safety Zenica. All participants signed questionnaire and they were maintained.

Declaration of Competing Interest
Declarations of interest: none.

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AI contributed to the data collection and processing of results, and writing and editing of the manuscript; SH were responsible for the organization and coordination of this idea; SSS, FB, and JD were responsible for analysis, data collection, and processing of results. All authors have approved the final manuscript.

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