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Professional paper

PREVALENCE OF SARS-CoV-2 IgG/IgM ANTIBODIES AMONG PATIENTS IN THE TETOVO REGION, NORTH MACEDONIA

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Abstract

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a global pandemic, including North Macedonia. However, there are only limited data regarding the precise prevalence of the COVID-19 pandemic in North Macedonia. Here, to estimate the magnitude of SARS-CoV-2 infection in Tetovo region, North Macedonia, we investigated the prevalence of immunoglobulins G (IgG and IgM) antibodies. The detection of IgM and IgG immunoglobulins was performed through the Ichroma II serological test, in the Laor laboratory in Tetovo. We enrolled 1582 individuals from June to December 2020 and January to June 2021 observed that the subjects' overall prevalence of IgG antibodies to SARS-CoV-2, for the female gender, it was 661 cases (42%), while for the male gender there were 921 immunized cases (58%). The highest prevalence among age groups was in the 20-40-year-olds, during 2020 (313 immunized persons), while during 2021, the age group 40-60 years had the highest prevalence (383 immunized persons), and the lowest prevalence was in the age group 0-20 years, for both years. Also, the younger population has shown less susceptibility to the disease. In conclusion, the COVID-19 outbreak among asymptomatic populations was characterized by a high prevalence of infection in Tetovo region.

Keywords: Coronavirus, SARS-CoV-2, immunoglobulins G (IgG and IgM), immunized, prevalence.

1. Introduction

Coronavirus 19 disease (COVID-19)—a severe, acute respiratory syndrome caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) - was first identified in Wuhan, China, in December 2019 [(Huang *et al*, 2020) (Zhu *et al*, 2020)], and spread within months to most nations of the world (Hick *and* Biddinger, 2020). The World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19), as a global pandemic on March 11, 2020 (4). The SARS-CoV-2 (2019-nCOV) is a member of the betacoronavirus genus, that also includes Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV). The causative agent was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to its genetic similarity with the SARS virus (Zhu, *et al*, 2020). COVID -19 has become a global pandemic and has spread rapidly to more than 195 countries/regions (World Health Organization, 2021).
The COVID-19 disease can present as either asymptomatic or symptomatic infections. Symptomatic infections can be mild, or moderate to severe (Huang *et al*, 2020). The diagnosis and management of COVID-19 are based on the detection of SARS-CoV-2 in nasopharyngeal swabs from patients presenting with clinical signs (including fever, dry cough, and/or shortness of breath), or in suspected cases, by reverse transcription polymerase chain reaction (RT-PCR) [(Chan *et al*, 2020) (Ai *et al*, 2020)]. Since the manifestation of SARS-CoV-2 infection ranges from asymptomatic to fatal, the surveillance of confirmed COVID-19 cases might not be representative of a particular community [(Xu *et al*, 2020) (Stringhini *et al*, 2020)]. The SARS-

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CoV-2 virus can induce specific humoral immune responses in most symptomatic and asymptomatic infections in which IgM antibodies appear after 3 to 10 days of infection as a primary immune response followed by IgG after 14 days of infection as a secondary immune response that lasts for months [(Tan *et al*, 2020) (Zhao *et al*, 2020)].

Although the RT-PCR technique is a gold standard for the diagnosis of COVID-19, this technique does not reflect the true spread of the disease in the community. Firstly, many clinically infected cases can give negative results during initial testing by RT-PCR (Arevalo-Rodriguez *et al*, 2020); secondly, the cost of the test is high and many clinically infected patients cannot afford the test; and thirdly, only patients with a clinical diagnosis of the disease are subjected to this test. Therefore, using serological tests with high sensitivity and specificity on a large scale is useful to gain insight into the dynamics of specific antibody responses during and after the spread of the virus and, if undertaken routinely, to inform health authorities, politicians, and policy-makers about seroprevalence at any given stage during an epidemic (World Health Organization, 2020) (Munster *et al*, 2020). The prevalence of specific serum antibodies (IgG and/or IgM) against SARS-CoV-2 can provide a sound indication of exposure to SARS-CoV-2 in a population [(Xu *et al*, 2020) (Pollán et al, 2020)]. Due to an apparent persistence of antibodies to SARS-CoV-2 (particularly IgG) after viral clearance (Xu *et al*, 2020), it is expected that serological monitoring and surveillance provide relevant datasets to estimate the cumulative prevalence of SARS-CoV-2 infection/exposure in a population [(Xu*et al*, 2020) (Thomas *et al*, 2020)], and may even indicate the immune status of individuals or populations [(Stringhini *et al*, 2020) (Pollán *et al*, 2020)].

Also, studies have shown that even though students of most school-going age may not be critically affected and die of the SARS-CoV-2, they may have higher viral loads as compared to adults, suggesting that they may still be transmitters of the disease, similar to other respiratory viruses [(Bobrovitz et al, 2021) (Glezen, 2006)]. This is one of the key factors that has caused many countries worldwide to implement school closures as an important component of SARSCoV-2 transmission mitigation strategies. According to the United Nations Educational, Scientific and Cultural Organization (UNESCO), in over 160 countries Nord Macedonia implemented nationwide school closures, impacting over 87% of the world's student population (United Nations Educational, Scientific and Cultural Organization, 2022). Interestingly, a published work has shown that there are very rare cases of SARS-CoV-2 reinfection (Lumley *et al*, 2021). It is therefore, more important to identify those who have had SARS-CoV-2 infections and had a successful seroconversion and develop the antibody against SARS-CoV-2 to best streamline the workforce and implement logical policies that can best mitigate the Covid-19 pandemic but with minimal economic impact.

Several commercial and in-house immunoassays are being used for the detection of IgG and/or IgM serum antibodies to SARS-CoV-2; these are mainly enzyme-linked immunosorbent assays (ELISAs), chemiluminescence immunoassays (CLIAs) or lateral flow assays (LFIAs) [(Van Elslande *et al*, 2020) (Isabel *et al*, 2020)]. The diagnostic specificity and sensitivity of these methods vary and depend on the use of recombinant or purified protein antigens—e.g. spike (S), envelope (E), membrane (M), nucleocapsid (N), or receptor binding domain (RBD) proteins—and the rigor of assay optimization (Peiris *et al*, 2004) (Jiang *et al*, 2020).

Therefore, the present study aimed to detect seropositivity against the SARS-CoV-2 virus among outpatients who visited the private diagnostic laboratory for COVID-19 RT-PCR tests, as well as other symptomatic, and asymptomatic individuals. We undertook a meta-analysis to estimate the seroprevalence of SARS-CoV-2 in people from the Tetovo region.

Molecular tests are generally not suited for public health screening, antibody tests are more suited, and the more portable, the better. Recently, Boditech launched a Fluorescence Immunoassay (FIA) for the qualitative determination of IgG/IgM antibodies against "Novel Coronavirus" in human whole blood, serum, and plasma. There is no gold standard assay to

evaluate antibody tests with, so we decided to evaluate the Abbott Architect SARS-Cov-2 IgG/IgM assay which is a Chemiluminescent Microparticle Immunoassay (CMIA) used for qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in human serum and plasma on the Architect 1 System recently evaluated by Public Health England (PHE) (Mahase, 2020). We conducted a study with patients' blood samples during the Covid-19 pandemic period, with their consent for Covid-19 testing, to determine the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between the Boditech iCHROMA™ IgG/IgM antibody assay. iCHROMA™ COVID-19 Ab test system is a rapid in vitro diagnostic test that qualitatively detects anti-SARS-CoV-2 IgG/IgM antibodies in venous whole blood, serum, or plasma samples with high sensitivity. The test results are important in detecting infections with few or no symptoms. Hence, the detection of anti-SARS-Cov-2 antibodies in patient specimens has clinical significance for prevention or effective control of community spread of COVID-19. Moreover, periodic serological tests after confirmation of SARS-CoV-2 infection in a patient may help determine the course of treatment.

2. Methodology

In this cross-sectional study, a total of 1582 blood samples were collected from individuals from the region of Tetovo who visited the private laboratory Laor from June 2020 to December 2020, and from January to June of 2021. Sera were separated and tested immediately for IgG and IgM antibodies using the Ichroma COVID-19 test. All individuals with and without clinical diagnoses of COVID-19 visited the laboratory on their own to check their immune response against the SARS-CoV-2 virus. The age of our sample population ranged from 1 to 75 years with a mean of 38.0 ± 17 SD. All serum samples from the study population were tested for anti-SARS-CoV-2 virus IgG and IgM antibodies using the Boditech iCHROMA™ IgM/IgG in conjunction with ichroma™ II assay.
Boditech iCHROMA™ Method Principle, the test uses a sandwich immunodetection method; fluorescence labeled conjugates in a dried detection buffer bind to antibodies in the sample, forming antibody-antigen complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized anti-human IgG on the test strip. More antigen-antibody complexes lead to a stronger fluorescence signal by the detector antigen which is processed by the iCHROMA™. The iCHROMA™ processes the signal using a cut-off index of 0.9-1.1, results <0.9 are interpreted as negative, results between 0.9 and 1.1 are interpreted as indeterminate and results >1.1 are interpreted as positive.

3. Results and Discussion

3.1. Results: The results of this study are reflected in the tables below. These results are categorized by year, gender, and age group. A total of 1582 individuals were tested in this study. From June to December 2020, 738 individuals were tested, while in 2021, 844 individuals were tested during the January-June period. The results of this study are reflected in the following tables. These results are categorized in Table 1. General results of the frequency of SARS-CoV-2 antibodies in the region of Tetovo during the year 2020-2021-year, gender and age group; Table 2. Comparison of study participants by gender during the years 2020/21; Table 3. Presence of antibodies by age group during 2020/21.

Table 1. General results of the frequency of SARS-CoV-2 antibodies in the region of Tetovo during the year 2020-2021

Month/year 2020	*Tested*	IgG		IgM		*Month/year 2021*	*Tested*	IgG		IgM	
		>0.9	<0.9	>0.9	<0,9			>0.9	<0.9	>0.9	<0.9
June	21	13	8	18	3	January	154	81	73	48	106
July	77	61	16	73	4	February	64	34	30	19	45
Aguste	63	41	22	62	1	March	157	72	85	38	119
September	27	17	10	25	2	April	275	169	106	65	210
October	57	39	18	56	1	May	133	90	43	37	96
November	254	148	106	160	94	June	61	45	16	9	52
December	239	93	146	118	121	**Total**	**844**	491	353	216	628
Total	**738**	412	326	512	226	**Percent**		**58%**	**42%**	**26%**	**74%**
Percent		**56%**	**44%**	**69%**	**31%**						

In Table 1, the data reflect the presence of antibodies in persons immunized by SARS-CoV-2 in the region of Tetovo from 2020 to 2021. From the preliminary data, the results show that the people who underwent the screening at the Laor laboratory in Tetovo, during this period, were a total of 1582 individuals, of which 679 were positive with IgG, while 854 were positive with IgM. In November 2020, the IgM value was higher compared to other months, while during 2021, the highest IgM value was in April, which means current infection with SARS-CoV-2.

Table 2. Comparison of study participants by gender during the years 2020/21

Month/year 2020	Tested	Tested female	Tested male	Month/year 2021	Tested	Tested female	Tested male
June	21	12	9	January	154	68	86
July	77	29	48	February	64	21	43
Aguste	63	13	50	March	157	58	99
September	27	**17**	10	April	275	113	162
October	57	28	29	May	133	62	71
November	254	106	148	June	61	27	34
December	239	107	132	**Total**	**844**	**349**	**495**
Total	**738**	**312**	**426**	**Percent**		**41%**	**59%**
Percent		**42%**	**58%**				

Table 2 shows the results of testing by gender during the years 2020 and 2021. The study is focused on the year 2020 from June to December, and on the year 2021 from January to June. The largest number of those tested during the year is male, out of 738 tested, 426 (58%) were male. It should be noted that only during September the number of women tested was higher. Throughout 2021, the same trend has been with a narrowing of the difference of 1%. During this year, in the months where the study was focused, a total of 844 SARS-CoV virus-2 were tested, of which 51%c (495) were men and 41% (349) were women.

Table 3. The presence of antibodies by age group during 2020/21

Age group /2020	Total number	Female	Male	Age group /2021	Total number	Female	Male
0-20	40	20	20	0-20	22	12	10
20-40	313	138	175	20-40	324	146	178
40-60	261	106	155	40-60	383	144	239
>60	124	42	82	>60	115	45	70
Total	738	306	432	**Total**	844	347	497
Percent		**42%**	**58%**	**Percent**		**41%**	**59%**

According to the aforementioned results, we observe the prevalence of immunoglobulins according to different age groups during the year 2020. The age group 20-40 years has the highest titer of immunoglobulins, according to gender, the largest number was in the male

gender (175 patients) out of 313 tested. Then follows the age group 40-60 years, from 261 people tested, in 155 male patients, the titer of immunoglobulins was detected. While the age group over 60 years has the lowest titer of immunoglobulins, the presence of immunoglobulins was detected in 82 male persons out of 124 persons tested.
According to the results of the prevalence of immunoglobulins according to different age groups during 2021, we can conclude that the 40-60 age group was most affected by the infection, with 239 male persons and 144 female persons. Then follows the age group 20-40 years, 324 people who have been infected, of which 178 were men and 146 women. The age group over 60 resulted in 115 people who passed the infection, of which 70 were men and 45 were women. As for the age group 0-20 years, it can be seen that it is the age group with the fewest cases that were affected by the infection, 12 were women and 10 were men.

3.2. Discussion: Serological examination in the region of Tetovo, North Macedonia, confirmed the presence of infection with a different level in different genders and age groups during 2020 and 2021. Of the 1582 participants, 661 (42%) were female and 921 (58%) were male; the mean age was 39.76 (SD 16.83) years old.738 tests were considered valid for 2020 and 844 for 2021. In 2020 of the valid samples, 512 tested positive for IgM and 412 tested positive for IgG. In 2021 of the valid samples, 216 tested positive for IgM and 491 tested positive for IgG. During two years, 903 tests were considered seropositive for IgG and 728 for IgM. During 2020, the overall prevalence of anti-SARS-CoV-2-IgG was 56% (412 immunized persons), while that of IgM was 69% (512 immunized persons). As of 2021, the overall prevalence of anti-SARS-CoV-2-IgG was 58% (491 immunized persons), while that of IgM was 26% (216 immunized persons).
Comparison of the prevalence according to sampling gender (2020/2021), revealed that the prevalence of anti-SARS-CoV-2- IgM was higher in the male gender (58%; 426/2020, 495/2021) compared to the female gender (42%; 312/2020, 349/2021), although the differences were not significant.
It should be noted that there was also a significant difference in the prevalence of anti-SARS-CoV-2 antibodies between age groups. During 2020, the 20-40 age group had the highest prevalence (with 313 immunized persons), while the 0-20 age group showed the lowest prevalence (40 positive cases). During 2021, the 40-60 age group had the highest prevalence (383 positive cases) and the 0-20 age group showed the lowest prevalence (with 22 positive cases).
In the SARS epidemic, the detection of IgM and IgG allowed serological diagnosis. Similar serological responses have been observed in patients with COVID-19, and the dynamic pattern of these responses is consistent with acute viral infection. Antibody testing against SARS-CoV-2 is rapid and sensitive for the adjunctive diagnosis of COVID-19. In the present study, serological responses, that is, IgM and IgG antibody levels, were retrospectively analyzed in COVID-19 patients with different disease severities and outcomes. During viral infection with SARS-CoV-2, the production of specific antibodies against the virus is stable in most patients, except immunocompromised patients. IgM can be detected as early as 3 days after infection and provides the first line of defense of humoral immunity, after which high-affinity IgG responses begin and play a key role in long-term immune memory.
Our data showed that IgM was generated in patients with COVID-19 one week after the onset of symptoms, then reached its peak level in 2-3 weeks, after which the level decreased. Meanwhile, IgG levels increased rapidly starting slightly later compared to IgM, and were maintained at high levels for 2 months. Therefore, detectable levels of IgM and IgG antibodies can provide information about the serological convention during the disease, as the detection of IgM antibodies indicates recent exposure to SARS-CoV-2 and the detection of IgG antibodies in the absence of detectable IgM antibodies. indicates previous exposure to the virus.

A few limitations should be mentioned. First, false-negative and false-positive results of antibody detection may affect the analysis of patients with different disease severities. Second, the time from symptom onset to admission can be long, and continuous monitoring data in a patient. Third, the relationship between antibody levels and viral copies within the same patients is unknown. In conclusion, this study found that anti-SARS-CoV-2 antibody levels differ significantly among COVID-19 patients with different disease severity and outcomes. Quantitative IgM and IgG assays may play an important role in the diagnosis and prognosis of COVID-19.

After SARS-CoV-2 caused a serious global pandemic, posed a great threat to human health, and seriously affected the normal order of social life, it is a "real troublemaker". Humans have made considerable efforts in the fight against COVID-19 and have achieved several results, including completing the task of developing vaccines and specific drugs. But we still face the threat of the "resurgence" of the virus. Despite the many past challenges, through the efforts of all humanity, we will eventually overcome this virus.

4. Conclusions

From the analysis, comparison, and discussion of the data we can come to the following conclusions:

Antibody testing against SARS-CoV-2 is rapid and sensitive for the adjunctive diagnosis of COVID-19. In the present study, IgM and IgG antibody levels were retrospectively analyzed in COVID-19 patients with different disease severity and outcomes. During viral infection with SARS-CoV-2, the production of specific antibodies against the virus was stable in most patients, except immunocompromised patients.

IgM can be detected as early as 3 days after infection and provides the first line of defense of humoral immunity, after which high-affinity IgG responses begin and play a key role in long-term immune memory. Our data showed that IgM was generated in patients with COVID-19 one week after the onset of symptoms, then reached its peak level in 2-3 weeks, after which the level decreased. Meanwhile, IgG levels increased rapidly starting slightly later compared to IgM, and were maintained at high levels for 2 months.

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Comparison of the prevalence according to sampling gender (2020/2021), revealed that the prevalence of anti-SARS-CoV-2- IgM was higher in the male gender (58%; 426/2020, 495/2021) compared to the female gender (42%; 312/2020, 349/2021), although the differences were not significant.

It should be noted that there was also a significant difference in the prevalence of anti-SARS-CoV-2 antibodies between age groups. During 2020, the 20-40 age group had the highest prevalence (with 313 immunized persons), while the 0-20 age group showed the lowest prevalence (40 positive cases). During 2021, the 40-60 age group had the highest prevalence (383 positive cases) and the 0-20 age group showed the lowest prevalence (with 22 positive cases).

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face the threat of the "resurgence" of the virus. Despite the many past challenges, through the efforts of all humanity, we will eventually overcome this virus.
In conclusion, this study found that anti-SARS-CoV-2 antibody levels differ significantly among COVID-19 patients with different disease severity and outcomes. Quantitative IgM and IgG assays may play an important role in the diagnosis and prognosis of COVID-19.

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References

[1] Ai T., Yang Z., Hou H., Zhan C., Chen C., Lv W., 2020. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: *a report of 1014 cases Radiology*, 296 (2020), pp. E32-E40.

[2] Arevalo-Rodriguez I., Buitrago-Garcia D., Simancas-Racines D., Zambrano-Achig P., Del Campo R., Ciapponi A., Sued O., Martinez-Garcia L., Rutjes A.W., Low N., 2020. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. *PloS One 15*: e0242958.

[3] Bobrovitz N., Arora R.K., Cao C, Boucher E., Liu M., Donnici C., 2021. Global seroprevalence of SARSCoV-2 antibodies: A systematic review and meta-analysis. Khudyakov YE, editor. *PLoS One* [Internet]. 2021 Jun 23 [cited 2022 May 29];16(6): e0252617. *Available from*: https://dx.plos.org/10.1371/journal.pone.0252617.

[4] Chan J.F.-W., Yip C.C.-Y., To K.K.-W., Tang T.H.-C., Wong S.C.-Y., Leung K.-H., 2020. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. *J Clin Microbiol*, 58, pp. e00310-e00320.

[5] Glezen W.P., 2006. Herd protection against influenza. *J Clin Virol Off Publ Pan Am Soc Clin Virol*. 2006 Dec;37(4):237–43.

[6] Hick J.L., Biddinger P.D., 2020. Novel coronavirus and old lessons—preparing the health system for the pandemic N Engl J Med, 382 (2020), p. e55.

[7] Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., Zhang L., Fan G., Xu J., Gu X., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 395: 497-506.

[8] Isabel M., Damien G., Benoit K., Hafid D., Soleimani R., Vincenzo C., 2020. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. *J Clin Virol*, 128, p. 104413.

[9] Jiang S., Hillyer C., Du L., 2020. Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. *Trends Immunol*, 41, pp. 355-359.

[10] Lumley S.F., O'Donnell D., Stoesser N.E., Matthews P.C., Howarth A., Hatch S.B., 2021. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. *N Engl J Med*. 2021;384(6):533–40.

[11] Mahase E., 2020. Covid-19: Two antibody tests are "highly specific" but vary in sensitivity, evaluations find. *BMJ 369*: 2066.

[12] Munster V.J., Koopmans M., van Doremalen N., van Riel D., de Wit E., 2020. A novel coronavirus emerging in China—key questions for impact assessment N Engl J Med, 382, pp. 692-694.

[13] Peiris J., Guan Y., Yuen K., 2024. Severe acute respiratory syndrome. *Nat Med*, 10, pp. S88-S97.

[14] Pollán M., Pérez-Gómez B., Pastor-Barriuso R., Oteo J., Hernán M.A., Pérez-Olmeda M., 2020. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet, 396, pp. 535-544.*

[15] Stringhini S., Wisniak A., Piumatti G., Azman A.S., Lauer S.A., Baysson H., 2020. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP). *A population-based study Lancet*, 396, pp. 313-319.

[16] Tan W., Lu Y., Zhang J., Wang J., Dan Y., Tan Z., He X., Qian C., Sun Q., Hu Q., 2020. Viral kinetics and antibody responses in patients with COVID-19. *MedRxiv*: 20042382.

[17] Thomas S.N., Altawallbeh G., Zaun C., Pape K., Peters J.M., Titcombe P.J., 2020. Initial determination of COVID-19 seroprevalence among outpatients and healthcare workers in Minnesota using a novel SARS-CoV-2 total antibody ELISA. *Available at SSRN*: https://doi.org/10.2139/ssrn.3627313, Accessed 15th Aug 2020.

[18] United Nations Educational, Scientific and Cultural Organization (UNESCO), 2022. COVID-19 Educational Disruption and Response [Internet]. 2022 [cited 2022 Jul 15]. *Available from*: https://en.unesco.org/node/320920.
[19] Van Elslande J., Houben E., Depypere M., Brackenier A., Desmet S., André E., 2020. Diagnostic performance of 7 rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. *Clin Microbiol Infect*, 26, pp. 1082-1087.
[20] World Health Organization (WHO), 2020. Coronavirus disease 2019 (COVID-19): *situation report*, vol. 43. Available: nhttps://apps.who.int/iris/handle/10665/331354. Accessed 10 August 2020.
[21] World Health Organization (WHO), 2021. *Available*: https://www.who.int/emergencies/diseases/novel coronavirus-2019/situation-reports/. Accessed 15 August 2021.
[22] World Health Organization, 2020. Global surveillance for COVID-19 disease caused by human infection with the 2019 novel coronavirus *Available from*: https://www.who.int/publications-detail/global surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov), Accessed 18th Aug 2020.
[23] Xu X., Sun J., Nie S., Li H., Kong Y., Liang M.,2020. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China. *Nat Med*, 26, pp. 1193-1195.
[24] Zhao J., Yuan Q., Wang H., Liu W., Liao X., Su Y.., Wang X, Yuan J., Li T., Li J., 2020. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis* 71: 2027-2034.
[25] Zhu N., Zhang D., Wang W., Li X., Yang B., Song J., Zhao X., Huang B., Shi W., Lu R., 2020. A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med* 382: 727-733.