Validation of SARS-CoV-2 Serological Essay, Bahrain Experience

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Background: The standard test for diagnosing Coronavirus disease (COVID-19) is the polymerase chain reaction (PCR) test. Since the start of the pandemic, there has been a rush in the development of tests that can detect the presence of antibodies produced by COVID-19 cases as a response to the exposure to the SARS-CoV-2 virus.

Objective: To evaluate the validity of the serology tests for detecting SARS-CoV-2 protective IgG antibodies.

Design: Cross-Sectional Prospective Study.

Setting: COVID-19 Testing and Caring Facilities, Kingdom of Bahrain.

Method: From 22 June to 1 July 2020, healthcare workers, non-national laborers, symptomatic and asymptomatic patients were included in the study. All patients underwent PCR and serology tests. The presence of IgG antibodies among participants were measured. The sensitivity and specificity of the serology tests were evaluated.

Result: Three hundred eighty-eight participants were included in the study, the mean age was 40±13 years. Two-hundred thirty-two (59.7%) were males and 242 (62.3%) were Bahrainis. Seventy-three (18.8%) were healthcare workers, 87 (22.4%) were non-national laborers, 109 (28.1%) were symptomatic and 119 (30.7%) were asymptomatic. One hundred sixty-four (42.2%) participants were COVID-19 positive. Ninety-six (24.7%) had a positive serology test with IgG level >1.4. The sensitivity of the serology test at <7 days was 28% (CI: 19.4%-38.4%), at 7-13 days was 77.8% (CI: 60.9%-89.9%) and >14 days was 84.4% (CI: 67.2%-94.7%). The specificity of the test was 93.3% (CI: 89.2%-96.2%).

Conclusion: The sensitivity of the serology test to detect the IgG antibodies 14 days after testing positive with COVID-19 was 84% and the specificity was 93.3%. The result supports the use of the test in a serosurvey study.

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Since the beginning of the Coronavirus disease (COVID-19) outbreak in China in late December 2019, the number of cases had climbed to approach six million cases across the globe. On 4 August 2020, the number of COVID-19 cases in the Kingdom of Bahrain had exceeded 40 thousand cases[1].

COVID-19 is a newly emerging infectious disease caused by the SARS-CoV-2 virus. Generally, all ages and all races are susceptible to the disease. Close contacts with patients are at a higher risk of getting the infection. The main mode for transmission of the disease is through inhalation and respiratory droplets. The mean incubation period of the disease is 5.2 days[2].

As the pandemic was rapidly accelerating, there has been a rush in the development of tests that can detect the presence of antibodies produced by COVID-19 cases as a response to the exposure/infection to the SARS-CoV-2 virus. The specificity of these tests depends on the chosen target antigen. The level of the developed immunity and cut-off concentration value

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of SARS-CoV-2 immunoglobulin G (IgG) antibodies have not yet been determined. Moreover, the interpretation of the results of those tests will need to be linked to some of the clinical data collected from cases such as being symptomatic or asymptomatic and the duration of symptoms[3].

During the period of the lifting of the coronavirus pandemic restrictions, it is important to identify people who had immunity against the virus and are potentially protected from re-infection. The randomly selected SARS-CoV-2 antibody testing may be a necessity to lift the restrictions.

Immunity to SARS-CoV-2 "infection has been equated to the presence of antibodies to the crown protein or spike and more specifically to the receptor-binding domain (RBD) able to neutralize the virus"[4]. The immunity against SARS-CoV-2 is based on the assumption that the infection will create sufficient protective antibodies. To establish a population immunity, enough number of people will need to be infected to have sufficient immunity. A mass serological testing will help in determining how many individuals are immune and how far the community is from achieving the population immunity[5].

The aim of this study is to evaluate the validity of the serology tests for detecting SARS-CoV-2 protective IgG antibodies in people who had or had not been infected by COVID-19.

METHOD

In Bahrain, there is one centralized testing facility located in the Exhibition Center where all suspected cases of COVID-19 infection are referred. Out of 18 treatment facilities, three treatment facilities were selected for the study, Sitra COVID Treatment Facility, which accommodates non-national laborers, Abdulla Bin Ali Kanoo Center which accommodates mainly asymptomatic COVID positive cases, and Ebrahim Khalil Kanoo which accommodates symptomatic COVID positive cases.

The participants were recruited from the COVID-19 testing and treating facilities from 22 June to 1 July 2020. The study population was stratified into four groups. The groups were healthcare workers (HCW) including nationals and non-nationals, non-national laborers, asymptomatic patients and symptomatic patients. COVID-19 symptoms were adopted from the World Health Organization (WHO) COVID-19 case definition[1]. Each group was further divided according to the results of COVID-19 real-time reverse transcription-polymerase chain reaction (rt-PCR) test into "tested positive" or "tested negative". Healthcare workers in the COVID-19 caring facilities undergo regular rt-PCR to diagnose COVID-19 weekly during which they were invited to participate in the study and to give blood for the serology test. HCW who were previously infected with COVID-19 were also invited to participate and to donate blood for serology through a phone call.

The non-national laborers in the whole country were randomly subjected to the rt-PCR test in the centralized testing center. The first sixty who agreed to give written consent for a blood sample for serology were included. Positive cases for non-national laborers were recruited from the Sitra COVID-19 Treatment Facility which accommodates mainly non-national.

Asymptomatic cases were recruited from patients who were attending the testing center as a contact for positive cases. The definition of COVID-19 contacts was adopted from the World Health Organization (WHO)[1]. The symptomatic and asymptomatic cases with positive COVID-19 tests are recruited from the treatment facilities. All participants were required to fill a written consent and a short questionnaire for the study. The data were collected between 22 June and 1 July 2020 where all patients participating in the study underwent the rt-PCR and the serology tests.

A serum sample was collected from each participant with the COVID-19 rt-PCR test. The serum samples were screened for the presence of COVID-19 virus-specific antibodies using a serological test named SARS-CoV-2 IgG for use with ARCHITECT; reference B6R8620 (Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, USA). The serology test used detects the IgG antibodies to SARS-CoV-2 in the human serum and plasma using the chemiluminescent micro particles immunoassay technology. The amount of IgG antibodies in the sample is measured as a relative light unit which results from the chemiluminescent reaction and had a direct relationship with the IgG antibodies level in the sample. This relationship is reflected in a calculated index. The index cut off point is 1.4, below which the IgG is considered negative. The mean level of serum IgG among positive cases was calculated for each week post-COVID-19 diagnosis.

Data collected were entered into a specifically designed excel sheet and then analyzed using statistical package (SPSS) version 21. The mean and standard deviation (SD) for age were calculated for each group while the frequency and percentages were calculated for sex and nationality. The results from rt-PCR and IgG tests were presented as frequency and percentage of negative and positive tests. The sensitivity and specificity of the serology test were calculated for the group as a whole and the sub-groups. The days between the rt-PCR positive test and the positive serology test results were calculated and grouped by weeks. In each week, the mean and the SD level of the IgG positive patients was calculated. The presence of antibodies and the level of the antibodies were compared between the groups.

The levels of antibodies were compared between positive COVID-19 and negative. The ANOVA test was used to compare the ages between the groups. A Chi-square test was used to compare the categorical variables. The sensitivity and specificity of the serology tests were calculated based on the PCR test result as the gold standard for diagnosis and at a prevalence level of 1.25%. The sensitivity and specificity of the test were recalculated for patients who underwent a serology test one week and two weeks from the date of the rt-PCR test.

RESULT

A total of 388 participants were included in the study from 22 June to 1 July 2020. The mean age was 40±13 years ranging from 9 to 99 years. Two-hundred thirty-two (59.7%) were males and 242 (62.3%) were Bahrainis. Participants in the study were divided into four groups: 73 (18.8%) healthcare workers, 87 (22.4%) non-national laborers, 109 (28.1%) symptomatic and 119 (30.7%) asymptomatic. Each group consisted of both positive and negative patients to COVID-19 PCR tests, see table 1. Healthcare workers were mainly females, 50 (12.9%) and they had the lowest mean age with 339± years. Non-national laborers were mainly males, 83 (21.4%) and mean age 40 years.

The percentage of participants with positive COVID-19 tests varied between the groups (P<.0001). Twelve (3%) healthcare workers had the lowest positive COVID-19 tests and 63 (16%) symptomatic patients had the highest positive COVID-19 tests.

Ninety-six (24.7%) patients showed positive results to

Table1: Personal Characters of the Study Population and the Four Subgroups Based on Work Status and the Presence of Symptoms

Characteristics	Total	Healthcare Worker	Non-national Laborer	Asymptomatic	Symptomatic
Total	**388 (100%)**	**73 (18.8%)**	**87 (22.4%)**	**109 (28.1%)**	**119 (30.7%)**
Age, year, mean (SD)	40 (13)	33 (9)	40 (11)	40 (13)	43 (16)
Gender					
Male	232 (59.7%)	23 (31.5%)	83 (95.4%)	68 (62.3%)	58 (48.7%)
Female	156 (40.3%)	50 (68.5%)	4 (4.5%)	41 (37.7%)	61 (51.2%)
Nationality					
Bahraini	242(62.3%)	69 (94.5%)	3(3.4%)	92 (84.4%)	78 (65.5%)
Non-Bahraini	146 (37.7%)	4 (5.5%)	84 (96.5%)	17 (15.5%)	41 (34.4%)
Patient with positive COVID-19 test	165 (42.2%)	12 (16.4%)	40 (45.9%)	50 (45.8%)	63 (52.9%)

HCW= health care worker, SD= standard deviation, COVID-19= Corona virus disease

IgG testing, 82 (21%) had been previously diagnosed with COVID-19 infection. Fourteen (3.6%) participants with no previous history of exposure to the infection had developed IgG against the virus. The IgG positive results among patients with no exposure history varied between the groups (P<.0003). The highest frequency of IgG positive was among non-national laborers, 87 (22.4%). The sensitivity of the serology test was calculated to be 49.4% (CI 41.5% to 57.3%) and the specificity of the test was 93.3 % (CI 89.2% to 96.2%). One-week post-rt-PCR test, the sensitivity increased to 79.7% (CI 68.3-88.4%) and the specificity to 93.7% (CI 89.7-96.5%). Two weeks from the rt-PCR positive test, the sensitivity reached 81.8% (CI 64-93%) and the specificity reached 93.7% (89.7%-96.5%).

One hundred eight (27.8%) symptomatic patients had documented symptoms onset date. The sensitivity of the serology test among symptomatic patients was 71.3% (CI: 61.4-79.9%). One week after the symptoms, the sensitivity reached 91.4% (CI: 81-97%). If the patients were tested two weeks from the onset of symptoms, the sensitivity reached 95.6% (CI: 78-99.9%). In asymptomatic patients 280 (72.2%), the calculated sensitivity was 15.6% (CI: 7.7-26.9%) and the specificity was 94.9% (CI: 91-94.7%), see table 2.

Table 2: Sensitivity and Specificity of the Test Comparing the Four Subgroups and the Period between the PCR Positive and Serology Test

Variables	All participants % (CI)	Asymptomatic % (CI)	Symptomatic % (CI)	HCW % (CI)	Laborer % (CI)
Sensitivity					
0-6 days	28 (19.4-38.4)	12.8 (4.3-27.4)	40 (22.7-59.4)	0 (0-97)	38.5 (20.2-59.4)
7-13 days	77.8 (60.9-89.9)	44.4 (13.7-78.8)	88.3 (63.6-98.5)	Nil	90 (55.5-99.8)
≥ 14 days	84.4 (67.2-94.7)	50 (1.3-98.7)	93.3 (68.1-99.8)	72 (39-93)	100 (39.8-100)
Specificity	93.3 (89.2-96.2)	100 (93.9-100)	89.3 (78.1-95.9)	100 (94.1-100)	81.3 (67.4-91)

CI= confidence interval, HCW= health care worker.

The levels of IgG of all positive cases were measured quantitively. The mean level of IgG among patients with negative rt-PCR was 0.36±1.13 while the mean level of IgG among patients with positive rt-PCR for COVID-19 was 2.99±3.2 (P <0.001). The mean level among symptomatic was 2.7±3.2 and among asymptomatic was 0.46±1.3 (P-value <0.001). The highest IgG level was reached at week four post-COVID-19 diagnosis with a mean IgG level of 6.6 ±1.7. After week four, the level of the IgG started to decline. The mean of the IgG levels per week post-diagnosis is shown in figure 1.

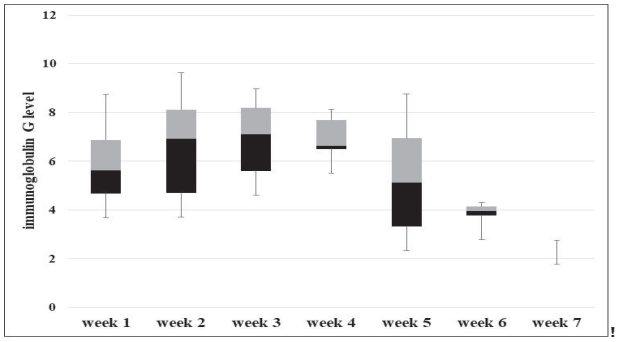

Figure 1: Box Plot Showing Immunoglobulin G Level among Patients with Positive COVID-19 Plotted by Weeks from Positive COVID Test

DISCUSSION

Many serological tests for the detection of the antibodies against the SARS-CoV-2 are commercially available. It is important to evaluate them before their use on a wide scale in the community[6]. The evaluation of these tests is mainly done against the PCR, which is the gold standard for the SARS-CoV-2 diagnosis. This method is not straightforward as the PCR detects viral nucleic acid while the serology test detects the host response for the infection (antibodies production). Serology is not recommended for diagnosing COVID-19[7]. Rt-PCR remained the assays of choice to diagnose the SARS-CoV-2 infection and the serology test can be used as supplementary tools[8]. The role of the serology test is still evolving. As the level of antibodies correlates to the immunity among individuals is still unclear, the serology test is not a guide for personal protective equipment use or social distancing policies[6].

The serology test in this study was found to have an overall sensitivity of 49.4% (CI 41.5% to 57.3%) and a specificity of 93.3% (CI 89.2% to 96.2%). The sensitivity of the test reached 81.8% and the specificity 93.7% when the test was done two weeks after the rt-PCR positive result. Tang et al found similar results with a sensitivity of 47.6% when the test was performed three days post positive rt-PCR test and 81.3% when the serology test was done 14 days or more after the rt-PCR[9].

Bryan et al found that when using the manufacturer's recommended index value cutoff point of 1.40 for determining positivity, the test specificity reached 99.9% which is similar to what is reported by the assay package insert. The sensitivity of the test performed 17 days after symptoms onset or 13 days after PCR positive have reached 100%; the test was performed on more than 1,000 patients[10]. The results from this study also showed that the sensitivity and specificity of the test increased as the time between the exposure to the virus and the performance of the test increased; however, it did not reach 100%. There are some concerns about the bias and applicability of the test when used in the clinical setting as the test sensitivity was mainly done in hospitalized symptomatic patients who are expected to have higher antibodies levels[11]. Considering this, the test under the study showed high specificity (100%) among asymptomatic patients involved in the study and had a sensitivity of 50%.

The test may be useful in patients who are symptomatic for ≥7 days with clinical presentation consistent with COVID-19 disease and had negative PCR test result[10]. In our study, the serology test showed higher sensitivity among symptomatic patients which increased from 71% to 96.6% if the patients were tested 14 days or more after the onset of symptoms.

A study had evaluated three different serological essays and found that all of the three tests performed poorly when used few days from symptoms onset and the sensitivity and specificity improved if the test was done 14 days post rt-PCR[12]. Another study had evaluated seven different serological tests and found a sensitivity level of >92% if the tests were used 14 days after the onset of symptoms[13]. A review of COVID-19 antibody testing found that the sensitivity of the antibody test in the first week of symptoms is too low to be used in the diagnosis of COVID-19 infection[11]. The detection of antibodies increased after two weeks from the onset of symptoms and can be a useful tool to detect previous COVID-19 infections[11]. The seroconversion to anti-SARS-CoV-2 IgG positive occurred mainly at the end of the second week after infection[6].

People with previous history of COVID-19 had a higher rate of positive antibodies compared to non-exposed[14]. In our study, only 14 (3.6%) patients with negative rt-PCR had positive serology tests.

Though the presence of antibodies indicates the exposure of the patients to the SAR-CoV-2 virus, it does not ensure the presence of immunity. To infer that the presence of antibodies gives protection from infection, the antibodies threshold level needs to be established first[6,14]. It is still an assumption that people with positive antibody tests who had recovered from COVID-19 infections had immunity to the disease[15].

In our study, all healthcare workers in the COVID-19 healthcare facilities with no previous history of COVID-19 infections were found to have no antibodies. A study found a low seroprevalence of 1.6%[16].

This validation study was carefully designed towards targeted populations. High-risk include patients with COVID-19 symptoms, contact of COVID-19 positive patient and healthcare workers[17]. This justifies the involvement of healthcare workers and household contacts as they have different exposure which may not be equal to the risk in the rest of the population[14].

CONCLUSION

The sensitivity of the test under the study was 84% when used 14 days after testing positive to the SARS-CoV-2 virus PCR test and the specificity of the test was 93.3%.

The serology test showed a high specificity rate across all the tested groups and can be used to rule that the patient had been exposed to SARS-CoV-2 previously. The test can be recommended to be used on a wider scale in serosurvey in the future.

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