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Immunological Features of COVID-19 in Hodeidah, Yemen

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Authors' contributions

This work was carried out in collaboration between both authors. Author MAAK analyzed the data, wrote, revised and edited the final manuscript and author TA implemented the experimental part (collection and analysis of samples) and collected the data, analyzed samples, and wrote the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Monitoring of the immunological status linked with coronavirus disease 2019 (COVID-19) infection in Yemen is practically absent. Several studies vary in study design, populations under study, serologic tests used, timing of sample collection, and quality.
Objective: Therefore, our study aimed to present the validation of immunological method namely rapid test for detection of immunoglobulin G (IgG) of COVID-19 infection immune response development in the blood of healthy participants (asymptomatic) were living in the COVID-19 pandemic area and of the patients who have undergone COVID-19 infection.
Methodology: Rapid test was validated that included the sensitivity, specificity, precision and accuracy parameters and used for sampling in research analysis. Participated volunteers of this

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study were provided written consent. The study was designed in one time cross sectional COVID-19 antibodies survey after three months of COVID-19 pandemic and implemented in four groups (N:72): the first group COVID-19 was recovered patients (n:18) that admitted in isolation department , Center of Tropical Medicine and Infectious Diseases (CTMID), Al Thawara Public Hospital Authority, Hodeidah, Yemen, the second group was contacts of severe patients (n:18), the third group was mild and moderate cases (n:18) that were treated at home ,and the fourth group was asymptomatic cases (n:18)". Data obtained were analyzed based on appropriate statistical tools.

Results: The results of rapid test validation showed that is sensitive (85.19%; CI: 72.88 to 93.38%), specific (83.33%;CI: 58.58 to 96.42%) , precise (93.88%; CI : 68.73 – 102.52%) and accurate (86.11%; CI : 84.72 to 92.12%) for detection of IgG of COVID-19 in Hodeidah, Yemen. In total, 49 of 72 participants were rapid test positive, giving a prevalence of COVID-19 of 68.05%. The COVID-19 IgG antibodies were detected in 18/18 cases (100%) of recovered severe patients (high prevalence); 17/18 cases (94.44%) of contacts (high prevalence). In addition, IgG were detected in 11/18 cases (61.11%) of mild and moderate patients (middle prevalence) and 3/18 cases (16.66%) of asymptomatic (low prevalence).

Conclusion: The study concluded that COVID-19 IgG antibodies become detectable after symptom onset of severe cases and their contacts (high prevalence) based on validated immunological method. On the other hand, the antibodies were developed in mild and moderate patients (middle prevalence). The IgG were developed in asymptomatic patients (low prevalence). However, additional data are needed before modifying public health recommendations based on serologic test results.

Keywords: COVID -19; Immunological; IgG; antibodies; prevalence; Hodeidah; Yemen.

1. INTRODUCTION

"Millions in the world were infected with the coronavirus disease 2019 (COVID-19) , the virus that causes COVID-19, they develop antibodies a few weeks after infection. The infection can be evened in the peoples who have even severe disease, mild disease, and even asymptomatic infection, do develop these antibodies" [1]. "Center for Diseases Prevention and Control – United States (CDC-US) reported that antibodies most commonly become detectable 1–3 weeks after symptom onset, at which time evidence suggests that infectiousness likely is greatly decreased and that some degree of immunity from future infection has developed. However, additional data are needed before modifying public health recommendations based on serologic test results, including decisions on discontinuing physical distancing and using personal protective equipment" [2,3].

World health organization (WHO) reported "There are now more than 200 peer-reviewed publications, pre-prints, manuscripts and government reports of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seroprevalence studies. These studies vary in study design, populations under study, serologic tests used, timing of sample collection, and quality. Overall, the population-based seroprevalence reported across available studies remains low, at below 10%. Some studies conducted in areas of known high virus transmission and studies of health care workers in areas of known high transmission have reported seroprevalence estimates over 20%" [4].

"Despite the great interest of the scientific community in the behavior of the human body after contact with COVID-19, monitoring of the immunological status of patients with COVID-19 having varying severity degrees and of the people with a low COVID-19 viral load is practically absent" [5]. The aim of this study was a detecting of COVID-19 infection immune response development using qualitative assessment of IgG in the blood of healthy donors (asymptomatic) were living in the COVID-19 pandemic and of the patients who have undergone COVID-19 infection (post-recovery of patient and post exposure of community).

2. METHODOLOGY

2.1 Study Area

The study was carried out in COVID-19 isolation department, Molecular Biological Unit, Center of Tropical Medicine and Infectious Diseases (CTMID), AL Thawara Public Hospital Authority, Hodeidah Yemen.

2.2 Study Design

The study was designed in one time cross sectional COVID-19 antibodies survey that included 72 participants , divided into four groups: the first group included 18 of recovered patients from COVID-19 who admitted in isolation department ,CTMID, AL Thawara Public Hospital Authority , Hodeidah Yemen. The second group was contacts of severe patients (n:18), the third group was recovered mild and moderate cases (n:18) that treated at home, and the fourth group was healthy peoples " asymptomatic" (n:18) (Fig. 1).

2.3 Real Time – Polymerase Chain Reaction (RT-PCR) for Detection the COVID-19 Infection

The RT- PCR of COVID-19 detection was re-validated partially in Molecular Biological Unit of CTMID, AL Thawara Public Hospital Authority of Hodeidah, Yemen. The assay for molecular detection of COVID-19 on nasopharyngeal swabs was performed using the RT-PCR Bio-System. The Norgen's COVID-19 TaqMan RT-PCR kit that was designed for the detection of COVID-19 specific RNA [12].

2.4 Rapid Test for Detection of Antibodies of COVID-19 Infection

Eighteen out of 72 samples have been tested positive using RT-PCR, and all of them were also positive based on rapid test IgG. The rapid test (indirect infection detection) was re-validated based gold bio-analytical method namely RT-PCR that detects the infection directly by detecting the viral RNA. The IgG were detected in 18 recovered severe patients that were confirmed with COVID-19 RT- PCR . The analytical efficiency including sensitivity, specificity, precision, accuracy, false positive and false negative rate. Finally, the study applied the rapid test for immunological response (detection of IgG only) post - recovery of patient and post exposure of community (antibodies detection in patients and community). On the other mean, IgG was detected after 3 months from infection (Fig. 1) [13-16].

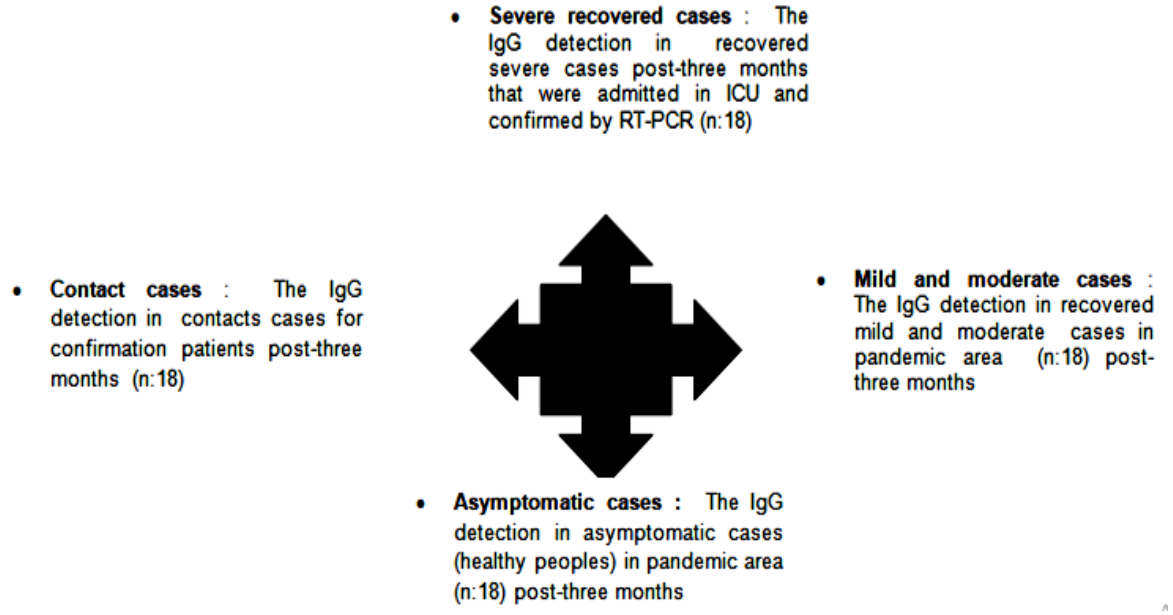

Fig. 1. Study design for diagnostic test evaluation of rapid test method for IgG detection in Hodeidah peoples, Yemen: Note: Mild Cases: Symptoms of respiratory infection (fever, cough, pharyngitis, headache, … etc) Symptomatic, meeting the case definition for COVID-19, without evidence of viral pneumonia or hypoxia. Moderate Cases: Clinical signs of non-severe pneumonia (cough or difficulty breathing and fast breathing and/or chest indrawing) and no signs of severe pneumonia. Severe cases: Clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO_2 < 90% on room air. Contacts cases: The persons contacted with confirmed cases. Note: Major symptom in mild and moderate cases were acute smell and/or taste loss [6-11]

2.5 Avoiding Cross Reaction

The study area was Hodiedah, Yemen and the dengue fever is an endemic in this area, therefore to avoid the cross reaction with IgG of dengue, all participants were diagnosed by IgG of dengue fever based on rapid validated method [17-20].

2.6 Data Management

The simple statistical process was used to partial validation of RT-PCR for detection of COVID-19 and rapid test assay for IgG detection. Data were collected ,checked and entered in an Excel format. Then the data was analyzed using tables, graphs, percentages, range, average, and standard deviation were the main descriptive tools.

3. RESULTS

3.1 Partial Validation of RT-PCR

The RT-PCR (Bio-system 7500) was validated partially for assessing the accuracy, precision and quantification with limit of different nasopharyngeal samples. Participated volunteers of this study were provided written consent and the results of re-validated method were precise to each analyze with percent relative standard deviations (RSD%) that was 3.36% (< 5.0%). Furthermore, the accuracy of validated method exhibit well recovery values of 98% - 102% (± 5%) (Table 1).

3.2 Diagnostic Test Evaluation of Rapid Test Method for IgG Detection

The results of rapid test validation showed that was sensitive (85.19%; CI: 72.88 to 93.38%), specific (83.33%; CI: 58.58 to 96.42%), precise (93.88%; CI: 68.73 – 102.52%) and accurate (86.11%; CI: 84.72 to 92.12%) for detection of COVID-19 IgG antibodies in Hodeidah, Yemen (Table 2, 3 and Fig. 2).

3.3 Detection of IgG in the Patients and Community

In total, 49 of 72 participants were positive with rapid test positive, giving a prevalence of COVID-19 of 68.05%. The IgG were detected in different groups, high prevalence was reported in recovered severe patients (18/18 cases; 100%) and their contacts group (17/18 cases; 94.44%. In addition, middle prevalence was reported in recovered mild and moderate patients (11/18 cases; 61.11%). Finally, low prevalence was reported in asymptomatic group (3/18 cases; 16.66%). On the other meaning , we observed a statistically higher frequency of IgG against COVID–19 in older patients, occurring in cases more than 50 year old, and the lowest frequency was in cases under 50 year old (Table 4 and Fig. 3).

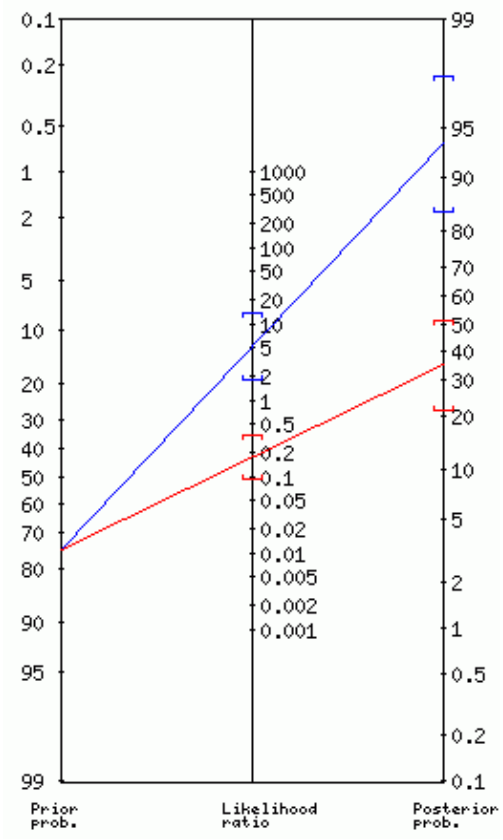

Fig. 2. Prior probability (odd): 75% (3.0); Positive test: Positive likelihood ratio: 5.11 with 95% confidence interval: [1.81,14]. Posterior probability (odds): 94% (15.3) with 95% confidence interval: [84%,98%] (~ 1 in 1.1 with positive test are sick). Negative test: Negative Likelihood ratio: 0.18 with 95% confidence interval: [0.09,0.35]. Posterior probability (odds): 35% (0.5) with 95% confidence interval: [21%,51%] (~ 1 in 1.5 with negative test

Table 1. Partial validation of RT-PCR method

Parameters	Limit of quantification	Limit of detection	Precision (RSD%)	Accuracy (recovery%)
Cycle Threshold (Ct)	10 – 39	7	3.36%	98 - 102%.

Table 2. Re-validation of rapid test method based on RT-PCR

n : 18	RT-PCR	Rapid Test
* Recovered severe patients	Confirmed	IgG detected (100%)
* Recovered patients: The IgG was detected after three months from discharge of patients		

Table 3. Diagnostic test evaluation of rapid test method for IgG detection

Parameters	Value (%)	95% CI (%)
Sensitivity	85.19	72.88 – 93.38
Specificity	83.33	58.58 – 96.42
Precision	93.88	84.44 – 97.74
Positive likelihood ratio	5.11	1.81 – 14.45
Negative likelihood ratio	0.18	0.09 – 0.32
Positive predictive value	93.88	84.44 – 97.74
Negative predictive ration	65.22	48.91 – 78.60
Accuracy	84.72	74.31 – 92.12

Table 4. Detection of IgG in Hodeidah, Yemen according to age and sex (N = 72)

	Mild and moderate		Severe		Contacts		Asymptomatic		
Age	Male	Female	Male	Female	Male	Female	Male	Female	Total
< 50 year	9	3	3	0	17	0	15	0	72
> 50 year	6	0	14	1	1	1	3	0	
	16	2	17	1	18	0	18	0	
Total	18		18		18		18		

Fig. 3. Detection of IgG against COVID-19 in symptomatic and asymptomatic of Hodeidah peoples, Yemen

4. DISCUSSION

Firstly, due to lack of formal guidance or regulatory requirements, several approaches are possible to select the experimental design, for choosing the statistical data treatment and hence for the decision process namely the rapid test of COVID-19 to detect of antibodies. The success of an analytical method validation of rapid test for detection of antibodies of COVID-19 is tested by comparing results of RT-PCR of COVID-19 as standard confirmation method. On the other meaning, the objective of this work is to demonstrate the applicability of the simple approaches with certain statistical models to a more variability domain bio-analytical methods namely rapid test (immune -chromatographic technique) and in the interpretation of acceptance criteria of validation of rapid test present the immunological response (detection of COVID-19 IgG antibodies) in Hodeidah peoples based on validated bio-analytical method.

Secondly, it was used to identify past COVID -19 infection in Yemeni people who were infected at 3 months previously. The present study showed that antibodies namely IgG were detected in Hodeidah, Yemen (68.05%), the IgG antibodies were detected in recovered severe patents (100%), contacts (94.44%), recovered mild and moderate (61.11%) “acute loss in their sense of smell and/or taste in a community and asymptomatic peoples (16.66%). In comparing with other study carried out in Aden, Yemen , the prevalence of IgG was 25% and the prevalence of asymptomatic COVID-19 in the entire study group was 7.9% , the prevalence of COVID-19 was significantly higher among females, housewives and subjects with a history of contact with a COVID-19 patient: 32%, 31% and 39%, respectively [21].

“On the other hand , in comparing with other studies carried out in different countries of the world, the present study results agreed with a study done by Makaronidis et al. in London , UK (77.6%) with acute smell and/or taste loss had SARS-CoV-2 antibodies” [22]. “IgG antibodies seroprevalence were recorded randomly by Stringhini et al. in Geneva, Switzerland “the first week was 4·8%, the estimate increased to 8·5% in the second week, to 10·9% in the third week, 6·6% in the fourth week, and 10·8% in the fifth week” [23]. Antibodies prevalence in England fell from 6.0% to 4.4% over three months, study finds [24]. In Wuhan, IgG antibodies prevalence were 89.8% in COVID-19 patients, 4.0% in healthcare providers, 4.6% in general workers, and 1.0% in other patients [25]. 8.3% tested positive for IgG in an asymptomatic population in Sergipe, Brazil [26]. “In Italy, a prevalence in symptomatic individuals and their family contacts was 23.1% and the highest prevalence was found in the age class 40 - 49 years. Overall, 34.4% of the participants reported at least one symptom and among the symptoms, anosmia and ageusia were strongly associated with seropositivity” [27]. In previous study included prospective longitudinal cohort study entitled "dynamics of IgG-avidity and antibody levels after COVID-19 " where Löfström E et al found a significant ongoing increase in avidity maturation after COVID-19 whilst the levels of antibodies were declining, suggesting a possible aspect of long-term immunity [28].

Finally, the question, what is the degree of susceptibility of previously infected individuals to reinfection by SARS-CoV-2? Alzaabi et al. indicated a sustained and prolonged positive immune response in COVID-19 recovered patients. The consistent rise in antibody and positive levels of IgG titers within the first 5 months suggest that immunization is possible, and the chances of reinfection minimal [29]. In Brazil dynamics of anti-SARS-CoV-2 IgG antibodies post-COVID-19 was studied and the authors showed a high frequency of loss of anti-SARS-CoV-2 IgG antibodies within 3 months after COVID-19 [30]. Previous study entitled “SARS-CoV-2 reinfection in patients negative for immunoglobulin G following recovery from COVID-19” concluded patients who recover from COVID-19 with no detectable anti-nucleocapsid IgG concentration appear to remain more susceptible to reinfection by SARS-CoV-2, with no apparent immunity. Also, the authors suggested the chance is lower, the possibility for recovered patients with positive anti-nucleocapsid IgG findings to be reinfected similarly exists [31]. CDC reported COVID-19 vaccination causes a more predictable immune response than infection with the virus that causes COVID-19. Getting a COVID-19 vaccine gives most people a high level of protection against COVID-19 and can provide added protection for people who already had COVID-19. One study showed that, “for people who already had COVID-19, those who do not get vaccinated after their recovery are more than 2 times as likely to get COVID-19 again than those who get fully vaccinated after their recovery” [32].

5. CONCLUSION

The study concluded that COVID-19 IgG antibodies become detectable after symptom onset of severe cases and their contacts (high prevalence) based on validated immunological method. On the other hand, the antibodies were developed in mild and moderate patients (middle prevalence). The COVID-19 IgG antibodies were developed in asymptomatic people (low prevalence). However, additional data are needed before modifying public health recommendations based on serologic test results.

6. LIMITATIONS OF THE STUDY

There are some limitations in this study that need to be considered. The small samples size in this study and other immunological features are not included.

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CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the authors. The raw data are secured in the Center of Tropical Medicine and Infectious Diseases (CTMID), Al-Thawara Public Hospital Authority, Hodeidah, Yemen.

ETHICAL APPROVAL

The studies involving human participants were reviewed and approved by Ethics Committee of CTMES-HU and CTMID, Al-Thawara Public Hospital Authority, Hodeidah, Yemen.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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