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Prevalence and Classes of SARS-CoV-2 Antibodies among COVID-19 Suspected Patients who Attended a Health Care Setting in Sana'a, Yemen

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TAS and MAY designed the study and author MAY carried out the data collection. Authors TAS and AAB analysed and interpreted the data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigates SARSCoV-2 antibody prevalence and classes among COVID-19 suspected patients in Sana'a, Yemen. Antibody response to SARS-CoV-2 infection remains to be fully elucidated. Currently, no reports on SARS-CoV-2 antibody response from Yemen are available.
Study Design: This cross-sectional study investigates SARS-CoV-2 antibody prevalence and classes among COVID-19 suspected patients.
Place and Duration of Study: This study was conducted in Sana'a the capital of the Republic of Yemen from June 2020 through January 2021.
Methodology: Serological investigation for Anti-SARS-CoV-2 antibody tests was conducted for 259

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suspected COVID-19 patients who attended a health care facility for antibody testing to confirm the diagnosis on C.

Results: The mean age was 40.8 ±16.6 years. Of all subjects, 180 (69.5%) were males and 79 (30.5%) were females, 73% were < 50 years of age. A total of 133 (51.4%) had at least one anti-SARS-CoV-2 antibody class, 6 (2.3%) had isolated IgM, 80 (30.9%) had concomitant IgM and IgG and 49 (18.9%) had isolated IgG. Only the seropositivity of isolated anti-SARS-CoV-2 IgG significantly (p=0.002) differs among various age groups. There was a significantly higher (p=0.017) IgM seropositivity among females than among males.

Conclusions: Among subjects with suspected COVID-19, > 30% had concomitant IgM and IgG with a minority having isolated IgM or IgG suggesting concurrent or close seroconversion time of both antibody classes. In addition, around 50% of subjects were SARS-CoV-2 seropositive suggestion low SARS-CoV-2 seroconversion and consequently low community seroprevalence. An antibody dynamic study based on will characteristics of COVID-19 patients is required. Also, a community-based seroprevalence study based on the detection of a combination of IgM, IgG, and IgA remains essential to determine the prevalence of SARS-CoV-2 infection in Yemen.

Keywords: COVID-19; IgG; IgM; SARSCoV-2; Seroprevalence; Yemen.

1. INTRODUCTION

Immune response to SARS-CoV-2 infection remains to be fully elucidated. However, the available data, although inconsistent, have shown that SARS-CoV-2 infection induces antibody response 4 to 15 days post infection[1-6] with rising levels in severe disease [7,8] and increasing seroconversion rates with progress of time [2,9,10]. Although anti-SARS-CoV-2 antibodies response apparently follows conventical serological dynamic with sequential IgM and IgG, the concomitant appearance of both antibody isotypes has been reported [11,12] Immunoglobulin M first appears 5-10 days post onset of symptoms in most patients, rising about 2 to 3 weeks rapidly to reach a level maintained for 1 to 4 weeks and begin to decline thereafter [8,13]. However, IgG antibody has been reported to appear 10 days after infection [13], and remains detectable up to 6 months after infection [14]. Reports on decline time frame of anti-SARS-CoV-2 antibody levels are inconsistent as they vary from 20 [11,12] to 63 days [15-17] for IgM and 35 to > 49 days.[8,11,18,19] and even to as long as 199 days [18] in the case of IgG. This variation in seroconversion time and duration of antibodies reflects variations in different studies, in patients' populations, in age and gender of the population enrolled, in disease severity, in clinical course, and in the serological assays used. These together seem to negatively impact the attempt to reliably establish the dynamic of the antibody response and hinder a genuine estimate of seroprevalence and the herd immunity level.

So far epidemiological studies have shown conflicting low seroprevalence rates of SARS-CoV-2 antibodies, in different parts of the world. Various reports have shown seroprevalence rates of 4 to 7.3% in several part of Europe, 4.2% in Northern America, 22% in Central and southern Asia [17], 0.07% in South Korea [16], 3.8% in Wuhan geographic regions [19] and 7.8% in Malaysia [20]. This variation suggests varying time of testing through the pandemic, demographic and geographic variations and variation of serological assays used. The low seroprevalence rates is attributable to the fact that majority of the studies were mainly based on detection of IgG and IgM whereas IgA was found in COVID-19 patients in isolation of IgM and IgG [20,21]. Thus inclusion of IgA detection in seroprevalence studies detects more COVID-19 infections. Yemen is one of the countries where confinements were inadequately implemented during spread of COVID-19 because of the current political turmoil and the collapse of the health care system. Based on latest estimate of the Ministry of Public Health and Population in Yemen, COVID-19 might expand and potentially infect 90% of the population [2,22]. Despite this gloomy scenario the other side of the story is vowing as the absence of containment measures will promote the development of natural herd immunity.

Despite the frequent reports of SARSCoV-2 antibody prevalence and insights on antibody response from a number of countries around the globe but not from Sana'a, Yemen. Yemen as one of the countries where confinement of COVID-19 was ineffectively implemented since the emergence of the disease because of the current political turmoil and the collapse of the health care system. This is one of the very few studies if not the first on antibody response and

seroprevalence of SARS-CoV-2 in the major city of Sana`a, northern part of Yemen. Moreover, this study will provide insights on the antibody response to SARS-CoV-2 in Yemen where the study was conducted relatively early during SARS-CoV-2 pandemic as well as it could work as a baseline to further studies that measure the level of herd immunity to COVID-19 in the country. Therefore, the aim of this study was to investigate the antibody response and to estimate the seroprevalence of SARS-CoV-2 among COVID-19- suspected patients who attended a health care setting for COVID-19 testing in Sana`a, Yemen.

2. MATERIALS AND METHODS

2.1 Study Design and Settings

This cross- sectional study was conducted retrospectively in the Sana`a region in Yemen during the second week of June 2020 through January 2021. On an informed consent the anti-SARS-CoV-2 antibody tests results and the demographic data that included age and gender of 259 COVID-19 suspected patients who were referred to a health care facility for antibody testing to confirm the diagnosis were anonymously enrolled in this study.

2.2 Antibody Testing

The sera were tested for anti-SARS-CoV-2 IgM and IgG antibodies using iFlash –SARS-CoV-2 IgM and IgG paramagnetic particle chemiluminescent (CLIA) (Shenzhen yhlo biotech co., ltd. China) for qualitative determination of IgM and IgG according to the manufacture instructions. In summary prediluted serum samples were incubated with a recombinant SARS-CoV-2 antigen that was coated to paramagnetic microparticles to allow the formation of antigen- antibody complex. Then complex was washed under magnetic field during which magnetic particles were absorbed into the inner wall of the reaction tubes while the unbound materials were washed away from the solid phase of the magnetic field. Then the complex was incubated with Acridinium-labeled anti-human antibody conjugate. The newly formed complex that consisted of SARS-CoV-2 antigen- antibody and acridinium-labeled anti-human antibody was washed. Then a pre-trigger and a trigger solution were added to trigger the signal and the resulting chemiluminescent reaction was measured as relative light units (RLUs) the detection of which by the iFlash optical system was proportional to the antibody concentration present in the sample. The results are determined through a calibration curve by 2-point calibration. Antibody level ≥ 10 Absorbance units per milliliter (AU/ml) was considered positive.

2.3 Statistical Analysis

Data entered in a statistical package (SPSS version 22) for analysis. Categorical variables were used for the description of the demographic characteristics of the participants (age and sex). Association between the seroprevalence findings of the IgM and IgG with the characteristics of the participants were undertaken using a chi-square test, where *p* value findings of < 0.05 was considered significant.

3. RESULTS

The mean age of the 259 subjects was 40.8 ±16.6 years, ranging between 3-85 years. Most of the participants 180 (69.5%) were males and 79 (30.5%) were females, young adults (≤18 -49 years), who were mostly infected in week 2 of June 2020 (Fig. 1). A total of 133 (51.4%) were positive for at least one anti-SARS-CoV-2 antibody class, and of these 6 (2.3%) had isolated IgM, 80 (30.9%) had concomitant IgM and IgG, 49 (18.9%) had isolated IgG, and 128 (49.4%) had IgG with or without IgM (Table 1).

Table 1. Seropositivity rate of various anti-SARS-CoV-2 antibody classes among COVID-19-suspected subjects who attended a health care facility in in Sana`a, Yemen

Variable	Category	n	%
Antibody response	At least one Antibodies	133	51.4
	Absence of antibodies	126	48.6
	Isolated IgM	6	2.3
	Concomitant IgG/IgM	80	30.9
	Isolated IgG	49	18.9
	IgG with/without IgM	128	49.4

Table 2. Seropositivity rate of anti-SARS-CoV-2 antibody classes among males and females and different age groups of COVID-19- suspected subjects who attended a health care facility in in Sana`a, Yemen(n=259)

Variable		At least 1 antibody class n= 133		Isolated IgM (n= 6)		Isolated IgG (n= 49)		IgG with/without IgM (n=128)		Concomitant IgM & IgG (n= 80)	
		n (%)	*p*	n (%)	*p*	n (%)	*p*	n (%)	*p*	n (%)	*p*
Sex	Male	88 (69.8)	0.907	1 (17.0)	0.017	144 (68.6)	0.503	89 (67.9)	0.581	125 (69.8)	0.861
	Female	38 (30.2)		5 (83.0)		66 (31.4)		42 (32.1)		54 (30.2)	
Age (years)	3-18	8 (6.3)	0.069	0 (0.0)	0.721	16 (7.6)	0.002	8 (6.1)	0.112	8 (4.5)	0.074
	19-49	85 (67.5)		5 (83.3)		147 (70.0)		89 (67.9)		117 (65.4)	
	50-64	13 (10.3)		1 (16.7)		23 (11.0)		14 (10.7)		29 (16.2)	
	≥ 65	20 (15.9)		0 (0.0)		24 (11.4)		20 (15.3)		25 (14.0)	

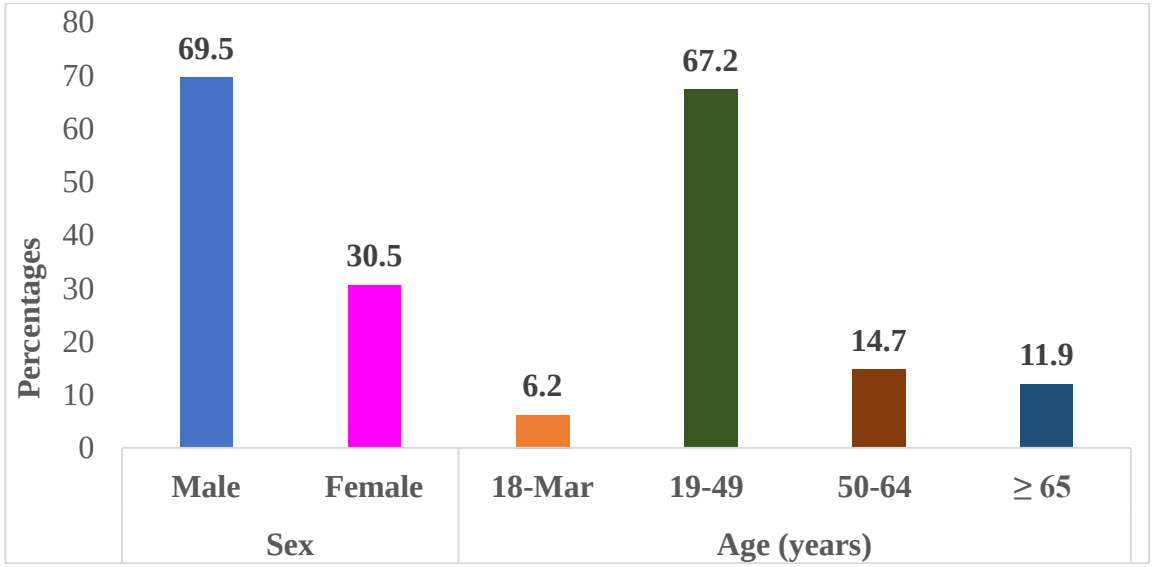

Fig. 1. Demographic characteristics of subjects COVID-19 -suspected who attended a health care facility in in Sana`a, Yemen (n=259)

3.1 Seropositivity Rate According to Antibody Classes by Age and Sex

Although, the seropositivity rate of the isolated anti-SARS-CoV-2 IgG significantly (*p*=0.002) differs among various age groups, the seropositivity of the IgG and the IgM in combination or in isolation did not differ significantly (p>0.05) between males and females or between various age groups with the highest seropositivity rate detected being among the 19-49 years age group and the lowest detected was among ≥65 years age group (Table 2).

4. DISCUSSION

To the best of our knowledge, this is one of very few studies if not the first on seroprevalence of SARS-CoV-2 in the major city of Sana`a, northern part of Yemen. Of all patients who were suspected of having COVID-19 around half of them had at least one antibody marker, either IgG or IgM or both, suggesting exposure to SARS-CoV-2 infection. This is obviously because these patients were not clinically well characterized as cases of COVID-19 but were referred for testing on suspension of the disease having presented with suggestive signs and symptoms. This explain the lower seroprevalence than has been reported among well characterized patients who were clinically diagnosed with COVID-19 early in epidemic where almost 100% became antibody positive 2 to 3 weeks post disease onset [7,12]. However, owing to the attendance of the majority of the cases in a single health care facility for testing in only two weeks' time indicating the peak of epidemic, the contagious nature of COVID-19 and the lack of adequate confinement measures, a higher seropositive can be expected. One reason for the low seroprevalence rate is that the subjects may have produced transient systemic antibodies because of less severe or mild infection. It has been reported that systemic antibody production against SARS-CoV-2 develops mainly in severe COVID-19, whereas mild disease may be associated with transient serum anti- SARS-CoV-2- specific antibodies [9]. Additionally, our subjects may have responded with more localized respiratory antibody response. It has been reported that some SARS-CoV-2-exposed cohorts were negative for SARS-CoV-2-specific serum IgA and IgG but showed detectable SARS-CoV-2-specific IgA in nasal fluids and tears without serum antibody response [9]. Additionally symptomatic patients who may not seroconvert despite developing persisting T cell responses have also been reported [7]. Several reports have shown underestimated seroprevalence when investigation was based on IgM and IgG alone [20].

Over 70% of our seropositive subjects were below the age of 50 years, which reflects the main age stratum of the Yemeni population (< 50 years) constituting 90% of the population [23], rather than suggests that this age groups were particularly susceptible. The low seroprevalence among those 3-18 years may reflect the mild or asymptomatic infection that usually occur in this age group and thus do not seek health care. Various reports have shown that less children (<18 years) than adults presented with mild to less severe and lower death rate [24-26]. The attendance of small proportion of our subjects of this age for testing further supports this inference. The low level of SARS-CoV-2 infection among children has been explained by the less expressed angiotensin-converting enzyme 2 (ACE2) receptor of the virus, better containment of the virus in the upper tract or to the less adverse immune mechanisms arising from infection in <18 years than in adults [22] .

Among our subjects less females than males presented for testing which could suggests low exposure among females. However this possibly because of stay-at-home custom of Yemeni women rather than due to low susceptibility to infection among females. Supporting this is the seroprevalence rate among females which did not significantly differ from that among males indicating equal risk of infection. Small minority of seropositive subjects (2.3%) had an isolated IgM and 18.9% had isolated IgG while over 30% had concurrent IgM and IgG. This could suggest the narrow window period of the antibody class switching from IgM to IgG making it difficult to detect patients with isolated IgM and to less extent isolated IgG. The possibility of simultaneous occurrence of both antibody classes cannot be excluded as simultaneous appearance of both antibody classes in some of SARS-CoV-2 infected subjects has been reported elsewhere [7,9,10,12-22,25-31] Furthermore, 3 patterns of SARS-CoV-2 seroconversion have been proposed so far. These include sequential seroconversion of IgM followed by IgG, concurrent seroconversion of both IgG and IgM, and an unusual pattern of IgG preceding IgM [30]. This probably reflects the variation in different serological assays. Therefore, validation and standardization of

SARS-CoV-2 serologic assays in large clinical cohort is required before coming to a final inference of serological dynamic of SARS-CoV-2. Majority of our subjects with isolated IgM were females (83%). The significance of this is difficult to point out due to the small sample size (6 subjects). However, this merit further investigation.

5. CONCLUSIONS

Among subjects with suspected COVID-19 over 30% had concomitant IgM and IgG with minority having isolated IgM or IgG suggesting concomitant or close seroconversion time point of both antibody classes. In addition, around 50% of subjects were SARS-CoV-2 seropositive suggestion low SARS-CoV-2 seroconversion and consequently low community SARS-CoV-2 seroprevalence in Yemen. As the systemic immune response to SARS-CoV-2 currently is not well established, the seroprevalence of SARS-CoV-2 cannot be reliably determined hence herd immunity seems to be hard to establish at this stage. A large-scale community-based seroprevalence study based on detection of combination of IgM, IgG, and IgA seems essential to determine the magnitude of SARS-CoV-2 infection in Yemen. Antibody response study based on will characterized COVID-19 patients is also required.

CONSENT

Data presented in this study were obtained in an informed consent with a clear declaration on the confidentiality of the obtained data and will not be used out of the research dimension.

ETHICAL APPROVAL

This study attested and approved (# REC-77-2020) by relevant academic institution.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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