ORIGINAL ARTICLE

COVID-19: MOLECULAR AND SEROLOGICAL PROFILE OF MOROCCAN PATIENTS AFTER CLINICAL RECOVERY

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ABSTRACT

Background: In this paper we focused on Corona virus Disease 2019 (COVID-19). The pandemic is spreading and 22,170 cases were confirmed worldwide as of August 18, 2020, in 218 territories, with 780,792 deaths and 14.841.838 recoveries. **Methods**: we studied a cohort of Moroccan COVID-19 patients, with confirmed SARS-CoV-2 infection, after clinical recovery and hospital discharge. Here, we present their molecular and serological profile after hospital discharge in a single-center experience in Casablanca. **Results**: The study shows that in the during the convalescent phase of the disease, detecting antibodies in serum specimens is more important than detecting viral RNA. Typical antibody response is induced during the SARS-CoV-2 infection. The serology testing provides important complementation to RNA test for pathogenic specific diagnosis and helpful information to evaluate the adapted immunity status of patient.
Keywords: *Sexual Dysfunction, Survey methodology, Urology, Smartphones.*

1. INTRODUCTION

Corona virus Disease 2019 (COVID-19) is an infectious condition caused by a new strain of coronavirus, SARS-CoV-2. The pandemic is spreading and 22.170 cases were confirmed worldwide as of August 18, 2020, in 218 territories, with 780.792 deaths and 14.841.838 recoveries [1]. In Morocco, the first case was diagnosed on March 2 in Casablanca. As of August 18, 2020, 44,803 cases were confirmed, with 714 deaths and 31, 002 recoveries [1].

In front of this new virus whose pathophysiology is little known and the limited number of studies concerning the anti-SARS-CoV-2 antibody response in COVID-19 patients, we studied a cohort of Moroccan COVID-19 patients, with confirmed SARS-CoV-2 infection, after clinical recovery and hospital discharge. Here, we present their molecular and serological profile after hospital discharge in a single-center experience in Casablanca.

2. METHODES

2.1 Patients and samples

Patients with confirmed or suspected SARS-CoV-2 infection from March 25th to April 9th 2020 were admitted in Cheikh Khalifa International University Hospital. Case definition was determined by ministerial circular as patient showing clinical features of COVID-19 or close contact of a confirmed case, with a positive real time RT-PCR on nasopharyngeal swab specimen.

Hospital discharge was granted after completion of 10 days treatment, normalization of biological tests, clinical recovery and either two consecutive negative RT-PCR, or after 24 days of hospitalization

2.2 Virological monitoring

Nasopharyngeal swabs were collected for patient monitoring and sent to Reference National Laboratory (LNR, Casablanca). All consecutive patients (n=50) with confirmed or suspected SARS-CoV-2 infection have been tested by real-time RT-PCR for viral infection (GeneFinder, Osang Healthcare), and were treated in Cheikh Khalifa International University Hospital.

Three genes were detected: RdRp gene, E gene and N gene. In monitored COVID-19 patients, the detection of one of those 3 genes was considered as a positive result.

2.3 Serology

Patients serum were collected after their clinical recovery and hospital discharge, between 24 and 43 days after their first COVID-19 positive RT-PCR result.

SARS-CoV-2 IgG and IgM antibodies have been assessed, in the LNR, using an indirect chemiluminescent assay (Maglumi, Snibe Diagnostics). According to manufactor's declaration, those antibodies are directed against both CoV-S and CoV-N proteins. Sensitivity and specificity have been evaluated at 95.6 % and 96 %, respectively.

The antibody levels were expressed as arbitrary unit per mL (AU/ml). The results ≥ 1 AU/ml are considered positive and the results < 1 AU/ml are considered negative.

2.4 Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 24.0 software, with a confidence interval of 95 %. A two-sided α of less than 0.05 was considered statistically significant ($P < 0.05$).

3. RESULTS

- Demographics and patient characteristics

The serum samples were collected from 50 morrocan admitted hospital patients with confirmed SARS-CoV-2 infection in Cheikh Khalifa International University Hospital, from March 25th to april 9th 2020. The clinical characteristics of the patients were shown in **Table 1**.

Table 1: Demographics and clinical characteristics of 50 patients in this study.

Variable	**All Patients (n = 50)**
Age (median, years)	55
Sex ratio (M/F)	2.57
Presenting symptoms	**Number patients (%)**
Fever	23 (46)
Cough	13 (26)
Dyspnea	5 (10)
Myalgia	8 (16)
Chest pain	1 (2)
Headache	1 (2)
Sore throat	9 (18)
Diarrhea and/or vomiting	3 (6)
Anosmia and/or dysgeusia	13 (26)
Chronic medical conditions	
Hypertension	13 (26)
Type 2 Diabetes Mellitus	8 (16)
Asthma	1 (2)
Cardiovascular disease	2 (4)

The median age was 55 years (IQR, 12-86) and 68% of the patients were men. Eighty-two percent (82%) of patients had one or more of the following symptoms: fever, cough, dyspnea, myalgia, chest pain, headache, sore throat, diarrhea, vomiting, anosmia and dysgeusia. 18% of patients were asymptomatic. Most of the patients (62 %) had no medical history. The rest (38%) of the patients were treated for one or more of the following diseases: arterial hypertension (26%), type 2 diabetes mellitus (16%), asthma (2%) and cardiovascular disease (4%).

3.1 Virological results

Out of the 50 patients, 66 % were declared cured after two consecutive negative RT-PCR test results at least 24 hours apart. The rest of the patients remained positive in the RT-PCR tests, with only the N gene still detected.

3.2 Serological results

The results of the laboratory tests were classified into 4 categories regarding the presence of IgG and IgM antibodies. Thus, 22 % of the results were negative for IgG and IgM, 2 % were negative for IgG and positive for IgM, 40 % were positive for IgG and IgM, and 36 % were positive for IgG and negative for IgM **(Figure 1).**

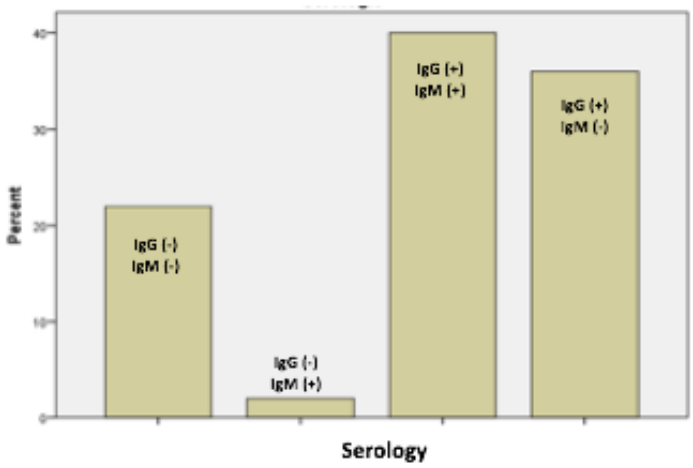

Figure 1: IgG and IgM serology results in SARS-CoV-2 patients.

The comparison between the serological and virological findings showed that there is no statistically significant association (P = 0.118) between the detection of SARS-CoV-2 IgG and IgM antibodies and the results of SARS-CoV-2 RT-PCR test (presence or absence of N gene) after clinical recovery. Our results also showed that there is no correlation (P = 0.311) between the initial symptoms of the patients and the detection of SARS-CoV-2 IgG and IgM antibodies at least 24 days after the appearance of the first symptoms or after patients first COVID-19 positive RT-PCR result.

Thus we have classified the patients in four groups according to their ages: patients below 20 years, patients between the age of 20 and 40, patients between the age of 40 and 60 and patients above 60 years. We then analyzed the serological results according to these different categories **(Table 2)**.

Table 2: Serological results according to patients age.

Years	IgG (-) IgM (-) (%)	IgG (-) IgM (+) (%)	IgG (+) IgM (+) (%)	IgG (+) IgM (-) (%)
< 20	33.33	0	0	66.67
≥ 20 < 40	50	0	0	50
≥ 40 < 60	22.2	0	27.8	50
≥ 60	9.5	4.8	71.4	14.3

In our study, 40 % of the patients were positive for both IgG and IgM. The 20 patients concerned were all aged over 40 years (43-85 years). Their average age was 65,9 years and their average IgG level was 36,39 AU/ml. None of the patients under the age of 40 was positive for both IgG and IgM (Table 2).

Only one patient was positive for IgM and negative for IgG. It was a 84 years old male, treated for type 2 diabetes mellitus, who presented as initial symptoms a fever and diarrhea. His IgM level was 1.145 AU/ml.
Also, our study showed that there is a statistically significant association (p = 0.045) between the age and the IgG levels in the COVID-19 infection (figure 2).

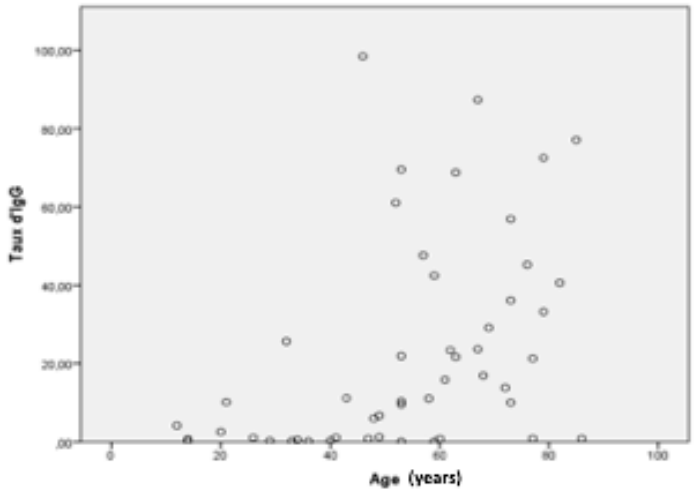

Figure 2: IgG antibodies levels according to patient's age.

We calculated the average level of anti-SARS-CoV-2 IgG in the four different categories of age. For the patients below 20 years (N = 3), the average IgG level was 1.679 AU/ml. For the patients between 20 and 40 years (N = 8), the

average IgG level was 5.08 AU/ml. For the patients between 40 and 60 years (N = 18), the average IgG level was 22.194 UA/ml. In patients above 60 years (N = 21), the average IgG level was 33.146 AU/ml (Figure 3).

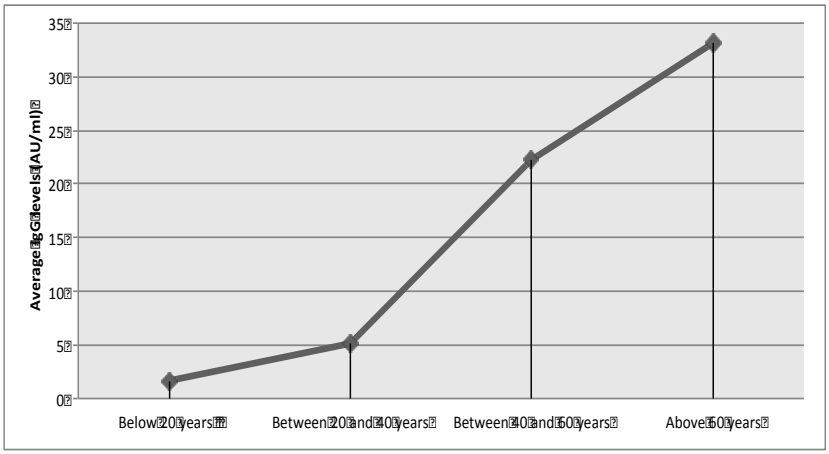

Figure 3: the average level of anti-SARS-CoV-2 IgG according to patient's age.

4. DISCUSSION

Little is known about the antibody response to SARS-CoV-2 infection. Antibodies against SARS-CoV-2 have been reported to be detected as early as a few days, to as many as three weeks after onset of symptoms [2,3], with the median time from symptom onset to detectable levels of IgG reported as six days [4]. The presence of SARS-CoV-2 IgG antibodies generally are indicative of current or previous infection by SARS-CoV-2, and are thought to confer some degree of immunity [5], however the extent and duration of immunity conferred by SARS-CoV-2 IgG antibodies remains unknown [4,6].

The kinetics of SARS-CoV-2 antibodies production is still poorly known, especially in asymptomatic patients [7]. Scientists from around the world are currently conducting studies to answer these questions. Most of these studies were conducted on hospitalized symptomatic patients and are therefore not giving answers about the antibody response of patients suffering from a mild or subclinical infection. These patients currently represent at least 80 % of all SARS-COV-2 infections [8]. Therefore, it is essential to evaluate the SARS-COV-2 antibody response of patients presenting a mild form of the disease.

In this study, carried out between March 25th and April 9th 2020, on 50 patients with confirmed or suspected SARS-CoV-2 infection and treated at Cheikh Khalifa International University Hospital, we analyzed the SARS-CoV-2 antibody response (IgG/IgM) using indirect chemiluminescent assay (Maglumi, Snibe Diagnostics). Patients' demographics and clinical characteristics were also studied.

In our study, the median age was 55 years (IQR, 12-86). It is similar to the one found by Chuan Qin and al in Wuhan « 58 years old » (China), [9]. In Morocco, the Ministry Of Health reports in its May 11th COVID-19 weekly newsletter, that the average age of active cases until March 16th was 55 years old. This average increased to 34,5 years old on May 4th. Also, over time, a rejuvenation of the patients was noted in our country [10]. Regarding the clinical symptoms of COVID-19, patients infected may present none or few symptoms, which may ressemble flu symptoms. The most common symptoms include cough, fever, breathing difficulties and bilateral pneumonia. They may appear two to 14 days after exposure. It is the longest incubation period known for this disease.

In table 1, 18 % of the patients recruited in this study were asymptomatic. The 82 % remaining presented one or more of the following symptoms: fever (46%), cough (26 %), dyspnea (10 %), myalgia (16 %), chest pain (2 %), headache (2 %), sore throat (18 %), diarrhea and/or vomiting (6 %), anosmia and dysgueusia (26 %). Our results showed that most of the patients (62 %) had no medical history, while the 38 % remaining were treated for one or more of the following diseases: arterial hypertension (26%), type 2 diabetes mellitus (16%), asthma (2%) and cardiovascular disease (4%).

In Morocco, the Ministry Of Health reports in its May 11th COVID-19 weekly newsletter, that at admission, 84,50 % of COVID-19 cases were asymptomatics or presented mild symptoms, 12,66 % presented moderate symptoms and 2,84 % were admitted in a severe or critical state. And the most frequent symptoms were cough (63,4% vs 26%), fever (53% vs 46%), myalgia (33% vs 16%) and sore throat (32% vs 18%) [10].

Regarding the results of the laboratory tests, on the 50 patients tested, 78 % (39/50) were positive for SARS-Cov-2 IgM and/or IgG. This result is similar to those obtained by Lei Liu and al, in China, in a preliminary study where 81,5 % (194/238) of the patients were positive to at least one of the two SARS-CoV-2 antibodies (IgM and/or IgG) [11].

Furthermore, 40 % of our patients were positive for IgG and IgM, 36 % were positive for IgG and negative for IgM, and 2 % were negative for IgG and positive for IgM. Also, our results showed that there is a strong correlation between the age of the patients and their serological results, especially the IgG levels.

Indeed, all of the patients that were positive for both SARS-CoV-2 IgG and IgM, were aged over 40 years. Likewise, we noted that the IgG levels tended to increase with age. Furthermore, of the 50 patients tested, only one was positive for IgM and negative for IgG, 43 days after his first COVID-19 positive RT-PCR result. Two weeks laters, his IgM antibodies disappeared (0.992 AU/ml vs 1.145 AU/ml), and his IgG levels were still negative. It was a 84 years old male, treated for type 2 diabetes mellitus, who presented as initial symptoms a fever and diarrhea.

We tried comparing him with the two other patients of our study that were above 80 years. The first patient is a 82 years old female, treated for hypertension, who presented as initial symptoms a fever and myalgia. She was positive for IgG and and negative for IgM, 33 days after her first COVID-19 positive RT-PCR result. The second patient is a 85 years old mal, with no medical history, who presented as initial symptom a dyspnea. He was positive for both IgG and IgM, 34 days after his first COVID-19 positive RT-PCR result. We were not able to find an explanation regarding this patient's serological result.

At last, the comparaison between the serological and virological results, showed that there is no correlation between the results of SARS-CoV-2 RT-PCR test after clinical recovery, and the detection of SARS-CoV-2 IgG and IgM antibodies.
This way, serological testing appears to be an interesting method that could be used to declare biological recovery for patients in whom the N gene persists in the RT-PCR. Also, serological testing appears to be the most realiable diagnostic methode in surveillance, comparing to RT-PCR because of its inability to detect past infection [12].

5. CONCLUSION

The study shows that in during the convalescent phase of the disease, detecting antibodies in serum specimens is more important than detecting viral RNA. Typical antibody response is induced during the SARS-CoV-2 infection. The serology testing provides important complementation to RNA test for pathogenic specific diagnosis and helpful information to evaluate the adapted immunity status of patient.

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