SARS-CoV-2 Antibody Responses in Newly Recovered Patients from Covid-19 Infection

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Abstract

Background: The coronavirus disease 2019 pandemic continues, causing considerable morbidity and mortality worldwide. Real-time reverse transcription polymerase chain reaction (RT-PCR) diagnostic assays are a goal standard for case ascertainment and diagnosis. Comparing to PCR, serological testing is advantageous with faster turn-around time, high-throughput and less workload. However, the clinical value of antibodies largely depends on the understanding of host antibody responses during infection.

Objective: To investigate the dynamics of (Immunoglobulin M (IgM) and Immunoglobulin G (IgG)) in response to SARS-CoV-2 in patients newly recovered from different severity Covid-19 infection in Baquba-Iraq.

Patients and Methods: In this cross sectional study a (93) patients with recent covid-19 infection who came to the central blood bank of Diyala for convalescent plasma donation for the period from the 1st June 2020 till 1st August 2020 were enrolled. The questionnaire form were filled regarding the time of infection, clinical manifestations, severity and polymerase chain reaction (PCR) results. While blood were taken for antibody titer measurement using the SARS-CoV-2 IgM and IgG VIDAS test.

Results: Male constitute about 91% of the study population. About 74% of the cases were below 40 years old. Fifty four of the patients had mild disease, 28% had moderate severity while the other 18% had severe type of the disease. fatigue and fever were the most common symptoms and seen in 87%,84% respectively. IgM Antibodies start to be positive before 21 days post infection mainly in mild and moderate cases 100%, While 0% of severe cases were positive at this time. After 49 day from the time of the infection 67%and 75% of the mild and moderate cases where IgM positive respectively and 100% of the severe cases where IgM positive. IgG Antibodies were positive in 100%, 0% of the mild and severe cases respectively at 21 day post infection but it's positive in 100% of both mild and severe cases after 49 day post infection.

Conclusion: : Although the serological response for SARS-CoV-2 appears in the first three weeks from the disease onset this response differs from patient to another according to the time from the infection and the severity of the infection.

Keywords: SARS-CoV-2, serological response, Immunoglobulin G Antibodies.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic continues, causing considerable morbidity and mortality worldwide. The severity of COVID-19 ranges from asymptomatic to fatal pneumonitis, with mildly symptomatic patients accounting for approximately 80% of all cases according to current understanding [1]. Full-genome sequencing and phylogenic analysis indicated that SARS-CoV-2 belongs to the betacoronavirus 2b lineage, the same group as Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), another highly virulent pathogens in humans. Bats are suspected to be the reservoir and pangolins are suggested to be an intermediate host for SARS-CoV-2 [2, 3]. Initial clinical manifestations of the disease include fever, fatigue, and dry cough, while patients with severe disease may exhibit pneumonia and acute respiratory distress syndrome (ARDS) [4].

Apart from supportive care, specific drugs for this disease are still being researched [5,6]. Real-time reverse trans cription polymerase chain reaction (RT-PCR) diagnostic assays are a goal standard for case ascertainment and diagnosis [7].

Many cases that were strongly epidemiologically linked to SARS-CoV-2 exposure and with typical lung radiological findings remained RNA negative in their upper respiratory tract samples. The performance of RT-PCR depends on many factors such as the sample types [8], different stage of infection in patients [9] the skill of sample collection, and the quality and consistency of the PCR assays being used. A nasopharyngeal specimen is the preferred choice for swab-based SARS-CoV-2 testing, but oropharyngeal sample should also be collected whenever feasible [9,10].

These problems lead to a noteworthy delay of early diagnosis and following management and propose serious challenge to providing timely life support treatment and preventive quarantine. Comparing to PCR, serological testing is advantageous with faster turn-around time, high-throughput and less workload. However, the clinical value of antibodies largely depends on the understanding of host antibody responses during infection. Given that SARS-CoV-2 is a newly emerging virus, the antibody response in COVID-19 patients remains largely unknown [11].

The aim of this study is To investigate the dynamics of the (Immunoglobulin M (IgM) and Immunoglobulin G (IgG)) in response to SARS-CoV-2 in patients newly recovered from different severity Covid-19 infection.

Patients and Methods

This cross sectional study where done in the Central blood bank of Diyala for the period from 1st June 2020-1st August 2020. (93) newly recovered patients with documented SARS-CoV-2 infection by using reverse transcriptase polymerase chain reaction test (RT-PCR) who came for convalescent plasma donation were enrolled in this study. The questionnaire form was filled including the patient name, age, address, gender, clinical manifestations at time of the infection, the severity and the RT-PCR results.

VIDAS SARS-CoV-2 IgM and VIDAS SARS-CoV-2 IgG test: This test done by taking (5ml) blood from the patient, then

centrifuged to separate the serum. Then we take a small amount of the serum and test for IgG and IgM antibodies by using combines a two-step sandwich enzyme immunoassay method with a final fluorescence detection. This test done by using Minividas machine made by France at 2016 which is available in the central blood bank-Diyala.

Statistical Analysis

Statistics is an advanced analytical software and solutions package originally developed by StatSoft. The program includes a combination of data analysis, data management, statistics, machine learning, text analytics and data visualization procedures, and data mining procedures; In addition to a variety of predictive modeling, clustering, classification, and exploratory techniques.

Results

Male constitute about (91%) of the study group. Only 26% cases while 74% cases below (40) years old. (54%) of the patients had mild disease, (28%) had moderate severity while the other (18%) had severe type of the disease. About comorbidities (6%) of the cases had chronic bronchitis, (4%) of the cases had diabetes mellitus, (3%) had hypertension and (1%) had chronic renal disease. The demographic characteristics of them were shown in Table (1).

Table (1): : The demographic characteristics of the study group

	COVID (N=93) (100%)	Mild (N=50) (54%)	Moderate (N=26) (28%)	Severe (N=17) (18%)
Median Age (IQR)	34 (20-57)	33 (20-53)	32 (23-47)	36 (23-57)
Age group, n (%)				
20-29	36 (39)	22 (44)	11 (42)	3 (18)
30-39	33 (35)	16 (32)	9 (35)	8 (47)
40-49	21 (23)	11 (22)	6 (23)	4 (23)
>50	3 (3)	1 (2)	0 (0)	2 (12)
Male, n (%)	85 (91)	46 (92)	22 (85)	17 (100)
Female, n (%)	8 (9)	4 (8)	4 (15)	0 (0)
Comorbidities				
Ch.bronchitis, no. (%)	6 (6)	2 (4)	4 (15)	0 (0)
Hypertension, no. (%)	3 (3)	1 (2)	1 (4)	1 (6)
Diabetes, no. (%)	4 (4)	2 (4)	1 (4)	1 (6)
Kidney disease, no. (%)	1 (1)	0 (0)	0 (0)	1 (6)

The most common symptoms of the patients under the study during the time of infection were fatigue and fever and seen in (87% and 84%) respectively, while asymptomatic patients were seen in (2%) only. As shown in Table (2).

Table (2): The main symptoms of the patients at time of infection

Symptoms (n=93)	No. (%)
Fever	78 (84)
Cough	62 (67)
Difficulty breathing	45 (48)
Fatigue	81 (87)
Headache	61 (66)
Anosmia	58 (62)
Loss of taste	52 (56)
Diarrhea	55 (59)
Anorexia	50 (54)
No symptoms	2 (2%)

The IgM Antibodies starts to appear in the circulation before (21) days post infection mainly in mild and moderate types (100%). While no one of the severe cases were positive at this period. After (49) day from the time of the infection (67% and 75%) of the mild and moderate cases where IgM positive and (100%) of the severe cases were IgM positive. The Antibody response to SARS-CoV-2 was shown according to the severity of the illness in Table (3).

Table (3): the seroconversion of the antibodies stratifies by day of illness and severity (N= 93 tests)

	PCR positive	ELISA IgM ≥ 1			
		Total	Mild	Moderate	Severe
Day < 21	4	4/4 (100%)	3/3 (100%)	1/1 (100%)	0 (0%)
Day 22-28	20	15/20 (75%)	8/9 (88.9%)	6/8 (75%)	1/3 (33.3)
Day 29-35	23	17/23 (74%)	7/11 (64%)	6/8 (75%)	4/4 (100%)
Day 36-42	24	19/24 (79%)	12/16 (75%)	3/4 (75%)	4/4 (100%)
Day 43-49	9	6/9 (67%)	3/5 (60%)	1/1 (100%)	2/3 (67%)
Day > 49	13	10/13 (77%)	4/6 (67%)	3/4 (75%)	3/3 (100%)
	93	71/93 (76%)	37/50 (74%)	20/26 (77%)	14/17 (82%)
	PCR positive	ELISA IgG ≥ 1			
		Total	Mild	Moderate	Severe
Day < 21	4	4/4 (100%)	3/3 (100%)	1/1 (100%)	0 (0)
Day 22-28	20	20/20 (100%)	9/9 (100%)	8/8 (100%)	3/3 (100%)
Day 29-35	23	21/23 (91%)	10/11 (91)	7/8 (88%)	4/4 (100%)
Day 36-42	24	24/24 (100%)	16/16 (100%)	4/4 (100%)	4/4 (100%)
Day 43-49	9	9/9 (100%)	5/5 (100%)	1/1 (100%)	3/3 (100%)
Day > 49	13	13/13 (100%)	6/6 (100%)	4/4 (100%)	3/3 (100%)
	93	91/93 (98%)	49/50 (98%)	25/26 (96%)	17/17 (100%)

Although mild cases show early seroconversion (before 21 day) but only (67%) had IgM Antibodies after (49) day post infection, while severe type show late appearance of IgM Antibodies (after 21 day) and at (49) day post infection all patients with severe type of the disease (100%) were IgM positive.

IgG Antibodies were positive in (100%, 0%) of the mild and severe cases respectively at (21) day post infection but it's positive in (100%) of both mild and severe cases after (49) day post infection. This indicate that although the IgG antibodies appear slowly and later than IgM antibodies but it last longer in the circulation mainly in the severe type of SARS-CoV-2 infection.

Discussion

In this study the male patients constitute (91%) of the cases and this agree with the study done in Iraq in 2021[12], another study done in Iraq in 2021[13],a study done in Bangladesh in 2020 [14] and a study done in Sweden in 2020 [15] which show that male constitute about (67%,63%, 69% and 62%) respectively. While in other studies like a study from China 2020 [16] and a study from Thailand in 2020 [17] which show that male constitute only (39.5% and 40%) respectively. And this may be due to the circumstances under which the study were done. Also many factors, including innate immunity, regulatory T cells, expression of angiotensin-converting enzyme 2 (ACE2), or other mechanisms related to sex hormones might explain the difference in immunity between both sex.

The median age group affected in this study were (34) and the most affected age group was (20-29). This is lower than the finding in many studies like that from Sweden in 2020 [15],a study from China in 2020 [16] and a study from Thailand in 2020[17] which show a median age were(45, 47 and 38% respectively)and this may be due to the fact that this is the age of most workers and others who participate in social activities and easily get the infection there.

The main chronic disease in our study patients were chronic bronchitis which is found in about (6%). While in other studies like that from Sweden in 2020[15] and from China in 2020[16] it was hypertension in (9% and 23% respectively). This may be due to the prevalence of these chronic illnesses in our community. So many patients were smokers and had chronic bronchitis which put them at risk for Covid-19 infection.

The main symptoms were fatigue and fever and seen in (87% and 84%) respectively, this agree with a previous study done in Iraq in 2021 [12] and a study done in Bangladesh in 2020 [14] which show the main symptom was fever and seen in (79.5% and 76%) respectively.

The IgM Antibodies starts to appear in the circulation before (21) days post infection mainly in mild and moderate types (100%) and in the severe cases (0%) were positive at this period and this agree to some degree with the study done in China in 2020 [18] which show that the positive rate for IgM in the mild group was 100%, compared to 75% in the severe group at (15-21) day post infection. And these findings go with the suggestion that the appearance of noticeable levels of SARS-CoV-2 IgM antibodies in severe cases of COVID-19 is delayed.

While after (49) day from the time of the infection (67%and 75%) of the mild and

moderate cases respectively where IgM positive and (100%) of the severe cases were IgM positive. So, this suggest that the IgM Antibodies appear early and decline early in mild-moderate cases while in severe cases it raises slowly and decline later in the course of the illness.

The IgG Antibodies were found to be positive in (100%,0%) of the mild and severe cases respectively at (21) day post infection but it's positive in (100%) of both mild and severe cases after (49) day post infection. This disagree with the study done in Thailand in 2020 [17] which show that only (50%) of the mild cases and (100%) of both moderate and severe cases were positive at day (15-28) post infection. But it goes with the findings in other studies like that from Sweden in 2021[15]which show that clinical severity of the disease is associated with higher SARS-CoV-2-specific serum-IgG antibodies that although it appears late in the course of the disease but it lasts longer and further studies with long follow up period needed to prove it.

Conclusions

Although the serological response for SARS-CoV-2 appears in the first three weeks from the disease onset this response differs from patient to another according to the time from the infection and the severity of the infection.

Recommendations

Further studies were needed to follow up post SARS-CoV-2 patients for longer periods to assess the exact duration of these antibodies

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Diyala Journal of Medicine

استجابات الأجسام المضادة لـلمايكروب التاجي الثاني المسبب لمتلازمة الجهاز التنفسي الحادة لدى المرضى المتعافين حديثًا من عدوى المايكروب التاجي-19

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الملخص

خلفية الدراسة: لا تزال جائحة مرض فيروس كورونا 2019 مستمرة، مما يتسبب في معدلات مراضة ووفيات كبيرة في جميع أنحاء العالم. تعد فحوصات تشخيص تفاعل البلمرة المتسلسل العكسي (RT-PCR) في الوقت الحقيقي معيارًا هادفًا للتأكد من الحالة وتشخيصها. بالمقارنة مع تفاعل البلمرة المتسلسل ، يعد الاختبار المصلي مفيدًا مع وقت دوران أسرع وإنتاجية عالية وعبء عمل أقل. ومع ذلك، فإن القيمة السريرية للأجسام المضادة تعتمد إلى حد كبير على فهم استجابات الأجسام المضادة المضيفة أثناء العدوى.

اهداف الدراسة: لدراسة ديناميكيات (الجلوبيولين المناعي (IgM والجلوبيولين المناعي (IgG في الاستجابة لـلمايكروب التاجي الثاني المسبب لمتلازمة الجهاز التنفسي الحادة في المرضى المتعافين حديثًا من عدوى Covid-19 مختلفة الخطورة في بعقوبة-العراق.

المرضى والطرائق: في هذه الدراسة المقطعية، المرضى الذين يعانون من إصابة حديثة بفيروس كورونا والذين جاءوا إلى بنك الدم المركزي في ديالى للتبرع ببلازما النقاهة للفترة من 1 يونيو 2020 حتى 1 أغسطس 2020. تم ملء استمارة الاستبيان بخصوص وقت الإصابة، والمظاهر السريرية، وشدتها، ونتائج تفاعل البلمرة المتسلسل (PCR). بينما تم أخذ الدم لقياس الأجسام المضادة باستخدام اختبار SARS-CoV-2 IgM وIgG VIDAS.

النتائج: يشكل الذكور حوالي 91% من مجتمع الدراسة. وحوالي 74% من الحالات كانت أعمارهم أقل من 40 عامًا. وكان 54 من المرضى يعانون من مرض خفيف، و28% منهم يعانون من شدة معتدلة، بينما كان 18% الآخرون يعانون من نوع شديد من المرض. كان التعب والحمى من أكثر الأعراض شيوعًا وشوهدت في 87% و84% على التوالي. تبدأ الأجسام المضادة IgM في أن تكون إيجابية قبل 21 يومًا من الإصابة بالعدوى بشكل رئيسي في الحالات الخفيفة والمتوسطة بنسبة 100%، في حين أن 0% من الحالات الشديدة كانت إيجابية في هذا الوقت. بعد 49 يوم من وقت الإصابة 67% و 75% من الحالات الخفيفة والمتوسطة يكون IgM إيجابيا على التوالي و 100% من الحالات الشديدة يكون IgM إيجابيا. كانت الأجسام المضادة IgG إيجابية في 100%، 0% من الحالات الخفيفة والشديدة على التوالي عند 21 يومًا بعد الإصابة ولكنها إيجابية في 100% من الحالات الخفيفة والشديدة بعد 49 يومًا بعد الإصابة.

الاستنتاجات: على الرغم من أن الاستجابة المصلية لـلمايكروب التاجي الثاني المسبب لمتلازمة الجهاز التنفسي الحادة تظهر في الأسابيع الثلاثة الأولى من بداية المرض، إلا أن الاستجابة تختلف من مريض إلى آخر حسب وقت الإصابة وشدة العدوى.

الكلمات المفتاحية: SARS-CoV-2، الاستجابة المصلية، الأجسام المضادة للغلوبيولين المناعي G.

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