Detection of SARS-COV-2 Antibodies in Pediatric Kidney Transplant Patients

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Abstract

Background

The seroprevalance of SARS-COV-2 infection have been studied in immunocompetent children. However, data in pediatric Kidney Transplant population (PKT) is lacking.

Methods

We screened 72 PKT patients who came for routine blood work in the outpatient clinic using two commercial immunoassays that measures IgG antibodies against SARS-COV-2 spike protein and IgG against the Nucleocapsid protein. Majority of patients with positive serology had their serology test repeated at least once during subsequent clinic follow up. Patients were confirmed to have SARS-COV-2 infection if they only have both tests positive.

Results

Eight patients out of the 72 screened (11.1%) had positive SARS-COV-2 IgG antibodies in both serological tests. Of those tested positive, 4 had positive SARS-COV-2 PCR before screening. All patients were asymptomatic or had history of mild symptoms. All tested patient had persistently positive antibodies at median follow-up time of 75 days (IQR, 44.5, 86.5). One patient remained to have positive PCR at 75 days and positive serology test at 120 days post infection.

conclusion

The Serooprevalence of SARS-COV-2 was relatively high (11.1%) in our population. SARS-COV-2 PCR seems to remain positive in PKT population for longer duration than general population. Although, all patients were asymptomatic or mildly symptomatic, they mounted a strong humoral immune response that persisted for few months despite being on triple immunosuppressants. These findings will have positive implications on vaccination efficacy in this group.

1. Introduction

Coronavirus disease 2019 (CVOID-19) pandemic is considered a real public health emergency. Despite all efforts and policies to contain it, the severe acute respiratory syndrome (SARS-COV-2) infection continued to spread rapidly. As of October 8, 2020, COVID-19 has led to over 1 million deaths and 3.5 million cases worldwide ¹. Around 85% of cases will run a mild course, while 10% will have moderate disease requiring hospitalization, and 5% will run severe cause requiring ICU admission ².

As the pediatric solid organ transplant recipients are considered a high-risk group and are prone to more severe viral, bacterial, and fungal infections, this pandemic has raised concerns about managing this vulnerable group among the transplant community. In the early days of the pandemic, COVID-19 thought to run a more severe course among immunocompromised. The fear of more severe disease has limited kidney transplant surgeries to urgent and to the closure of many living transplant programs ³, including ours. This conceptual thought is an extrapolation from other virus's data. Unlike influenza and adenovirus ⁴, SARS-COV-2 seems not to affect immunocompromised patients preferentially. There is an emerging evidence that immunosuppressed children exhibit mild disease ^{5,6,7}. This mild disease course could be well explained by immunosuppressants' role in damping the dysfunctional or hyperimmune response that occur in the later stages of the infection leading to lung tissue injury and picture of acute respiratory distress syndrome (ARDS)⁸. Although we are almost one year into the pandemic, little is known about the effect of COVID-19 on the pediatric kidney transplant (PKT) population.

To better understand the SARS-COV-2 infection prevalence, clinical presentation and the rate of seroconversion in the PKT population, we aim to perform universal SARS-COV-2 serological testing for all our pediatric kidney transplants attending outpatient clinics.

2. Methods

2.1 Study Design and Subjects enrollment

This study was approved by the institutional review board of King Fahad Specialist Hospital -Dammam/Saudi Arabia. Verbal consent was taken from parents of children ≥ 7 years of age and ascent from those who are more than seven years.

This study is a single-center cross-sectional study of all pediatric kidney transplant patients ≥ 17 years of age who had an outpatient clinic visit or came to the hospital laboratory at King Fahad Specialist Hospital-Dammam for routine post-transplant blood work between August 15th 7 till October 12, 2020. Data on demographics, immunosuppressants, time since transplant and SARS-COV-2 PCR results were collected from electronic records. Two extra ml of blood for serology testing was drawn from all patients during routine blood work extraction.

All children were screened for COVID-19 symptoms through the clinic triaging system or phone call surveys.

Symptoms checklist included: fever $> 38^{\circ}\text{C}$, cough, fatigue, shortness of breath, sore throat, runny nose, headache, abdominal pain, diarrhea, anosmia, and ageusia.

As the SARS-COV-2 seroprevalence in the pediatric transplant population is not well determined and expected to be low, the positive predictive value of a single serological assay might be not accurate. To improve the diagnostic accuracy of the serological test, we used two different FDA approved commercially available serological assays that test different SARS-COV-2 protein parts. Assays with dual target antigen have shown to have better predictive abilities⁹.

2.2 Serological Essay for SARS-COV-2 Antibodies:

1-Diasorin Liasion ® SARS-COV-2 S1/S2 IgG assay:

This assay is a quantitative chemiluminescent immunoassay (CLIA) against SARS-COV-2 Spike protein S1 and S2 with a reported sensitivity of 97.6 % at > 15 days and specificity of 99.3%, a positive predicted value (PPV) of 87.5%¹⁰. The test was performed as per manufacture manual. Readings > 15 AU/ml is considered positive and < 12 AU/ml as negative and the upper detectable limit is 400 Au/ml as per the manufacture manual.

2-Abbott Architect SARS-CoV-2 IgG

This assay is a qualitative chemiluminescent microparticle immunoassay (CMIA) intended to measure IgG antibodies against SARS-COV-2 nucleocapsid (N) protein. As per the last EUA authorized serology test performance, the Abbot test has 100% sensitivity, 99.6% specificity, 93.4 % PPV¹⁰. All assays were performed according to manufacturing protocols . Samples were interpreted as positive or negative according to the manufacture instructions¹¹.

Serology test was repeated for follow up titers if the patient had initial positive serology and had a follow-up clinic visit or follow-up laboratory work during the study time frame and for those with positive PCR who did not show evidence of seroconversion in the initial sample. Patients/ parents were informed about the positive SARS-COV-2 serology results and were asked again about any history of COVID-19 symptoms in the past 3 months or history of close contacts with confirmed COVID-19 cases. They were also asked to do PCR test for their children if one of the serology tests was positive.

3. Results

3.1 Demographics and clinical characteristics

Of the 72 PKT patients screened by serological testing for SARS-COV-2, 12.5% (9 of 72) were found to have positive SARS-COV-2 IgG in at least one test. 88.9% (8 of 9) patients had both tests positive. Worth mentioning, 4 out of the total tested series had history of a positive PCR before serological testing. Two out of the four (50%) patients who had positive PCR were completely

asymptomatic and test was performed for elective admissions, one for kidney biopsy for new onset de-novo donor specific antibodies (case 1) (Table.1) and the other for investigations for rising creatinine.

The third patient presented to the ER with history of fever (38.5 C°), cough, shortness of breath, myalgia and headache. PCR taken at that time was positive for COVID-19 (Case 2). The fourth patient (case 3) had symptoms and had positive PCR that was not disclosed before serology testing.

The median age of patients with detectable COVID-19 antibodies was 9 years (IQR, 6- 13). 77.8% (7 out of 9) were male (Table 1.). Seven patients were on triple maintenance immune suppressant consisting of Tacrolimus, Mycophenolate Mofetil (MMF) and every other day prednisone. One patient was on Azathioprine instead of MMF and one patient was not on prednisone. The median time from transplant to testing positive was 3.8 years (IQR, 1.5-3.8). All but one patient, had household contact with confirmed COVID-19. Upon asking the eight asymptomatic children or their parents at the time of serology about history of COVID-19 symptoms in the last 3 months, 75% (6 of 8) of them reported positive mild symptoms. 62.5% (5 of 8) reported history of fever. 50% (4 of 8) had history of cough. Two had history of diarrhea (25%). Headache and loss of taste and smell were reported in one patient (12.5%) (Table.1).

3.2 SARS-COV-2 antibodies test results

Total of 85 serological tests using both Diasorin Liasion ® and Abbott were performed on 72 PKT patients. We reported serological tests agreement between Abbott and Diasorin in 8 patients (88.9%). All patients with positive PCR test except one had serological evidence of seroconversion (3/4). The patient who did not seroconvert has received Rituximab 4 months before her positive PCR. She was first tested after 70 days of her first positive PCR and tested again at 90 days and was serologically negative. Her profound B cell lymphopenia at the time of infection which was confirmed by very low CD19 count, could explain her negative seroconversion. Another explanation for her negative seroconversion could be that she lost her immunoglobulins to SARS-COV-2 by the time she was tested. In one patient (case 9) Abbott test was negative but Diasorin assay was positive. In order to make sure that this is not a false positive result, we repeated both test after 1 week and the results were the same. This patient PCR was negative, and the patient was completely asymptomatic, and parents denies any history of close contact with COVID-19 patient.

From previous reports we learned that most COVID-19 patients are IgG antibodies positive by three weeks¹² and antibodies start to decline after two to three months¹³. However, this is not the case with our young patients. Surprisingly, we had two patients whom their IgG titers were above the test upper limit of detection (400 AU/ml). One patient had his test repeated at 70 days post his mild symptoms (case 6) and the other at 88 days (case 8). Overall, all patients have an increasing titers overtime (Table.2). Lowest titer detected was 32 AU/ml (Case 9). All patients with positive serological test had their test repeat at least once except for two patients (case 5,7). There was a clear increase in the serology titer overtime and all of them had positive antibodies until the end of the study. Median time to first serological test was 31 days (IQR 19.5-49.5) and the median time from symptoms/ positive PCR to last serological test was 75 days (IQR, 44.5-86.5).

We compared the antibodies titer of our patients to the antibody titer of their immunocompetent parents or adult household contacts who requested to check their antibody titers using the same quantitative assay (Table 2.) and interestingly their results were much lower than their children except in (case 1).

4. Discussion

Most SARS-COV-2 infection in children with intact immune response is mild. However, severe infection can rarely occur¹⁴. The difference in diseases severity between adult and pediatric might be related to more robust innate immune response and higher levels of interleukin- 17 and Interferon gamma in children¹⁵. Immunocompromised patients are always at a higher risk of contracting severe viral and bacterial infections. Understanding the host immune response of transplant adult and children to SARS-COV-2 is evolving but remains unclear.

Herein, we reported our experience in using serological testing as screening and confirmatory tool for COVID-19 patients. Of the 72 pediatric kidney transplant patient's cohort who underwent serological testing, the prevalence of COVID-19 based on two positive serological tests for SARS-COV-2 IgG was 11.1% (8 Of 72). In our series all patients with positive serology including the one who tested positive for PCR and did not seroconvert were either asymptomatic or had mild symptoms not requiring hospital admission. None of them developed pneumonia or required oxygen supplementation. Our findings are consistent with what is reported so far.

After reviewing the current literature about SARS-COV-2 infection in pediatric and adult transplant population, we found no clear evidence that transplantation increases the risk of ICU admission and mortality. It is well known that early adaptive humoral response with the development of neutralizing anti-viral antibodies is an important mechanism in eliminating viral replication¹⁶. Our patients demonstrated high titer against Spike proteins (which correlate with neutralizing antibodies) suggesting that the adaptive humoral response is the dominating response in PKT, and it is sufficiently intact to overcome severe disease. However, such finding needs to be confirmed by larger studies. Moreover, there is growing evidence from multiple case series showing that immunosuppressants medications might have a beneficial effect. Bush et al. have reported a case of pediatric kidney transplant recipient who had a mild course of the disease despite being on immunosuppressant medications¹². In a series of 200 pediatric liver transplant patient in a big transplant center in Italy, they reported 3 cases of covid-19 none of them had severe pneumonia or severe course¹⁷. This observation has led the author to conclude that immunosuppressant medications might have protective role. In one adult liver transplant study of 111 long term (> 10 years) stable liver transplant patients, three died because of severe COVID-19 pneumonia. Worth mentioning all of them had other risk factors associated with more severe course of COVID-19 like (male > 65 years, hypertension, DM, and hyperlipidemia) other than being immunocompromised¹⁸. They were also on very low doses immunosuppressant, making it difficult to be blamed. On the other hand, there are some case series from the adult transplant population showing high mortality rate (28%) compared to the general population¹⁹.

Furthermore, it has been shown in a systematic review of 16 articles including children and adult with cancer, kidney, heart, liver and immunodeficiency patients that immunocompromise patients has similar and sometimes more favorable outcome when compared with general population. However this favorable outcome was not observed in cancer patients²⁰

In our study, serological testing identified five patients with COVID-19 who otherwise would have been missed because of false negative PCR results or because of silent disease. Although none of those five patients had severe symptoms or complications that required hospital attention, identifying them helped us better understand the full clinical spectrum of the disease in this vulnerable group. Now we learned that asymptomatic COVID-19 is not uncommon in the PKT population. Identifying positive cases could also help in contact tracing and reduce disease spread. Moreover, we realize the stigma associated with COVID-19 in our community which led them to not reach out to us when their children developed symptoms. This will make us more vigilant in assessing our transplant patients during admission and out-patient clinic visits when we fully operate.

Out of the four patients with documented positive PCR, 75% seroconverted with relatively high antibody titers. Two patients had readings above the detectable upper limits of the test (> 400 AU/ml). Although these levels are very high, yet, no one really know how these levels translate into protection from the virus. These findings were unexpected as we already know that transplant patients who developed infection have blunted immune response and low rate of seroconversion when compared to regular population²¹. More interestingly, all those patients who mount vigorous immune response to COVID-19 were still positive at median time of 75 days (IQR ,69–83). One patient (Case 2) was still positive for SARS-cov-2 IgG at 120 days. More Interestingly, most parent's antibodies titers taking around the same time as their children were significantly lower than their children except in one patient. These findings are in contrast to what have been reported by Pierce et al. were they show that serum neutralizing SARS-COV-2 IgG antibodies were higher in adults when compared to children¹⁵

Important to note that, despite no major modifications was made on the immunosuppressant medication for all PCR positive patients except for one patient (Case 3) in whom MMF dose was reduced by half for one week. All patient but one, developed humoral immune response to COVID-19. Two adult kidney transplant patients reported to have seroconversion between day 19–23 days after reduction of immunosuppressants²². There was also a documented seroconversion in another two adult kidney transplant patients who developed SARS-COV-2 antibodies at 30 days post infection²³ Moreover, study done by Hartzell et al.

reported 100% seroconversion in 16 SARS-COV-2 positive adult kidney transplant patients²⁴. Furthermore, Choi et al. have also reported 100% seroconversion rate in 5 adult KTR²⁵. Most of the current transplant guidelines suggest reduction or complete withdrawal of immunosuppressant. However, this suggestions was challenged by multiple studies that showed the ability of CNIs to inhibit in vitro viral replication of SARS-COV-2 infection independent of its immunosuppressants effects²⁶. Furthermore, cyclosporine was successfully used to treat cases secondary hemophagocytic lymphohistocytosis (HLH) that overlaps with the cytokine-release storm which seems to be the leading cause of mortality in COVID-19 infection²⁷

To the best of our knowledge, our study is the first study to report a relatively high seroprevalence of COVID-19 in PKT population. And it is the first study to test the humoral immune response to SARS-COV-2 infection using quantitative serological assay that correlate with neutralization antibodies in this population. However, as all pediatric kidney transplant studies we are limited by the small sample size especially in this highly protected group. Moreover, results cannot be generalized to all PKT patients as majority of the patients enrolled were more than one year post transplants and on low maintenance immunosuppressants. Immunoassays used were IgG based, so acute infection could have been missed. Important to mention, all samples obtained were from Saudi children who might have different immunological background that can affect the immunoassays performance characteristics. For all these mentioned reasons, further larger studies with wider geographical presentation, and longer duration of antibodies titer follow up are needed.

Conclusion

The Seroprevalence of SARS-COV-2 infection in our study is the highest reported among PKT population so far. Screening PKT patients using serological testing was effective in identifying the whole spectrum of the disease including asymptomatic patients. On the other hand, Symptoms-based screening for SARS-COV-2 infection might be not effective tool in identifying who should go for testing as asymptomatic infection is not uncommon and can lead to disease spread in hospital settings and in public. Most importantly, Humoral immune response to SARS-COV-2 infection was rigorous and persistent in PKT population and this will have positive implications on future vaccination. Immunosuppressants seems to not exert an additional risk for more severe course in this population and reduction in immunosuppressants might not be necessary.

Declarations

Ethics approval and consent to participate

This study was approved by the local ethics committee (KFSH-D, Saudi Arabia). As this study does not carry more than minimal risk to subjects enrolled, only verbal consent was obtained from parents and additional assent from children more than 7 years of age. The patients and their family participation was voluntary and their anonymity was preserved by de-identifying the data. This study was conducted according to the principles of the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Patient consent for publication was not required as all data are anonymized.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.A. Generated the research idea, collected data , draft the manuscript, supervised the research

R.A. Performed the serological test, manuscript editing

A.AZ. Collect data, reviewed the manuscript

A.M. Performed the serological tests, reviewed the manuscript

Aknowledgment:

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Tables

Table.1. Demographics and Characteristics of SARS-COV-2 Serology positive patients

Cases	TST(m)	IS Regiment	Tac level (μ/L)	MMF/M2/d (mg)	Symptoms
Case 1	45	Tac/MMF/Pred	5.2	625	Asymptomatic
Case 2	32	Tac/MMF/Pred	5.4	620	Fever, cough, headache, SOT, diarrhea
Case 3	53.3	Tac/MMF/Pred	5.7	937.5	Fever, headache, cough, runny nose
Case 4	15	Tac/MMF/Pred	7.7	648	Fever, diarrhea
Case 5	46	Tac/MMF/Pred	4.4	595	Asymptomatic
Case 6	40	Tac/MMF/Pred	4.8	769	cough
Case 7	45.9	Tac/MMF/Pred	4.8		Cough, runny nose, diarrhea
Case 8	45.6	Tac/AZA/Pred	6.9	652	Asymptomatic
Case 9	3.3	Tac/MMF/Pred	5.4	694	Asymptomatic

TST= Time Since Transplant, m=months,, Tac= Tacrolimus, MMF= Mycophenolate Mofetil, M2=surface area, d=day, Pred= Prednisone, AZA= Azathioprine IS= Immunosuppressant,

Table 2. Kinetics of SARS-COV-2 IgG antibodies positive PKD patients using Diasorine Liasion ® serological assays

Cases	PCR Status	Duration of +ve PCR	Serology Test 1 (Au/ml)	Duration(days) post PCR/ Symptoms	Serology Test 2 (Au/ml)	Duration 2	Serology (3/4) (Au	Duration 3	Households contact serology (Au/ml)
1	Positive	24	153	30	238	53	104	85	186
2	Positive	37	negative	7	negative	31	116/192	69/120	135
3	Positive	75	negative	44	31.1	75	N/D	N/D	20.4
4	Negative	—	185	27	135	83	N/D	N/D	25
5	N/D	—	223	57	N/D	N/D	N/D	N/D	38
6	Negative	—	216	12	297	33	>400	70	38.3
7	N/D	—	98	32	N/D	N/D	N/D	N/D	N/D
8	Negative	—	234	55	311	67	>400	88	38.3
9	Negative	—	41	U/K	32	N/D	N/D	N/D	N/D

PCR= Polymerase Chain Reaction, N/D = Not Done, U/K= unknown