Impaired anti-SARS-CoV-2 antibody response in non-severe COVID-19 patients with diabetes mellitus: A preliminary report

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ABSTRACT

Background and aims: Patients with diabetes mellitus (DM) often demonstrate impaired antibody response to influenza/hepatitis B vaccines. Hence, we compared anti-SARS-CoV-2 antibody response in non-severe COVID-19 patients with and without type 2 diabetes mellitus (T2DM).
Methods: Records of non-severe COVID-19 patients admitted at our institution between April 10, 2020 and May 20, 2020 were retrieved. Qualitative detection of total (IgG + IgM) anti-SARS-CoV-2 antibody was performed using electrochemiluminescence immunoassay in plasma samples collected at least 14 days post-polymerase chain reaction (PCR) confirmation of diagnosis.
Results: Thirty-one non-severe COVID-19 patients were included. Nine patients (29%) had T2DM with mean HbA_{1c} at admission of $8.3 \pm 1.0\%$. Anti-SARS-CoV-2 antibody was estimated at a median of 16 (14 −17) days post-PCR confirmation of COVID-19 diagnosis. Only three patients (10%) were seronegative, and all had T2DM. Patients with T2DM were more likely to have non-detectable anti-SARS-CoV-2 antibodies than those without DM ($p = 0.019$).
Conclusions: COVID-19 patients with T2DM may not undergo seroconversion even after two weeks of diagnosis. Impaired seroconversion could theoretically increase the risk of reinfections in patients with DM. However, the finding requires validation in large-scale studies involving serial estimations of anti-SARS-CoV-2 antibodies in patients with and without DM.

1. Introduction

Diabetes mellitus (DM) is associated with a high risk of severe disease, acute respiratory distress syndrome, intensive care unit admissions, and eventual mortality from the novel coronavirus disease (COVID-19) [1,2]. It has also been hypothesized that DM *might* increase the chances of reinfections with COVID-19 [3]. In line with this hypothesis, several cases of clinical recurrences of COVID-19 and/or positive real time-polymerase chain reaction (RT-PCR) re-positives have been reported in patients with DM [4–8].

Infection with SARS-CoV-2 leads to the generation of neutralizing antibodies essential for preventing reinfections [9,10]. A robust adaptive immune response and immunological memory are critical for the generation of neutralizing antibodies. Patients with DM have compromised adaptive immune system and often demonstrate impaired antibody response to influenza and hepatitis B vaccines, particularly those with poor glycemic control [11,12]. Similarly, seroconversion might also be impaired in COVID-19 patients with DM.

Hence, we planned to compare the anti-SARS-CoV-2 antibody response in non-severe COVID-19 patients with and without type 2 diabetes mellitus (T2DM).

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Table 1

Showing comparison of demographic/clinical parameters and anti-SARS-CoV-2 antibody status in non-severe COVID-19 patients with and without T2DM.

	With T2DM (n = 9)	Without T2DM (n = 22)	p value
Age (years) [median (IQR)]	30.0 (24.5—40.5)	55.0 (46.0—61.5)	0.001
Male:Female	4:5	1:1	1.000
Fever	7 (78%)	13 (59%)	0.429
Cough	3 (33%)	3 (14%)	0.320
Sore throat	1 (11%)	6 (27%)	0.639
Shortness of breath	2 (22%)	1 (4%)	0.195
Asymptomatic	2 (22%)	7 (32%)	0.689
Disease severity *			
• Mild	7 (78%)	21 (96%)	0.195
• Moderate			
	2 (22%)	1 (4%)	
HTN	6 (67%)	1 (4%)	0.001
CKD	1 (11%)	0 (0%)	0.290
HbA$_{1c}$ (%)	8.3 ± 1.0%.	–	–
(mean ± SD)			
Day of antibody testing post-PCR confirmation of diagnosis [median (IQR)]	15.0 (14.0—17.0)	16.0 (14.0—17.2)	0.450
Anti-SARS-CoV-2 antibody status			
• Positive	6 (67%)	22 (100%)	0.019
• Negative			
	3 (33%)	0 (0%)	

*Disease severity as advocated by the Government of India, Ministry of Health and Family Welfare.

T2DM: Type 2 diabetes mellitus; HTN: Hypertension; CKD: Chronic kidney disease; PCR: Polymerase chain reaction; HbA1c: Glycated hemoglobin; SD: Standard deviation; IQR: Interquartile range.

2. Methods

In this retrospective observational study, the records of patients with non-severe COVID-19 (mild/moderate disease) [13] admitted at our institution between April 10, 2020 and May 20, 2020 were initially retrieved. The diagnosis was based on the detection of viral RNA using real-time reverse transcription polymerase chain reaction (TaqPath COVID-19 RT-PCR, Applied Biosystems) in nasopharyngeal swab specimens. Patients who had undergone detection of anti-SARS-CoV-2 antibody in plasma samples collected at least 14 days post-PCR confirmation of diagnosis were finally selected. The anti-SARS-CoV-2 antibody was measured using Electrochemiluminescence Immunoassay (Elecsys Anti-SARS-CoV-2 assay, Elecsys Cobas e 801 analyzer, Roche Diagnostics, Mannheim, Germany). The assay has a sensitivity and specificity of 100% and 99.81%, respectively, and is approved by the US-FDA and the Indian Council of Medical Research for the *qualitative detection* of total anti-SARS-CoV-2 antibodies (IgM + IgG) [14]. A cutoff index ≥1.0 qualified as a reactive test (positive for anti-SARS-CoV-2 antibodies). In addition, glycated hemoglobin (HbA$_{1c}$) was measured in patients with a history of T2DM using a DCCT-standardized HPLC based analyzer (Variant II Turbo, Bio-Rad). The study was approved by the Institute Ethics Committee, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

Statistical analysis was carried out using the Statistical Package For The Social Sciences (SPSS) version 23.0 software (SPSS Inc., Chicago, IL, USA). The normality of data was checked using the Shapiro-Wilk test. Normally distributed data were expressed as mean ± SD while non-parametric data were expressed as median (interquartile range, IQR). Comparisons between patients with and without T2DM were made using Independent Samples *t*-test/ Mann-Whitney *U* test (for continuous variables) or Pearson Chi-square/Fisher's Exact test (for categorical variables). A *p* value < 0.05 was considered statistically significant.

3. Results

Thirty-one non-severe COVID-19 patients with antibody assay performed at least 14 days after confirmation of diagnosis were included. The baseline characteristics have been summarized in Supplementary Table 1. Of note, the median (IQR) age was 38 (27—55) years, with 15 patients being male. Nine patients (29%) had

Table 2

Showing comparison of non-severe COVID-19 patients with T2DM having a positive anti-SARS-CoV-2 antibody response and those having a negative antibody response.

Parameter	Anti-SARS-CoV-2 antibody positive (n = 6)	Anti-SARS-CoV-2 antibody negative (n = 3)	p value
Age (years)	54.0 ± 7.6	55.7 ± 11.8	0.804
(mean ± SD)			
Male:Female	1:1	1:2	1.000
Disease severity *			
• Mild	6 (100%)	1 (33%)	0.083
• Moderate			
	0 (0%)	2 (67%)	
HTN	4 (67%)	2 (33%)	1.000
CKD	0 (0%)	1 (33%)	0.333
HbA$_{1c}$ (%)	7.9 ± 0.5	9.1 ± 1.4	0.193
(mean ± SD)			
Duration of T2DM (years)	4.0 ± 2.5	7.0 ± 1.7	0.109
(mean ± SD)			
Day of antibody testing post-PCR confirmation of diagnosis (mean ± SD)	16.5 ± 3.0	14.6 ± 1.1	0.285

*Disease severity as advocated by the Government of India, Ministry of Health and Family Welfare.

T2DM: Type 2 diabetes mellitus; SD: Standard deviation; HbA1c: Glycated hemoglobin; PCR: Polymerase chain reaction.

a history of T2DM with a median (IQR) duration of the disease being 5 (3–8) years. The demographic and clinical parameters of patients with and without T2DM have been summarized in Table 1. Notably, patients with T2DM were older and more likely to have hypertension. The mean HbA$_{1c}$ at admission was 8.3 ± 1.0%. The anti-SARS-CoV-2 antibody was estimated at a median (IQR) of 16 (14–17) days post-PCR confirmation of COVID-19 diagnosis (range 14–22 days). Only three patients (10%) were negative for the SARS-CoV-2 antibody, and all had T2DM. Patients with T2DM were more likely to have non-detectable anti-SARS-CoV-2 antibodies than those without DM ($p = 0.019$, Fisher's Exact test). Although patients with negative antibody status had higher HbA$_{1c}$ and a longer duration of T2DM, we found no statistically significant differences between the two groups of T2DM patients (Table 2).

4. Discussion

In this retrospective preliminary study involving 31 patients with non-severe COVID-19, we found that patients with T2DM were more likely to be negative for anti-SARS-CoV-2 antibodies than those without DM. However, larger studies are required to validate the finding.

Patients with DM are at an increased risk of poor prognosis with COVID-19, which is partly explained based on immune dysfunction seen in DM [2]. Both the innate and adaptive arms of the immune system are compromised in patients with DM. The status of adaptive humoral immunity in DM is debatable; while some studies have reported normal plasma immunoglobulin levels, others have shown reduced levels of IgG/IgM [15,16]. B-lymphocytes require accessory signals from T-helper cells to activate antibody-producing plasma cells. T-helper cells have been reported to be abnormally differentiated in individuals with T2DM [17]. Besides, diminished pathogen-specific memory CD4$^+$ T-cell number and function seen in patients with uncontrolled DM are also likely to adversely affect humoral immune response [18,19]. This is coupled by the fact that COVID-19 is associated with T-cell exhaustion and a significant decrease of T-cell activation, as determined by CD25, CD28, and CD69 expression on CD4$^+$ and CD8$^+$ T-cell subsets [20]. Furthermore, an animal model has shown that IgM producing B-1 lymphocyte function is directly impaired by hyperglycemia [21]. Moreover, hypocomplementemia seen in patients with DM also leads to impaired B-cell function and antibody generation [19]. Supporting these hypotheses, studies have shown impaired antibody response in patients with uncontrolled DM following influenza and hepatitis B vaccinations [11,12].

Similarly, we found that patients with T2DM were more likely to be seronegative for the anti-SARS-CoV-2 antibody following initial infection than those without DM even after two weeks (median 16 days) of diagnosis. Those who were seronegative had higher HbA$_{1c}$ and a longer duration of T2DM, although statistical significance was not achieved likely because of the limited number of patients. Nevertheless, the absence of seroconversion would *theoretically imply* a high risk of reinfections with COVID-19. However, the presence of antibodies does not necessarily guarantee protection against reinfections, especially if the neutralizing activity of the antibodies is not known. Besides, the neutralizing antibodies titers may not be sufficient to counter the viral inoculum or that the infecting viral strain may be substantially different to the first infection and not recognized by the circulating antibodies [22].

We do respect the limitations of the study. First, the small sample size is a significant drawback. Second, we did not estimate IgG and IgM separately. However, the levels and the chronological sequence of IgM and IgG antibody appearance are highly variable, supporting the detection of both antibodies simultaneously, as in the present study. Third, we did not have the provision for

measuring antibody titers at our institution. Comparing absolute antibody titers would have made the study more robust. Fourth, the assay uses a recombinant protein representing the nucleocapsid antigen; hence it can only detect antibodies against the nucleocapsid antigen and not against the spike protein. Nevertheless, the detection of antibody against nucleocapsid antigen is more sensitive than the antibody to spike protein [23]. However, anti-spike antibodies that can block infection may be clinically more relevant [24]. Fifth, 10–20% of symptomatically infected patients with COVID-19 otherwise mount little or no antibody response [25]. Lastly, the possibility of delayed seroconversion in patients with T2DM cannot be ruled out. Serial evaluation of antibody status at weekly intervals might have been helpful in this regard.

5. Conclusions

In conclusion, COVID-19 patients with T2DM may not undergo seroconversion even after two weeks of diagnosis. However, the finding needs to be validated in large-scale studies involving serial estimations of anti-SARS-CoV-2 antibodies in COVID-19 patients with and without DM.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Post Graduate Institute Of Medical Education and Research).

Data availability statement

Anonymized data sheets are available from the corresponding author upon reasonable request.

Authors' contribution statement

RP is the primary author and had drafted the initial version of the manuscript. NS and SKB are the corresponding authors and had conceived the study concept. SM, VS, DZ, SR, GDP, AB, SLS, NP and AB had helped in data collection.

Declaration of competing interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2020.12.035.

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