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Association between SARS-CoV-2 seropositivity and disease activity in children with systemic lupus erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a long-lasting autoimmune disease with its involvement in several organs and varying levels of severity. Pediatric SLE is an uncommon condition, accounting for 10-20% of all SLE cases. Individuals with systemic autoimmune illness have a higher prevalence of severe forms of COVID-19. However, the underlying cause for the heightened severity of COVID-19 in these patients is unclear. **Objectives:** The aim of the current study was to evaluate SARS-CoV-2 antibodies in children with SLE and the association between SARS-CoV-2 seropositivity and SLE disease activity. **Methods:** A cross-sectional study enrolled 42 children with SLE diagnosed and followed-up with seropositive IgG for COVID-19 infection. Detection of antibodies was done by ELISA. **Results:** Forty-two children with SLE diagnosed and followed-up with seropositive IgG for COVID-19 infection and our study focused on 40 children with seropositive IgG. Regarding assessment of COVID-19 symptoms, 14 cases (35%) were symptomatic, and 26 cases (65%) were asymptomatic. There is significant difference between symptomatic and asymptomatic cases according to socio-demographic, laboratory investigation e.g. CRP, albumin level, Anti-dsDNA, Antinuclear antibody, C3, C4 and also score of the Physician Global Assessment (PGA) before COVID-19 infection and SLE Disease Activity Index (SLEDAI) before and after COVID-19 infection. Furthermore, Ig G level of COVID-19 was significantly associated with increased scores of either SELDAI or PGA when compared before and after infection. **Conclusion:** SARS-CoV-2 infection may predispose to SLE exacerbation and worsen disease activity that was found through increase of score of PGA and SELDAI after COVID-19 infection.

Introduction

Coronavirus disease 2019 (COVID-19) is a major public health problem, which results from an infection of the respiratory tract by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 31 March 2024, over 774 million confirmed cases and more than seven million deaths have been reported globally[1]. The clinical course of SARS-CoV-2 infection is highly variable, ranging from asymptomatic or mild manifestations to acute respiratory failure and shock. Moreover, patients with COVID-19 may develop long-term sequel, including respiratory, cognitive, cardiac problems. The negative impact of COVID-19 extends beyond its direct adverse health effects to include severe psychological, social, and economic consequences [2,3].

Accumulating body of evidence indicates that the multi-organ system affection in patients with SARS-CoV-2 infection is attributed to

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inappropriate immune response rather than direct viral cytopathic effects [4]. SARS-CoV-2 infection is associated with activation of the complement system and development of autoantibodies, which can be attributed to virus-induced hyper-stimulation of the immune system and the molecular mimicry between the virus and self-components of humans. Ultimately, these immune responses may trigger new or preexisting autoimmune diseases [5-8].

Studies have shown that SARS-CoV-2 infection may be associated with an increased flare rate in patients with rheumatic disease. Ye et al. (2020) reported that 19% of adult patients with rheumatic disease who were hospitalized for SARS-CoV-2 infection showed flaring of their underlying rheumatic diseases[9]. Naddei et al. (2021) and Hügle et al. (2021) reported that flares may occur after SARS-CoV-2 affection in children and adolescents with juvenile rheumatoid arthritis (JRA)[6,10]. In contrast, Sengler et al. (2021) and Sjöwall et al. (2021) showed mild effects of SARS-CoV-2 infection on children with rheumatic diseases and adults with SLE, respectively[11,12]. However, data on the effect of SARS-CoV-2 infection on the clinical course of children with SLE are scarce [12,13].

The current study aimed to evaluate SARS-CoV-2 antibodies in children with SLE and the association between SARS-CoV-2 seropositivity and SLE disease activity.

Methods

A cross sectional study was conducted in Pediatric Allergy, Immunology, and Rheumatology Clinic at the Pediatric Department of Sohag University Hospital as well as Department of Medical Microbiology and Immunology at Faculty of Medicine, Sohag University, Egypt from 1st March 2022 to 1st January 2023among 42 children with SLE diagnosed and followed-up with seropositive IgG for COVID-19 infection and our study focused on 40 children with seropositive IgG.The studied cases were enrolled in the study after fulfilling the eligibility criteria, **inclusion criteria**: age less than 18 years and established diagnosis of SLE according to the 1982 American College of Rheumatology (ACR), the 1997 updated ACR, or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE.The SLICC criteria for SLE classification require: fulfillment of at least four criteria, with at least one clinical criterion and one immunologic criterion or Lupus nephritis as the sole clinical criterion in the presence of ANA or anti-dsDNA antibodies. Clinical Criteria included acute cutaneous lupus, chronic cutaneous lupus, oral ulcers: palate, non-scarring alopecia (diffuse thinning or hair fragility with visible broken hairs), synovitis involving two or more joints, characterized by swelling or effusion or tenderness in two or more joints and thirty minutes or more of morning stiffness, serositis, renal, neurologic, hemolytic anemia, leukopenia (< 4000/mm3 at least once) and thrombocytopenia (<100,000/mm3) at least once.

Immunological Criteria included increased ANA above laboratory reference range, anti-dsDNA above laboratory reference range except ELISA: twice above laboratory reference range, anti-Sm, antiphospholipid antibody, low complement, direct Coombs test in the absence of hemolytic anemia[14]. **Exclusion criteria** included prior administration of SARS-CoV-2 vaccine, other autoimmune disorders (e.g., overlap syndrome), comorbid conditions (e.g., diabetes mellitus) and irregular SLE pharmacotherapy.

Data was collected from the studied participants or their caregivers and included four sections. **first section:** patients' medical data and clinical assessment: including sociodemographic data (e.g., age, gender, socioeconomic level), SLE disease details (e.g., age at onset, duration, presenting symptoms), management (steroids intake duration and intake of hydroxychloroquine), Suggestive manifestations of COVID-19 in the last 6 months (e.g., febrile illness, respiratory disease, diarrhea, hospital admission), anthropometric measures and vital signs. **Second section** included investigations: including complete blood count, ESR, CRP, renal and liver function tests, ANA, anti-dsDNA, C3, C4 and serum immunoglobulins. **Third section** included assessment of SLE disease activity using: SLE disease activity index-2000 (SLEDAI-2K) and Physician's global assessment (PGA). SLEDAI: this index measures disease activity within the last 10 days through 24 weighted clinical and laboratory variables of nine organ systems (ranging from 1 to 8) is used for each of the items and all the individual item scores are summated to provide a global score, with a possible maximum score of 105. Disease activity will be classified as no activity (0), mild activity (1–5), moderate activity (6–10), high activity (11–19), and very high activity (>20), with higher scores reflecting higher activity

of the disease [15] and PGA: This is a visual analog scale ranging from 0 to 3 (0 = inactivity; 1 = mild activity; 2 = moderate activity; 3 = severe activity)[16]. **Fourth section:** Evaluation of serum SARS-CoV-2 antibodies: Blood samples were obtained from patients under complete aseptic conditions. Serum samples separated and stored at −80°C until analysis for SARS-CoV-2 IgG antibodies using ELISA kits. (SEION ELISA agile SARS-Cov-2 IgG Lot: AM011, Germany). This essay is based on the detection of SARS-CoV-2 IgG against whole spike protein (S1/S2 ectodomain), which typically occurs within 12 to 14 days after infection. The following steps were done: at first, patient samples (V1) were diluted in dilution buffer (V2) as follows: V1 + V2 = 1+100. We Added each 100 µl of diluted sample or ready-to-use negative control/standard sera into the appropriate wells of microtiter test strips. Spare one well for substrate blank. Sample incubation was done for 60 minutes at 37 °C in moist chamber. After incubation we washed all wells with 300 µl of washing solution (Dilute washing buffer concentrate (V1) 1:30 with distilled water . to a final volume of V2) four times by automated washer. Then we Added 100 µl of the ready-to-use IgG conjugate to the appropriate wells (except substrate blank) and incubation was done for 30 minutes at 37 °C in moist chamber. After incubation we washed all wells with washing solution (4 x 300 µl DIL WASH). After that we Added 100 µl of ready-to-use substrate solution to each well (including well for substrate blank) then incubation for 30 minutes at 37 °C in moist dark chamber. Then we added 100 µl stopping solution to each well, we shaked microtiter plate gently to mix. Lastly, we Read optical density (OD) at 405 nm wave length by microplate reader (stat fax2100) against substrate blank. The results were interpreted according to the cut-off ranges. To fix the cut-off ranges multiply the mean value of the measured standard OD with the numerical data of the quality control certificate e.g.: OD = 0.559 x MV(STD) with upper cut-off. OD = 0.387 x MV(STD) with lower cut-off. In our work, positive is considered when OD>0.25 and negative <0.17. This essay has a reported sensitivity of 96.2% and specificity of 99.2%.

Statistical analysis

Statistical package of Social science (SPSS) version 25.0 was used for data entry and analysis. **Quantitative variables** were expressed as means and standard deviation for normally distributed data and as median and IQR for not normally distributed data. The normality of data distribution was tested using Kolmogrov-Sminrov test. **Qualitative variables** were described as frequencies (percentages).**Chi- square test** was used to compare between groups of qualitative data in testing relation between grades of HPLP II score and socio-demographic data(P value was significant if < 0.05).Student's T-test will be used in the comparison between two groups with quantitative data and parametric distribution, and Mann-Whitney test will be used in the comparison between two groups with quantitative data and non-parametric distribution(P value was significant if < 0.05). **Regression analyses** were conducted to predict factors affecting increased PGA score that were found to be associated in the univariate analyses. Adjusted P-value <0.05 was considered to be statistically significant.

Ethical considerations

Approval of the Ethical Committee of Faculty of Medicine, Sohag University was obtained, ***IRB*** Registration number: Soh-Med-22-02-30. Informed consent was obtained from parents or authorized legal representatives of all children included in this study.

Results

The current study enrolled 42 children with SLE diagnosed and followed-up with seropositive IgG for COVID-19 infection and our study focused on 40 children with seropositive IgG. Regarding COVID-19 symptoms assessment, it was found that 14 cases (35%) of them were symptomatic in the last 6 months (e.g., febrile illness, respiratory disease, diarrhea, hospital admission) and 26 cases (65%) of them were asymptomatic.

Table 1 illustrates that there is statically significant difference between the two groups according socio-economic level. However, there is statistically insignificant difference between symptomatic and asymptomatic cases of seropositive IgG COVID-19 infection regarding socio-demographic characteristics (age and gender), their physical assessment (weight, BMI), age of disease onset and disease duration, medication intake according to either steroid intake duration and intake of hydroxychloroquine (P-value > 0.05).

Table 2 describes laboratory investigation of the studied participants, there is statically significant difference between the studied groups according to CRP level, 57.1% of symptomatic

cases showed positive CRP in comparison to 11.5% of asymptomatic cases. Also, symptomatic cases show significant decrease in median (IQR) of albumin level in comparison to asymptomatic cases [3.1 (3.02-3.8) and3.5 (3.27-4)] g/dL. Moreover, half of symptomatic cases show positive Anti-dsDNA in comparison to 11.5% of asymptomatic cases with statistically significance. More than two thirds of symptomatic cases (64.3%) show positive anti-nuclear antibody in comparison to 26.9% of asymptomatic cases. Regarding to level of C3 and C4, 71.4% of symptomatic cases show low C3 level in comparison to 3.8% of asymptomatic cases and 57.1% of symptomatic cases show low C4 in comparison to 3.8% of asymptomatic cases. There is statically insignificant difference between the studied groups according to WBCs, platelets, ESR and IgG level for COVID infection.

Table 3 shows that symptomatic group show significant higher median (IQR) of score in comparison to asymptomatic group regarding either SELDAI before infection or SELDAI after infection. Furthermore, regarding PGA score, symptomatic cases before infection show significant higher score in comparison to asymptomatic cases but after infection there is statically insignificant difference between the studied groups (P-value > 0.05).

Regarding SELDAI, the studied cases had significant higher SLEDAI score after infection in comparison to before infection (10.57 ± 5.27 and 7.22 ± 4.34), as shown in figure 1. Moreover, seropositive IgG of COVID-19 cases had significant higher PGA score after infection in comparison to before infection (2.42 ± 0.71 and 1.87 ± 0.64), as shown in figure 2.

Table 4 illustrates those cases with increased SELDAI score had significant higher level of IgG of COVID-19 in comparison to cases with no increased SELDAI score. Also, regarding univariate regression analysis, there is only a significant association between level of IgG of COVID-19 and change of SELDAI after infection with odd ratio 0.9.

Table 5 describes the difference between cases with increased PGA after infection and cases with no increased PGA after infection among seropositive cases of COVID-19 infection. Moreover, cases with increased PGA score after infection had significantly higher level of IgG than cases with no increased PGA after infection with significant association and odds ratio 0.9.

Table 1. Demographic characteristics and medical history of the studied SLE seropositive IgG for COVID-19.

	Total (n = 40)	**Symptomatic (n = 14)**	**Asymptomatic (n = 26)**	**p. value**
Age, years Median (IQR)	12 (9.25-13)	11 (8.75-14)	12 (9.75-13)	0.9
Gender, n (%) Male Female	9 (22.5%) 31 (77.5%)	5 (35.7%) 9 (64.3%)	4 (15.4%) 22 (84.6%)	0.14
Weight (kg) Mean (SD)	41.65 (14.88)	41.57 (22.68)	41.69 (8.84)	0.5
BMI (kg/m^2) Mean (SD)	21.94 (6.13)	23.46 (8.91)	21.13 (3.9)	0.5
Socio-economic level, n (%) Poor Fair Good	6 (15%) 21 (52.5%) 13 (32.5%)	4 (28.6%) 3 (21.4%) 7 (50%)	2 (7.7%) 18 (69.2%) 6 (23.1%)	0.01
Age at onset, years Median (IQR)	10 (8-11)	10 (6.75-13)	10 (8-11)	0.9
Disease duration, years Median (IQR)	1 (1-2)	1.75 (1-2.12)	1 (1-2)	0.3
Hypertension, n (%)	15 (37.5%)	5 (35.7%)	10 (38.5%)	0.9
Nephritis/Nephrotic, n (%)	13 (32.5%)	5 (35.7%)	8 (30.8%)	0.8
Steroid duration, months Median (IQR)	24 (12-24)	24 (12-27)	24 (12-25.5)	0.7
Hydrochloroquine intake (yes), n (%)	25 (62.5%)	9 (64.3%)	16 (61.5%)	0.8

Table 2. Laboratory investigationof the studied SLE seropositive IgG for COVID-19.

	Total (n = 40)	**Symptomatic (n = 14)**	**Asymptomatic (n = 26)**	**P. value**
Hb level Mean (SD)	10.77 (1.55)	10.23 (1.74)	11.06 (1.39)	0.1
WBCs Mean (SD)	7.34 (3.01)	7.38 (3.98)	7.32 (2.44)	0.6
Platelet level Thrombocytopenia, n (%)	29 (72.5%)	11 (78.6%)	18 (69.2%)	0.5
ESR Median (IQR)	30 (20-53.75)	54 (18.25-80)	27.5 (20-36.25)	0.07
Positive CRP, n (%)	11 (27.5%)	8 (57.1%)	3 (11.5%)	0.002
High creatinine level, n (%)	4 (10%)	3 (21.4%)	1 (3.8%)	0.07
ALT Median (IQR)	28 (22-35)	22 (22-35)	30 (23.5-35.75)	0.2
Albumin, g/dL Median (IQR)	3.5 (2.1-3.65)	3.1 (3.02-3.8)	3.5 (3.27-4)	0.01
Anti-dsDNA positive, n (%)	10 (25%)	7 (50%)	3 (11.5%)	0.007
Anti-nuclear antibody positive, n (%)	16 (40%)	9 (64.3%)	7 (26.9%)	0.02
Low C3, n (%)	11 (27.5%)	10 (71.4%)	1 (3.8%)	< 0.001
Low C4, n. (%)	9 (22.5%)	8 (57.1%)	1 (3.8%)	< 0.001
COVID-19 IgG level Median (IQR)	119.75 (53.9-191.4)	119.2 (53.1-181.5)	121.2 (54.2-202.4)	0.6

Table 3. The Physician Global Assessment (PGA) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of the studied SLE seropositive IgG for COVID-19.

	Total (n = 40)	**Symptomatic (n = 14)**	**Asymptomatic (n = 26)**	**p. value**
SLEDAI 1 Median (IQR)	5.5 (4-10)	10.5 (9-13.25)	5 (4-6)	<0.001
SLEDAI 2 Median (IQR)	10 (1-2)	15 (11.5-20)	7 (5-10)	<0.001
Increased SLEDAI, n (%)	31 (77.5%)	10 (71.4%)	21 (80.8%)	0.5
PGA 1 Median (IQR)	2 (1-2)	2 (2-3)	2 (1-2)	<0.001
PGA 2 Median (IQR)	3 (2-3)	3 (2-3)	2 (2-3)	0.2
Increased PGA, n (%)	18 (45%)	5 (35.7%)	13 (50%)	0.4

SLEDAI 1: SLEDAI before COVID-19 infection, SLEDAI 2: SLEDAI after COVID-19 infection, PGA 1: PGA before COVID-19 infection, PGA 2: PGA after COVID-19 infection, Increased SLEDAI: Increase SLEDAI after COVID-19 infection in comparison to before infection. Increased PGA: Increase PGA after COVID-19 infection in comparison to before infection.

Table 4. Factors associated with increased SLEDAI among the studied SLE seropositive IgG for COVID-19.

	Summary statistics (n=40)			**Univariate regression**	
	Patients with increased SLEDAI (n = 28)	**Patients with no increased SLEDAI (n = 12)**	**P-value**	**Crud Odds ratio**	**p. value**
Age at onset, years Median (IQR)	10 (7.25-11)	10 (9.25-11.75)	0.4	1.1	0.5
Disease duration, years Median (IQR)	1 (1-2)	2 (1-2)	0.3	1.1	0.7
Hb level Mean (SD)	10.73 (1.45)	10.9 (1.97)	0.5	1	0.8
WBCs level Mean (SD)	7.28 (2.94)	7.54 (3.45)	0.9	1	0.8
Platelet level Thrombocytopenia, n (%)	21 (75%)	8 (66.7%)	0.6	3.8	0.2
ESR Median (IQR)	30 (20-47)	40 (20-66)	0.5	1	0.2
Positive CRP, n (%)	7 (25%)	4 (33.3%)	0.6	2.7	0.2
High Creatinine level, n (%)	2 (7.1%)	2 (16.7%)	0.4	0.2	0.2
Anti-dsDNA positive, n (%)	5 (17.9%)	5 (41.7%)	0.1	3.2	0.12
Anti-nuclear antibody positive, n (%)	9 (32.1%)	7 (58.3%)	0.1	2.95	0.12
Low C3, n (%)	7 (63.6%)	4 (33.3%)	0.6	0.66	0.6
Low C4, n. (%)	5 (17.9%)	4 (33.3%)	0.3	0.4	0.3
COVID-19 IgG level Median (IQR)	129.9 (94.92-201.9)	52.3 (39.42-134.6)	0.005	0.9	0.01
Steroid duration, months Median (IQR)	18 (12-24)	24 (12-28.5)	0.5	1.02	0.5
Hydrochloroquine intake (yes), n (%)	19 (67.9%)	6 (50%)	0.3	2.6	0.2

Table 5. Factors associated with increased PGA among the studied SLE seropositive IgG for COVID-19.

	Summary statistics (n=40)			**Univariate regression**	
	Patients with increased PGA (n=18)	**Patients with no increased PGA (n=22)**	**P-value**	**Crud Odds ratio**	**p. value**
Age at onset, years Median (IQR)	10 (7-11)	10 (8.75-12.25)	0.2	1.2	0.2
Disease duration, years Median (IQR)	1 (1-2)	1.75 (1-2)	0.6	0.9	0.9
Hb level Mean (SD)	10.83 (1.14)	10.71 (1.85)	0.7	0.9	0.8
WBCs Mean (SD)	7.82 (3.32)	6.95 (2.76)	0.5	0.9	0.4
Platelet level Thrombocytopenia, n (%)	12 (66.7%)	17 (77.3%)	0.5	1.7	0.45
ESR Median (IQR)	24 (12.5-38.75)	35 (20-68.5)	0.06	1.02	0.08
Positive CRP, n (%)	3 (16.7%)	8 (36.4%)	0.2	2.8	0.2
High Creatinine level, n (%)	2 (11.1%)	2 (9.1%)	0.8	0.8	0.83
Anti-dsDNA positive, n (%)	4 (22.2%)	6 (27.3%)	0.7	1.31	0.7
Anti-nuclear antibody positive, n (%)	7 (38.9%)	9 (40.9%)	0.9	1.08	0.9
Low C3, n (%)	4 (22.2%)	7 (31.8%)	0.5	1.63	0.5
Low C4, n. (%)	3 (16.7%)	6 (27.3%)	0.4	1.8	0.4
COVID-19 IgG level Median (IQR)	184.6 (112.45-216.7)	59.8 (42.95-132.5)	< 0.001	0.9	0.001
Steroid duration, months Median (IQR)	24 (12-30)	18 (12-24)	0.4	0.9	0.3
Hydrochloroquine intake, (yes), n (%)	11 (61.1%)	14 (63.6%)	0.9	1.11	0.87

Figure 1. SELDAI before and after COVID-19 infection among seropositive cases.

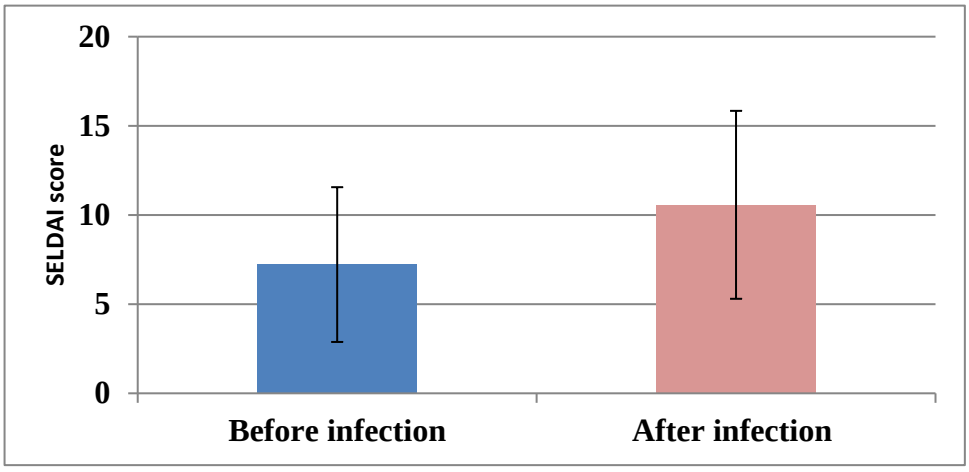
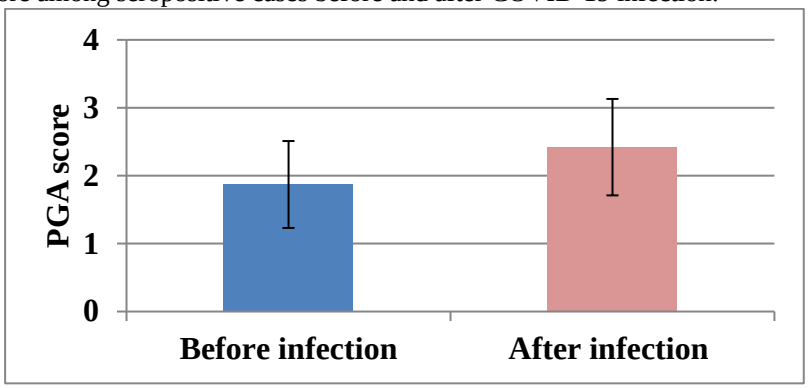


Figure 2. PGA score among seropositive cases before and after COVID-19 infection.



Discussion

Patients with systemic lupus erythematosus (SLE) are considered at higher risk of infections compared with the general population due to impaired immune functions, disease activity, and treatment[17]. This raised the question about whether patients with SLE might be at increased risk for contracting SARS-CoV-2 and a more severe clinical course once infected[18]. This could explain our findings in enrollment of cases at our departments, as we enrolled 42 SLE children diagnosed and followed-up with seropositive IgG for COVID-19 infection. Among those cases, 40 cases were seropositive IgG for COVID-19 infection and two cases were negative.

Our study aimed to evaluate SARS-CoV-2 antibodies in children with SLE and the association between SARS-CoV-2 seropositivity and SLE disease activity. To achieve those aims, a cross sectional study that was conducted among 40 SLE children at Pediatric Allergy, Immunology, and Rheumatology Clinic at the Pediatric Department of Sohag university hospitals and Department of Medical Microbiology and Immunology at Faculty of Medicine, Sohag University, Egypt from 1st March 2022 to 1st January 2023.

The issue of immunity against SARS-CoV-2 is especially significant for individuals with chronic inflammatory disorders due to the potential impact of therapies or the underlying disease on their ability to combat the infection, develop immunity, or react to immunizations. Diagnosing a flare of Systemic Lupus Erythematosus (SLE) after recovering from a COVID-19 infection may be difficult because to the same symptoms and laboratory results of both conditions. The precise mechanism by which COVID-19 infection triggers or exacerbates the severity of lupus nephritis remains unclear. The potential process involves viral infections, such as Epstein Barr Virus or Influenza, which may initiate Systemic Lupus Erythematosus (SLE) or exacerbate its symptoms by molecular mimicry and epitope transmission. This concept has been under consideration for the last four decades[19]. A hypothesis may be formulated based on the direct impact of the virus on the kidney and the indirect impact via the immune response known as cytokine storm, together with the abnormal functioning of the kidney in a patient with systemic lupus erythematosus (SLE). This hypothesis suggests that these factors may contribute to the development or worsening of lupus nephritis[20].

The acceleration of disease activity in our study was assessed by comparing the estimation of SELDAI and PGA score before and after infection. Regarding SLEDAI, the studied cases had significant higher SLEDAI score after infection in comparison to that before infection (10.57 ± 5.27 and 7.22 ± 4.34). Moreover, seropositive IgG of COVID-19 infection cases had significant higher PGA score after infection in comparison to before infection (2.42 ± 0.71 and 1.87 ± 0.64).Our study indicated flare of disease activity after infection which is in line with the results of the study that was done by Mousavi-Hasanzadeh et al who reported that COVID-19 may induce a flare of lupus nephritis among SLE patients [21]. In consistent with the results were obtained by Chattopadhyay et al. who revealed that viral infections are associated with an acceleration of rheumatological disorders [22]. Moreover, our findings were similar to those were obtained by Cheung et al. who retrospectively studied a group of SLE patients with COVID-19 infection and a group of SLE controls who did not have COVID-19 infection and revealed that clinical flares within 90 days were significantly more

common in patients infected with COVID-19 [23]. Also, in agreement with the study that was done by Naddei et al. on juvenile idiopathic arthritis (JIA) and revealed that COVID- 19 lockdown was associated with a higher rate of relapse in children with JIA [24]

Contradictory to our results, a study that was done by Petri et al. who reported there is insignificant change between SELDAI either before and after COVID-19 infection and between PGA either before and after infection[25].The difference can be attributed to prior vaccination by Pfizer and Moderna.COVID-19 vaccines produced satisfactory but impaired humoral response in SLE patients compared to controls and the effect of vaccine was dependent on the immunosuppressive medications use and type of vaccines received [26].

Also, our findings weren't in line with the findings of Sjöwall et al. who reported that clinical disease activity assessed by SLEDAI and PGA remained stable either before or after COVID infection among elderly SLE cases [12].The disparity between Sjowall and his colleagues may be ascribed to variations in the age range of the participants under investigation. They noted a notable decline in three distinct autoantibodies that target extrachromosomal antigens (La/SSB, U1RNP, and Sm/RNP) throughout the pandemic. The observed decreases were statistically significant even after accounting for variations in the technique used, and were not associated with any increased usage of immunosuppression in patients with decreasing antibody levels. However, they observed C3 decreased significantly during the pandemic and a similar trend was observed for C4. The latter usually represents increased activation of the complement pathway following immune complex deposition. If accompanied by positive anti- dsDNA in patients with SLE, this observation is referred to as "serologically active clinically quiescent".

Assessment of severity of SLE disease either before or after infection, in our study symptomatic group show significant higher median (IQR) of SELDAI score in comparison to asymptomatic group before infection [10.5 (9-13.25) and 5 (4-6)]. Also, SELDAI after infection, symptomatic group show significant higher score in comparison to asymptomatic group [15 (11.5-20) and 7 (5-10)].Furthermore, regarding PGA score, before infection symptomatic cases show significant higher score in comparison to asymptomatic cases [2 (2-3) and 2 (1-2)] but after infection there is statically insignificant difference between the studied groups. Our estimates weren't in line with the estimates that were done by Carvalho et al. on cases aged over 18 years, with history of COVID-19 were compared to those without history of COVID-19 and showed insignificant difference between symptomatic and asymptomatic cases according to either SLEDAI before infection [4.6 (7.7) and 4.9 (8.1)]and after COVID-19 infection[5.8 (6.8) and 4.5 (8)] and regarding PGA, there was insignificant difference between cases with history of COVID-19 and cases without history according to PGA before infection while there is significant difference according to PGA after infection[27]. The difference could be explained by change in selected age group which reflect change in immunity status.

The current study indicated that those cases with increased SELDAI score had significant higher level of IgG of COVID-19 in comparison to cases with no increased SELDAI score [129.9 (94.92-201.9) and 52.3 (39.42-134.6)]. Also, regarding univariate regression analysis, there is only significant association between level of IgG of COVID-19 and change of SLEDAI after infection with odd ration 0.9. Our findings weren't in agreement with the findings of Urowitz et al. who indicated by univariate and multivariate regression analysis that there was significant association between age at onset of disease and change of SLEDAI after infection [28]. This could be explained by genetic difference and personal protective habits between Canada and Egypt. Our cases had some sort of lack of knowledge of health protective measurements. Our study assessed disease flare using SELDAI and PGA score. We didn't find any research that assessed disease flare after COVID-19 using PGA score.

Regarding socio-demographic characteristics of the studied cases, there is statistically insignificant difference between symptomatic and asymptomatic cases of seropositive IgG COVID-19 infection regarding socio-demographic characteristics (age and gender) and their physical assessment (BMI). Our findings were in agreement with the findings of Carvalho et al. who reported that there is statistically insignificant difference between cases diagnosed with history of COVID-19 infection and without history of COVID-19 infection according to age, gender and BMI[27]

Regarding laboratory investigation of the studied participants, there is statically significant

difference between the studied symptomatic and asymptomatic cases according to CRP level, 57.1% of symptomatic cases showed positive CRP in comparison to 11.5% of cases. Also, symptomatic cases show significant decrease in median (IQR) of albumin level in comparison to asymptomatic cases [3.1 (3.02-3.8) and 3.5 (3.27-4)] g/dL. Moreover, half of symptomatic cases show positive Anti-dsDNA in comparison to 11.5% of asymptomatic cases with statistically significance. Also, 64.3% of symptomatic cases show positive anti-nuclear antibody in comparison to 26.9% of asymptomatic cases. Regarding to level of C3 and C4, 71.4% of symptomatic cases show low C3 level in comparison to 3.8% of asymptomatic cases and 57.1% of symptomatic cases show low C4 in comparison to 3.8% of asymptomatic cases with statistically significance. There is worsening of anemia and thrombocytopenia as well as elevated ESR of statistically insignificance.

Our results were in agreement with the results that were found by Maram et al. who reported that SLE cases who were infected with COVID-19 complained of low serum albumin, positive ANA, positive anti-dsDNA and low C3,C4 level [29]. The current study findings were in line with Rauf et al. who reported that symptomatic COVID-19 case had high level of CRP and ESR, positive ANA and anti-ds DNA and low C3, C4 but our results showed insignificant association with ESR [30]

This doesn't resemble the findings by Cheung et al. who showed that the changes in anti-dsDNA and complement C3 levels, however, were not significantly different between patients with history ofSARS-CoV2-infected than those without history of infection. [23]

Regarding assessment of antibodies against COVID-19, we prefer to assess IgG than IgM for comparison as IgM is expected to be produced earlier than IgG [31]. Notably, antibodies against SARS-CoV-2 (specifically IgA and IgM) were also found in samples obtained prior to the outbreak of the pandemic. The issue of whether this indicates a non-specific and persistent immune response, exposure to past coronaviruses, or interference with autoantibodies in the immunoassays, is yet unanswered. Given that Systemic Lupus Erythematosus (SLE) is a disorder marked by a wide range of autoantibodies in the bloodstream, it is plausible that this group of patients, as a whole, may have a higher likelihood of producing antibodies that target other antigens, including coronaviruses, even when not infected with COVID-19.Also, according to Sjöwall et al. who indicated that the SARS-CoV-2 IgG isotype was less often found in prepandemic samples and was in addition the only antibody that significantly associated with self-reported symptoms (body temperature >38.5°C) during the pandemic [12]

Our study indicated that there is insignificant difference between severity of symptoms of COVID and level of IgG which is in line with the results were reported by Nakano et al. who conducted the study on 105 participants, including 26 symptomatic COVID-19 patients to investigate the time courses of the anti-SARS-CoV-2 IgM and IgG titers and revealed that IgG remained stable at above 400 AU/mL after Day 13[31] which explain absence of difference between the studied symptomatic and asymptomatic cases. Also, symptomatic cases showed elevated level of IgG than normal level but with insignificant difference with asymptomatic cases which isn't in line with the study of Seow et al. who reported that nearly all participants had waning neutralising antibody levels afterwards, although patients who had more severe symptoms, and were thus likely to generate a more robust initial neutralizing antibody response, generally maintained elevated antibody titres over a follow-up period of up to 94 days [32]. The difference with our results and that of Seow et al. can be attributed to the difference in time of assessment of level of IgG.

Regarding medication intake, our results indicate there is statistically insignificant difference between symptomatic and asymptomatic cases according to steroid intake. This is in line with Carvalho et al. who reported there was insignificant association between history of COVID-19 symptoms and steroid intake[27].

Also,our study indicated that there isn't difference between severity of symptoms with hydroxycholoroquine intake which is in line with the results of Pinheiro et al. who reported that there is much evidence of a non-protective role of chronic hydroxychloroquine use concerning the severity of COVID-19 [33]. Also, our findings were in line with Horby et al., Rodrigo et al., Bozzalla Cassione et al., Gendebien et al.who failed to identify any benefit of HCQ in preventing or managing viral infections, including dengue, chikungunya, and SARS-CoV-2 [34-37].

Similar to our results, the results of D'Silva et al. who conducted the study on 52 rheumatic disease patients with COVID-19 on hydroxycholoroquine treatment and matched these to 104 non-rheumatic disease comparators and showed that patients with and without rheumatic disease had similar symptoms and laboratory findings and a similar proportion of patients with and without rheumatic disease were hospitalised but those with rheumatic disease required intensive care admission and mechanical ventilation more often [38]

Our findings weren't in line with that of Mousavi-Hasanzadeh et al., Gautret et al, Mathian et al.and Ruiz-Irastorza et al. who indicated that the HQC use in SLE patients may decrease the severity of COVID-19-associated pneumonia[21,39-41]. The difference could be explained as there are multiple genomes of corona virus resistant to hydroxycholoroquine, our studied participants may have combined viral and bacterial infection and small sample size of our study.

Strengths of the study included quantitative measurement of COVID-19 IgG level and assessment of SLEDAI and PGA before and after infection. We used two scores for evaluation of disease activity in children with SLE.

Constrains and limitations of the study included the cross-sectional design, small sample size and lack of control group were the main limitations. Also, we only evaluated the humoral and not the cell-mediated immune response to SARS-CoV-2. In our study we focused mainly on the role of covid-19 infection on activity of SLE but role of medications was not of our priority in our study.

Conclusion: SARS-CoV-2 infection may predispose to SLE exacerbation and worsen disease activity that was found through increase of score of PGA and SELDAI after COVID-19 infection. Also, it was found significant difference between severity of disease after infection and level of IgG of COVID-19 infection which highlights the role of COVID-19 in flare of disease activity.

Recommendation: Further studies should be done to investigate the fate of cases and organ dysfunction, severity of COVID-19 infection and to assess level of IgA and IgM of COVID-19. Further studies should be conducted with larger sample size of cases to obtain more clear results. Confirmation of diagnosis of infection of COVID-19 with PCR which make more reliable results.

Data Availability

The data will be available on request through authors themselves.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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