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Up-regulated serum levels of interleukin (IL)-17A and IL-22 in Egyptian pediatric patients with COVID-19 and MIS-C: Relation to the disease outcome

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A R T I C L E   I N F O

A B S T R A C T

Both IL-17A and IL-22 share cellular sources and signaling pathways. They have synergistic action on epithelial cells to stimulate their production of antimicrobial peptides which are protective against infections. However, both interleukins may contribute to ARDS pathology if their production is not controlled. This study aimed to investigate serum levels of IL-17A and IL-22 in relation to the disease outcome in patients with SARS-CoV-2. Serum IL-17A and IL-22 were measured by ELISA in 40 patients with SARS-CoV-2, aged between 2 months and 16 years, (18 had COVID-19 and 22 had multisystem inflammatory syndrome in children "MIS–C") in comparison to 48 age- and sex-matched healthy control children. Patients with COVID-19 and MIS–C had significantly higher serum IL-17A and IL-22 levels than healthy control children (P < 0.001). Increased serum IL-17A and IL-22 levels were found in all patients. Elevated CRP and serum ferritin levels were found in 90% of these patients. Lymphopenia, neutrophilia, neutropenia, thrombocytopenia and elevated ALT, LDH and D-dimer were found in 45%, 42.5 %, 2.5%, 30%, 32.5%, 82.5%, and 65%, respectively of these patients. There were non-significant differences between patients who recovered and those who died or had a residual illness in serum levels of IL-17A, IL-22 and the routine inflammatory markers of COVID-19. In conclusions, serum IL-17A and IL-22 levels were up-regulated in all patients with COVID-19 and MIS–C. Levels of serum IL-17A, IL-22 and the routine inflammatory markers of COVID-19 were not correlated with the disease outcome. Our conclusions are limited by the sample size.

1. Introduction

Cytokine storm has been observed in some COVID-19 patients that may progress to multiple organ dysfunction and death. Thus, the prevention and early treatment of cytokine storm in patients with COVID-19 are important. Measuring levels of serum pro-inflammatory cytokines have many potential applications in COVID-19 management, determination of prognosis and prediction of the response to treatment [1,2].

T helper (Th) 17 cells, a distinct lineage of effector CD4+ T cells, are characterized by the production of interleukin (IL)-17. These cells also express IL-22, at substantially higher amounts than Th1 or Th2 cells. IL-6 enhances the generation of Th17 cells and both IL-6 and IL-17 synergistically promote viral persistence by the protection of the virus-infected cells from apoptosis [3]. Similar to IL-17A, IL-22 expression was initiated by IL-6 and other pro-inflammatory cytokines [4].

IL-17 family of cytokines consists of six ligands from IL-17A to IL-17F. IL-17A is considered to be the effector and the classic cytokine of Th17 CD4+ T cells. IL-17A plays a protective role in the host defense against pathogens through eliciting an acute immune response at the epithelial and mucosal barriers to induce the tissue healing after injury and to maintain the epithelial tight-junction barrier during the process of inflammation [5,6]. However, uncontrolled and excessive production of IL-17A is one of the potential mechanisms underlying autoimmunity, chronic inflammatory conditions and neoplasms [6,7]. Although the mediated responses of IL-17A play a role in the killing of the pathogens via neutrophil recruitment, this may occur at the expense of the tissue damage. This "double-edged sword" paradigm has been involved in the

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lung injury caused by acute respiratory distress syndrome and H1N1 influenza infection [8,9].

IL-22 is a member of the IL-10 family. It mediates its effects via the IL-22 receptor complex which is expressed by non-hematopoietic cell lineages of lung, skin, intestine, liver, pancreas and kidney [10]. IL-22 has a dual role either protective or pathogenic functions during inflammatory, infectious and autoimmune diseases [11]. It has a role in tissue regeneration and regulation of host defense at barrier surfaces. However, IL-22 has also been linked to several conditions involving inflammatory tissue pathology [10].

Despite their protective role against extracellular pathogens and their active role in the regeneration of epithelial barrier organs, IL-17 and IL-22 may be detrimental when they are not tightly regulated. Indeed, these cytokines contribute to the pathology observed in several inflammatory and autoimmune diseases. They induce lung epithelial cells to express chemokines that attract neutrophils to the sites of infection [12]. Tocilizumab, a specific monoclonal antibody that blocks IL-6, has emerged as an alternative treatment for severe or critically ill COVID-19 patients with extensive lesions in the lungs and a confirmed elevated level of IL-6 [2]. A cytokine that may be related to IL-6 in the context of viral infection is IL-17 [13]. The debate whether blocking the other cytokines could reduce COVID-19 impact is growing. Targeting the TH17 pathway may benefit the patients with TH17 dominant immune profiles such as COVID-19 [12].

This study aimed to investigate serum levels of IL-17A and IL-22 in relation to the disease outcome in patients with COVID-19 and multisystem inflammatory syndrome in children (MIS–C).

2. Methods

2.1. Study population

This cohort study was conducted on 18 children with confirmed COVID-19 as defined by the Centre for Disease Control and Prevention (CDC) Case definition for COVID-19 [14,15] and 22 patients with MIS–C [16]. Patients were recruited from the Emergency Department, COVID-19 Isolation Section and Pediatric ICU at Children's Hospital, Ain Shams, Cairo, Egypt. Confirmed cases are defined as cases meeting confirmatory laboratory evidence which is the detection of SARS-CoV-2 RNA, in a clinical specimen, by using the molecular amplification detection test. We diagnosed COVID-19 from the date of first positive COVID-19 PCR swab. Based on clinical data and basic laboratory workup results, the degree of the disease severity was identified [14].

2.1.1. Exclusion criteria

- Patients with chronic inflammatory diseases, rheumatic diseases or other autoimmune disorders.
- Patients who had malignancies.
- Patients on corticosteroid therapy or other immune-modulatory drugs and patients who have received intravenous immunoglobulins.

The patients were compared with 48 age- and sex-matched healthy control children who were recruited from the Outpatients Pediatric Clinic, Faculty of Medicine, Ain Shams University, Cairo, Egypt. They were the healthy siblings of the children attending this clinic. They had no clinical evidence of a recent infection, previous COVID-19 infection, chronic inflammatory diseases or rheumatic disorders.

An informed written consent of participation in this study was signed by the parents or legal guardians of the study subjects. This work was approved by the local Ethical Committee of the Faculty of Medicine, Ain Shams University, Cairo, Egypt.

2.2. Study measurements

Clinical evaluation of the studied children: This was based on:

- Detailed history taking from caregivers including the personal data, history of contact with a COVID positive patient, presence of an underlying chronic illness. Parents were asked about symptoms at disease onset, duration of COVID illness, presence of fever, respiratory symptoms, gastrointestinal symptoms, anosmia, ageusia, skin rash and symptoms of organ dysfunction. Therapeutic interventions in the hospital were recorded.
- Detailed clinical examination was done for the detection of complexion color, temperature, vital signs, and signs of respiratory distress and skin examination for the presence of rash. A complete systemic examination was performed for the detection of organ involvement. O2 saturation in room air was measured by pulse oximetry.
- Outcome assessment: All patients were assessed on discharge from the hospital. They were classified according to their fate into patients with complete cure, patients with a residual illness in one or more of the major organs and those who unfortunately died.

2.2.1. Routine investigations to assess the levels of the routine inflammatory markers of COVID-19

- Complete blood picture (CBC): using coulter counter (Coulter MAXMUG- HL –CCI) and Leishman-stained peripheral blood film examination for differential white blood cell counting with special emphasis on neutrophil and lymphocyte counts.
- C reactive protein (CRP): by using "Latex agglutination test".
- Erythrocyte sedimentation rate (ESR): by using "Westergren Method".
- Lactate dehydrogenase enzyme (LDH): using Synchron CX7 auto-analyzer (Beckman Instruments, Bera, California, USA).
- Liver enzymes and serum creatinine: using Synchron CX7 auto-analyzer (Beckman Instruments, Bera, California, USA).
- Cardiac enzymes: including CK, CK-MB: Separation of CK into iso-enzymes can be accomplished by techniques like electrophoresis, column chromatography, or radioimmunoassay (ELISA). Troponin-I was measured by ELISA (if needed).
- D-Dimer assay: it is a monoclonal antibody based using ELISA.
- Serum ferritin level: using Synchron CX7 autoanalyzer (Beckman Instruments, Bera, California, USA).

2.2.2. Imaging studies

- Echocardiography: to assess the possibility of endocarditis, myocarditis, pericarditis, valvular lesions, coronary vasculitis, or any other changes related to COVID-19 or MIS-c.
- CT scanning of the chest: The findings were classified according to COVID-19 Reporting and Data System (CO-RADS)**.**

2.2.3. Assessment of serum concentrations of 17A and IL-22

Sampling: Samples were taken on the second day of presentation immediately after being confirmed as COVID-19 or MIS-C. Two ml whole blood samples were collected from all subjects. After clotting, samples were centrifuged for 20 min at approximately 1000*g*. Sera were separated and stored at −20 °C until time of assay.

Principle of the assay: Serum concentrations of 17A and IL-22 were measured by using commercially supplied ELISA kits (Wuhan Fine Biotech Co., Ltd.). Principally, a capture antibody is pre-coated onto 96-well plates. Biotin conjugated antibodies are used as detection antibodies.

Method of assay: At time of assay, samples were thawed to room temperature, vortexed and diluted as per the manufacturer's instructions; using the supplied dilution buffer, control samples were diluted 1:2 and patients' samples were diluted 1:5. The standards, diluted test samples and biotin conjugated detection antibody were subsequently added to the wells and washed with wash buffer after

incubation. Then, HRP-Streptavidin was added and the unbound conjugates were washed away with a wash buffer. The TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by using HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow color was proportional to the target amount of sample captured in plate. The O.D. absorbances of standards and samples were read at 450 nm in a microplate reader, and then the concentration of target in each sample was calculated by constructing a standard curve via plotting the O.D. at 450 nm of each standard solution on Y axis versus the respective concentration of the standard solution on X axis, then, target concentrations of samples were interpolated from the standard curve. The concentrations from interpolation were multiplied by the dilution factors to obtain the concentrations of targets in samples before dilution.

2.3. Statistical analysis

The results were analyzed by using the available software package (Statview, Abacus concepts, inc., Berkley, CA, USA). The parametric data were presented as mean and standard deviation (SD). The non-parametric data were presented as median and interquartile range (IQR) that is between the 25th and 75th percentiles. The parametric data was compared by using the Student's *t*-test, while the Mann–Whitney test was used to compare the non-parametric data. Chisquare test was used for comparison between qualitative variables of the studied groups. Spearman's rho correlation coefficient "r" test was used to determine the relationship between the all different variables. A probability (P) value of less than 0.05 was considered to be significant for all the tests. Patients were considered to have elevated serum levels of IL-17A and IL-22 if their levels were above the calculated highest cut-off values (the 95th percentiles of the healthy controls).

3. Results

Patients comprised 20 females and 20 males. Eighteen children had confirmed COVID-19 and 22 patients had MIS–C. Their ages ranged between 2 months and 16 years (mean ± SD = 6.5 ± 5.5 years). The control group included 24 females and 24 males. Their ages ranged between 2 months and 16 years (mean ± SD = 7.01 ± 4.1 years).

Five patients had moderate SARS CoV-2 and 35 patients had severe disease. In addition, two patients with SARS CoV-2 were presented without organ dysfunctions and 38 patients were presented with single or multiple organ dysfunctions in the form of cardiac, respiratory, gastrointestinal or neurological dysfunctions.

Regarding the outcome of the disease, 29 patients recovered (13 had COVID-19 and 16 had MIS–C), six patients died (2 had COVID-19 and 4 had MIS–C) and 5 patients had a residual illness (3 had COVID-19 and 2 had MIS–C) in the form of thyroid dysfunction, ophthalmological problems and neurological dysfunction (Table 1).

All patients with COVID-19 and MIS–C had significantly higher values of serum IL-17A and IL-22 than healthy control children (Table 2).

Patients were considered to have elevated serum values of IL-17A and IL-22 if their levels were above 546 ng/L and 450 ng/L, respectively which were the calculated highest cut-off values (the 95th percentiles of the healthy controls).

Increased values of serum IL-17A and IL-22 were found in all patients with COVID-19 and MIS–C. According to the pediatric normal reference range for age, elevated CRP and serum ferritin were found in 90% of these patients. Lymphopenia, neutrophilia, neutropenia, thrombocytopenia and elevated ALT, LDH and D-dimer were found in 45%, 42.5 %, 2.5%, 30%, 32.5%, 82.5%, and 65%, respectively of the studied patients (Table 3).

There were non-significant differences between patients who recovered and those who died or had a residual illness in serum levels of IL-17A, IL-22 and the routine inflammatory markers of COVID-19

Table 1
Basic clinical and laboratory data of the studied patients.

		All patients (n = 40)	Patients with COVID-19 (n = 18)	Patients with MIS-C (n = 22)
Age (years)	Range, Median (IQR)	2 month-16 yr, 7 (8.75)	2 month-16 yr, 6.50 (11.51)	10 month-14 yr, 7 (7.50)
Sex	Female Male	20 20	8 10	12 10
Outcome	Recovered Residual illness Death	29 5 6	13 3 2	16 2 4
TLC ($\times10^3$/uL)	Range, Median (IQR)	(2.6–32.7), 11.9 (10)	(2.6–26.8), 12.4 (10.8)	(2.8–32.7), 11.85 (8.7)
ANC ($\times10^3$/uL)	Range, Median (IQR)	(1–30), 2.65 (4)	(1–20), 7.05 (7)	(2–30), 7 (8)
ALC ($\times10^3$/uL)	Range, Median (IQR)	(0.2–16), 2.65 (4)	(1–16), 4.35 (5)	(0.2–11), 1.90 (3)
Hemoglobin (g/dl)	Range, Median (IQR)	(4–14), 10.95 (2)	(4–14), 11.00 (3)	(7–13), 10.95 (2)
Platelets ($\times10^3$/uL)	Range, Median (IQR)	(47–618), 200.5 (235)	(59–618), 213 (331)	(47–395), 190.50 (173)
CRP titer (mg/l)	Range, Median (IQR)	(2.5–423), 116.5 (198)	(6–290), 50 (158.9)	(2.5–423), 119 (218.3)
Ferritin (ng/ml)	Range, Median (IQR)	(33–4800), 367.5 (295)	(33–784), 233 (259)	(154–4800), 420 (394)
ALT (IU/L)	Range, Median (IQR)	(3–238), 25 (53)	(5–238), 19 (22)	(3–163),.35.5 (91)
Creatinine (mg/dL)	Range, Median (IQR)	(0.2–3.8), 0.7 (0.4)	(0.2–1.5), 0.6 (0.5)	(0.4–3.8), 0.7 (0.35)
LDH (IU/L)	Range, Median (IQR)	(3–1833), 366 (202)	(167–1833), 361.50 (206)	(3–1244), 397 (213)
D-dimer (ug/mIF)	Range, Median (IQR)	(0.4–19.8), 3.05 (4.5)	(0.4–16.5), 3.050 (2.6)	(0.6–19.8), 3.150 (21)
ESR (mm/hr)	Range, Median (IQR)	(10–110), 30 (30)	(10–110), 27.50 (30)	(11–95), 31.5 (45)
CK-T (IU/L)	Range, Median (IQR)	(14–12731), 70.50 (141)	(14–930), 74 (84)	(15–12731), 61 (202)
CK-MB (U/L)	Range, Median (IQR)	(10–353), 23 (19)	(13–210), 20.50 (16)	(10–353), 23 (25)
Troponin I (ng/mL)	Range, Median (IQR)	(0.001–1.3), 0.02 (0.054)	(0.001–0.6), 0.009 (0.029)	(0.001–1.3), 0.040 (0.094)

ALC: absolute lymphocytic count, ALT: Alanine transaminase, ANC: absolute neutrophil count, CK-MB: creatinine kinase-MB, CK-T: creatinine kinase- total, COVID 19: Corona virus disease 2019, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, IQR: Interquarantine rande, LDH: Lactate dehydrogenase, MIS–C: Multisystem inflammatory syndrome in children, TLC: total leucocytic count.

(Tables 4 and 5).

There were non-significant differences between patients who had COVID-19 and those who had MIS–C in serum levels of IL-17A and IL-22 (P > 0.05).

There were significant positive correlations between serum levels of IL-17A and IL-22 in the studied patients (Fig. 1).

Table 2
Comparison between the studied patients and healthy control children in serum levels of IL-17A and IL-22.

	IL-17A (ng/L) Range, Median (IQR)	Z (P-value)	IL-22 (ng/L) Range, Median (IQR)	Z (P-value)
All patients (n = 40)	(750–7200), 2730 (1980)	8.053 (<0.001)	(840–4950), 2025 (2018)	8.068 (<0.001)
Healthy controls (n = 48)	(60–840), 150 (113)		(75–525), 195 (90)	
Patients s with COVID-19 (n = 18)	(750–5400), 3000 (1950)	6.234 (<0.001)	(840–4950), 2400 (2040)	6.261 (<0.001)
Healthy controls (n = 48)	(60–840), 150 (113)		(75–525), 195 (90)	
Patients with MIS–C (n = 22)	(1200–7200), 2730 (2145)	6.706 (<0.001)	(840–4200), 1800 (2108)	6.717 (<0.001)
Healthy controls (n = 48)	(60–840), 150 (113)		(75–525), 195 (90)	

P < 0.001: highly significant.

Table 3
Percentage of the abnormalities of the levels of routine inflammatory markers of COVID-19, IL 17A and IL22 in the studied patients.

The inflammatory marker	All patients (n = 40)	Patients with COVID-19 (n = 18)	patients with MIS–C (n = 22)
Lymphopenia	45%	27%	59%
Neutropenia	2.5%	5.5%	0 %
Neutrophilia	42.5 %	38.5 %	45.5 %
Thrombocytopenia	30%	16.6%	40.9%
Elevated CRP	90%	88.8%	90.9%
Elevated Ferritin	90%	88.8%	100%
Elevated ALT	32.5%	16.6%	45.4%
Elevated LDH	82.5%	61.1%	100%
Elevated D-dimer	65%	61.1%	68.1%
Elevated IL-17A	100%	100%	100%
Elevated IL-22	100%	100%	100%

ALT: Alanine transaminase, C-reactive protein, IQR: Interquarantine rande, LDH: Lactate dehydrogenase.

Table 4
Comparison between patients who recovered and those patients who died in serum levels of the studied inflammatory markers of COVID-19.

		Cured Patients (n = 29)	Patients who died (n = 6)	(P-value)
TLC ($\times10^3$/uL)	Range, Median (IQR)	(2.6–32), 11.80 (8.9)	(6.4–32.7), 16 (16.3)	0.182
ANC ($\times10^3$/uL)	Range, Median (IQR)	(1–22), 6.70 (7)	(4–30), 8.85 (16)	0.274
ALC ($\times10^3$/uL)	Range, Median (IQR)	(1–16), 2.60 (5)	(2–11), 3.35 (4)	0.584
Hemoglobin (g/dl)	Range, Median (IQR)	(7–14), 11 (2)	(4–12), 10.85 (3)	0.273
Platelets ($\times10^3$/uL)	Range, Median (IQR)	(47–618), 173 (207)	(59–382), 263 (205)	0.431
CRP titer (mg/l)	Range, Median (IQR)	(6–423), 118 (195)	(2.5–370), 21.250 (292.4)	0.284
Ferritin (ng/ml)	Range, Median (IQR)	(33–4800), 369 (388)	(154–432), 386 (202)	0.710
ALT (IU/L)	Range, Median (IQR)	(3–238), 25 (46)	(12–140), 61 (76)	0.155
Creatinine (mg/dL)	Range, Median (IQR)	(0.20–3.80), 0.70 (0.40)	(0.50–3.60), 0.80 (1.23)	0.354
LDH (IU/L)	Range, Median (IQR)	(167–1833), 365 (179)	(3–1244), 588 (829)	0.054
D-dimer (ug/mIF)	Range, Median (IQR)	(0.4–19.8), 2.60 (4.1)	(1.8–9.5), 5 (23.6)	0.161
ESR (mm/hr)	Range, Median (IQR)	(10–95), 25 (29)	(15–70), 61 (40)	0.060
CK-T (IU/L)	Range, Median (IQR)	(15–930), 65 (104)	(57–12731), 150.50 (3310)	0.092
CK-MB (U/L)	Range, Median (IQR)	(10–210), 23 (17)	(20–353), 74.50 (149)	0.039
Troponin I (ng/mL)	Range, Median (IQR)	(0.001–0.600), 0.020 (0.053)	(0.001–0.210), 0.0205 (0.105)	0.861
IL-17A (ng/L)	Range, Median (IQR)	(750–7200), 2760 (2400)	(1200–3660), 2850 (1515)	0.430
IL-22 (ng/L)	Range, Median (IQR)	(840–4950), 2100 (2250)	(1080–4500), 1950 (1823)	0.965

ALC: absolute lymphocytic count, ALT: Alanine transaminase, ANC: absolute neutrophil count, CK-MB: creatinine kinase-MB, CK-T: creatinine kinase- total, COVID 19: Corona virus disease 2019, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, IQR: Interquarantine rande, LDH: Lactate dehydrogenase, TLC: total leucocytic count.
P > 0.05: non-significant.

4. Discussion

Beside the immune responses of Th1 and Th2 cells, a role of Th17 cells has been emerged in the pathogenesis of the inflammatory and autoimmune disorders. Th17 cells produce IL-17A, IL-17F, Il-21 and IL-22, which have a great role in the orchestration of the inflammatory response [7,17].

In the current study, patients with COVID-19 and patients with MIS–C had significantly higher values of serum IL-17A and IL-22 than healthy control children. COVID-19 patients have increased serum levels of plasma pro-inflammatory cytokines. Th1-Th17 cells are appearing to be the main source [13]. Clinical reports showed that both the mild and the severe forms of COVID-19 result in up-regulation of the cytokine secretion, particularly IL-1β, IL-6, IL-10 and IL-17 [18]. A recent study conducted on 63 adult patients with COVID-19 reported a significant increase of the serum levels of IL-17 in the patients than the levels observed in the studied 33 control group [19].

During the rapid progression phase of COVID-19, the immune and inflammatory responses induce a severe cytokine storm due to the release of cytokines such as IL-6, IL-7, IL-22, IL-17 and many others [20]. The expression of both IL-17A and IL-22 is initiated by IL-6 [3,4] which is increased in patients with COVID-19 [21]. Measurement of inflammatory markers may help in the diagnosis, evaluation of the severity and monitoring the treatment of COVID-19 [22]. Immune dysfunction, especially cytokine storm and lymphopenia, in some patients with COVID-19 is a fatal factor for these patients [23,24]. The elevated levels of inflammatory cytokines in COVID-19 patients is associated the decreased number and the increased exhaustion of lymphocytes [25,26].

Table 5
Comparison between patients who recovered and those patients with a residual illness in serum levels of the studied inflammatory markers of COVID-19.

		Cured Patients (n = 29)	Patients with a residual illness (n = 5)	(P-value)
TLC ($\times 10^3$/uL)	Range, Median (IQR)	(2.6–32), 11.80 (8.9)	(4.3–14.4), 6.40 (7.2)	0.076
ANC ($\times 10^3$/uL)	Range, Median (IQR)	(1–22), 6.70 (7)	(3–9), 3.70 (6)	0.233
ALC ($\times 10^3$/uL)	Range, Median (IQR)	(1–16), 2.60 (5)	(0.2–4), 1.30 (3)	0.173
Hemoglobin (g/dl)	Range, Median (IQR)	(7–14), 11 (2)	(10–13), 12 (2)	0.751
Platelets ($\times 10^3$/uL)	Range, Median (IQR)	(47–618), 173 (207)	(163–422), 242 (197)	0.215
CRP titer (mg/l)	Range, Median (IQR)	(6–423), 118 (195)	(27–243), 119 (182.5)	0.846
Ferritin (ng/ml)	Range, Median (IQR)	(33–4800), 369 (388)	(140–689), 323 (332)	0.752
ALT (IU/L)	Range, Median (IQR)	(3–238), 25 (46)	(7–39), 20 (23)	0.368
Creatinine (mg/dL)	Range, Median (IQR)	(0.20–3.80), 0.70 (0.40)	(0.50–1.00), 0.60 (0.35)	0.712
LDH (IU/L)	Range, Median (IQR)	(167–1833), 365 (179)	(179–549), 340 (296)	0.734
D-dimer (ug/mIF)	Range, Median (IQR)	(0.4–19.8), 2.60 (4.1)	(1.4–11), 2.50 (5.1)	0.971
ESR (mm/hr)	Range, Median (IQR)	(10–95), 25 (29)	(10–110), 17 (58)	0.609
CK-T (IU/L)	Range, Median (IQR)	(15–930), 65 (104)	(14–251), 65 (140)	0.983
CK-MB (U/L)	Range, Median (IQR)	(10–210), 23 (17)	(13–124), 16 (58)	0.381
Troponin I (ng/mL)	Range, Median (IQR)	(0.001–0.600), 0.020 (0.053)	(0.001–1.300), 0.003 (0.679)	0.368
IL-17A (ng/L)	Range, Median (IQR)	(750–7200), 2760 (2400)	(2160–3900), 2700 (1470)	0.575
IL-22 (ng/L)	Range, Median (IQR)	(840–4950), 2100 (2250)	(1350–3660), 2760 (1980)	0.733

ALC: absolute lymphocytic count, ALT: Alanine transaminase, ANC: absolute neutrophil count, CK-MB: creatinine kinase-MB, CK-T: creatinine kinase- total, COVID 19: Corona virus disease 2019, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, IQR: Interquarantine rande, LDH: Lactate dehydrogenase, TLC: total leucocytic count.
P > 0.05: non-significant.

IL-17A has been shown to play a protective role in host defense against pathogens through eliciting acute immune response at epithelial and mucosal barriers, to take part in tissue healing after injury. However, uncontrolled and excessive production of IL-17A is one of the mechanisms that have a key role in inflammatory conditions [6]. The up-regulated alveolar and circulating levels of interleukin-17A are associated with the increased percentage of alveolar neutrophils, alveolar permeability, and organ dysfunction in acute and chronic airway inflammation and in acute respiratory distress syndrome [9]. The IL-17 receptor antagonist knockout mice recruited fewer number of neutrophils to the airway in response to influenza A virus with a decreased

mortality. Thus, targeting IL-17 in acute lung injury due to viral infection could be beneficial [27].

IL-22, in collaborate with IL-17 and TNFα, is known to induce antimicrobial peptides in the mucosal organs. However, IL-22 may induce inflammatory responses [10]. IL-22 up-regulates mucins, fibrinogen, anti-apoptotic proteins, serum amyloid A, and LPS binding protein [28]. Therefore, IL-22 may contribute to the formation of life-threatening edema enriched with mucins and fibrin, seen in COVID-19 [29].

This study revealed increased values of serum IL-17A and IL-22 in all patients with COVID-19 and MIS–C. Elevated CRP and serum ferritin were found in 90% of these patients. Lymphopenia, neutrophilia, neutropenia, thrombocytopenia and elevated ALT, LDH and D-dimer were found in 45%, 42.5 %, 2.5%, 30%, 32.5%, 82.5%, and 65%, respectively of the all studied patients. Thus, serum IL-17A and IL-22 were the only inflammatory markers that were abnormally increased in the all studied patients. TH17 type response may contribute to the cytokine storm in some patients with pulmonary viral infection, including COVID-19, which results in tissue damage and likely promotes pulmonary edema [12].

In this work, there were non-significant differences between the patients who had COVID-19 and those who had MIS–C in serum levels of IL-17A and IL-22. Thus, both cytokines were elevated to the same extent in COVID-19 and MIS–C and the inflammatory response was the same in both diseases.

Despite the necessity, no reliable indicators are yet available to predict the disease progression and outcome in patients with COVID-19 infection [30]. The current study revealed non-significant differences between patients who recovered and those who died or had a residual illness in serum levels of IL-17A, IL-22 and the routine inflammatory markers of COVID-19. Thus, none of the studied inflammatory markers proved to be effective in the prediction of COVID-19 outcome. Our conclusions are limited by the sample size.

Infection is a complex series of interaction between the human host, the microbe and the environment. Host genomic variability has been linked to complications associated with infections. In COVID-19 pandemic, the identification of the host genomic factors that increase the resistance or the susceptibility to COVID-19 and translation of these findings to improve the patient care should be the goal [31]. Thus, genetic factors may determine the outcome of the disease rather than the level of the inflammatory markers. This may explain the lack of association between the studied inflammatory markers and the outcome of COVID-19 infection in this study.

In contrast to our study, elevated levels of serum IL-17A were reported to be associated with the disease severity and progression [32]. Although the inflammatory markers are considered non-specific in patients with COVID-19 infection, serum levels of IL-6 and CRP were reported to predict the outcome in patients with COVID-19 infection [33]. Significantly elevated white blood cell count and acute-phase reactants, including C-reactive protein, ferritin, serum amyloid A, and procalcitonin and marked lymphopenia were also reported as sensitive markers that may be used to predict disease severity, outcome, and mortality in patients with COVID-19 infection [34]. A *meta*-analysis suggested that elevated procalcitonin, CRP, D-dimer, and LDH and decreased albumin can be used for predicting severe outcomes in COVID-19 [35]. We couldn't trace data in literature regarding the relationship between elevated levels of serum IL-22 and the disease outcome in patients with COVID-19 to compare our results. The contradiction between the results of this study and the other studies may be attributed to the variations in the number and the age of the studied patients and the host genomic variability.

The current study revealed significant positive correlations between serum levels of IL-17A and IL-22 in patients with COVID-19 and MIS-C. This may be explained by the sharing of both cytokines with the cellular sources, signaling pathways, and some functional aspects. Both IL-17 and IL-22 are considered to be tissue-signaling cytokines that favor the protection and the regeneration of the epithelial barrier organs such as
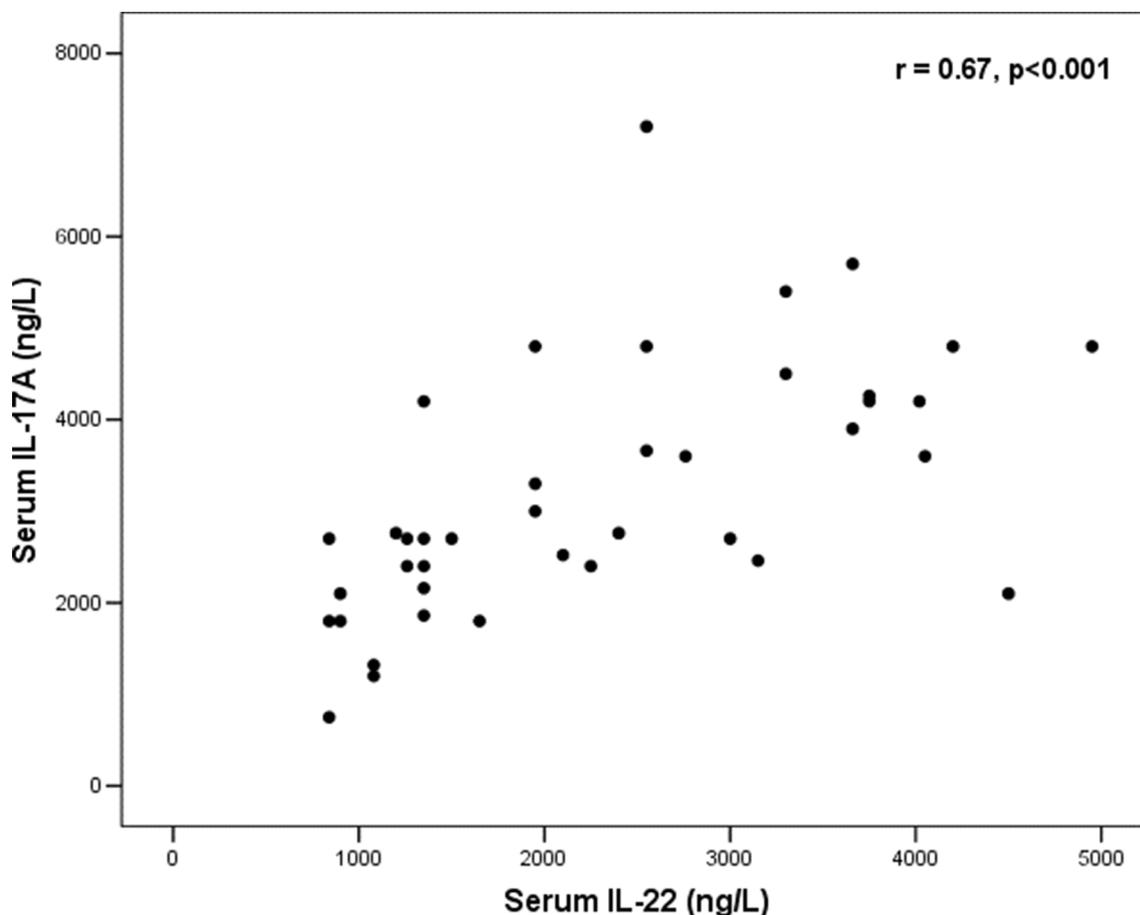
Fig. 1. A significant positive correlation between serum levels of IL-17A and IL-22 in patients with COVID-19 and MIS–C.

the lung, skin and gastrointestinal tract. IL-17 and IL-22 play essential roles in host defense against microbes and in the development of acute and chronic inflammatory diseases. Both cytokines enhance basic innate barrier defenses at mucosal surfaces and have a role in the rapid response to infectious agents, by recruiting neutrophils and by inducing the production of antimicrobial peptides that precede adaptive immunity [36–38].

Limitation of this study is the small number of the studied children. So, further studies, on large scales, that investigate the relationship between SARS-CoV-2 severity and outcome and both IL-17A and IL-22 are required.

5. Conclusion

Serum IL-17A and IL-22 levels were up-regulated in all patients with COVID-19 and MIS–C. Values of serum IL-17A, IL-22 and the routine inflammatory markers of COVID-19 were not correlated with the disease outcome. Our conclusions are limited by the sample size. The role of targeting TH17 pathway in patients with COVID-19 should be studied.

Authors' contributions

Gehan A. Mostafa: Conceptualization, Data Curation, Supervision, Writing the original draft, Writing the final version and editing. **Hanan M. Ibrahim:** Supervision, Data Curation. **Abeer A. Shehab:** Data Curation, Investigation. **Sondos M. Magdy:** Data Curation, Sharing in Writing the final version. **Nada A. Soliman:** Data Curation, Investigation. **Dalia F. El-Sherif:** Data Curation, Sharing in Writing the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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