Original Research Article

Assessment of Quantitative Serum SARS-CoV-2-IgM Antibodies in Febrile Children and It`s Relation to Radiological Findings in Tanta University Hospital

Abstract

Background: Serological testing is urgently required since COVID-19 is the pandemic that is spreading the fastest in recent times, Although RT-PCR is an effective and specific method for diagnosing acute patients, serological tools are urgently required for examining antibody responses and evaluating both individual and prospective herd immunity. The aim of this study was divided into primary objectives were to assess serum IgM antibodies for SARS-Cov-2 in febrile children attending ER in Tanta University Hospital and secondary objectives were to assess computed tomography (CT) findings in febrile SARS-Cov-2 IgM antibody-positive individuals.

Methods: This cross-section study was carried out on sixty children presented by fever with any respiratory symptom as cough and dyspnea and fever with non-respiratory and cutaneous symptoms. The patients were divided into three equal groups: group 1: included healthy children, group 2: included febrile children with respiratory symptoms as cough and dyspnea and group3: included febrile children with fever alone or with non-respiratory symptoms as Gastrointestinal symptoms as vomiting and diarrhea, cutaneous manifestations as rash, and CNS manifestations.

Results: IgM were significantly higher in group II compared to other groups, significantly higher in group III compared to group I (P value <0.001).CO-RADS 2,4 and 5 were significantly higher in group II compared to other groups, CO-RADS 3 was insignificantly

different between groups II and III. Patients with positive CXR at time of presentation were significantly higher in group II compared to other groups. (P value 0.005).

Conclusions: In children with COVID-19, Serum IgM to SARS-COV-2 was significantly higher in febrile children in Tanta University during the period from March 2021 to February 2022. According to CT findings, CO-RADS 2,4 and 5 were significantly higher in febrile patients with positive SARS-Cov-2 serum Igm Ab.

Keywords: Serum SARS-CoV-2-IgM, Febrile Children, Radiological Findings.

Introduction:

The 2019 coronavirus disease (COVID-19) is a contagious illness brought on by the most current coronavirus to be identified. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), It is a highly contagious virus and most individuals within the population are susceptible to the infection. It is transmitted via respiratory droplets and direct contact [1].

"The WHO on March 11, 2020, has declared the (COVID-19) outbreak a global pandemic" [2].

"The coronavirus disease 2019 has occurred in children, but they seemed to have a milder disease course and better prognosis than adults. Deaths were quite uncommon" [3].

The method of transmission is through intimate contact with family members or a history of exposure to the epidemic area, and both exposures in some patients [4].

Pediatric cases of COVID-19 are either asymptomatic cases or symptomatic. Fever is the commonest symptom followed by cough then rhinorrhea or pharyngeal congestion and less frequent diarrhea and sore throats. Other symptoms, including fatigue, headache and dizziness are rare [5].

"Patients with pneumonia had higher proportion of fever and cough and increased inflammatory biomarkers than those without pneumonia" [6].

Despite the fact that RT-PCR is an effective and specific method for diagnosing acute patients, the necessity for serological testing is particularly important given that COVID-19 is the pandemic that is spreading the fastest in modern times, additionally, we urgently require serological methods for monitoring antibody responses and evaluating both individual and possible herd immunity. [7].

"Sensitivity for the detection of IgG antibodies 14–25 days after the onset of symptoms is more than 92.1% for lateral flow assays (LFAs) rapid test compared to 89.5% for the IgG the Enzyme-Linked Immunosorbent Assay (ELISA)". [8].

"The early antibody response known as an IgM response starts and peaks within 7 days, and IgM persists as long as the acute phase of the disease does. In the course of COVID-19, an increase in virus-specific IgM during the acute phase, followed by an increase in virus-specific IgG during subsequent phases, has been noted" [9].

"There are other lab modalities can elevate suspicion for COVID-19 in both children and the close contact family as Lymphopenia was commonly observed at admission but did not differ significantly between those with and without severe disease". [10].

The aim of this study was divided into primary objectives were to assess serum IgM antibodies for SARS-Cov-2 in febrile children attending ER in Tanta University Hospital and secondary objectives were to assess computed tomography (CT) findings in febrile patients who have an IgM SARS-Cov-2 positive test result.

Patients and Methods:

This cross-section study was carried out on sixty children aged from 2 to15 years and presented by fever with any respiratory symptom as cough and dyspnea and fever with non-respiratory symptoms as gastrointestinal symptoms as vomiting, diarrhoea etc., cutaneous as rash and CNS manifestations or fever without focus presenting at ER. They attended at pediatric emergency room (ER) at pediatric department at Tanta University Hospital during the period from March 2021 to February 2022.

Exclusion criteria were children with confirmed COVID-19, chronic pulmonary diseases, age is less than 2 years or more than 15 years old, history of autoimmune disease, and history of chronic illness.

The patients were divided into three equal groups: group 1: included healthy children, group 2: included febrile children with respiratory symptoms as cough and dyspnea and group3: included febrile children with fever alone or with non-respiratory symptoms as

Gastrointestinal symptoms as vomiting and diarrhoea, cutaneous manifestations as rash, and CNS manifestations.

The clinical criteria for diagnosing COVID-19 in children according to latest systematic reviews and meta-analysis including the following in symptomatic patients: fever, respiratory symptoms (history, examination, investigation [radiological investigations: chest X. ray and CT chest and lab investigation: COVID -19 Ig M and CRP], non-respiratory symptoms

Non-respiratory symptoms

GIT symptoms (history, examination, investigation (lab investigation [COVID -19 Igm and CRP], radiological investigations [chest X. ray and CT chest if IgM +ve or +ve finding in chest X-ray]), cutaneous symptoms and CNS or Neurological symptoms (history, examination, investigation (lab investigation [COVID -19 Igm and CRP], radiological investigations [chest X. ray and CT chest if IgM +ve or +ve finding in chest X-ray]).

Laboratory investigations

COVID-19 quantitative serological test IgM antibodies detection in all children.

Name of the test use: iFlash Immunoassay Analyzer SARS-CoV-2 IgM (2019- nCoV IgM) or REF: C86095M

Method of use

The Pro-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RUS).

A direct relationship exists between the amount of SARS-CoV2 IgM antibody in the sample and the RLUS detected by the Flash optical system.

Results are determined via a calibration curve, which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent QR code.

The Flash-SARS-CoV-2 IgM assay is an indirect two-step immunoassay, first incubation: Anti-SARS-CoV-2 IgM in the sample, sample pretreatment solution and SARS-CoV-2

antigen-coated paramagnetic micro particles react to form a complex, under magnetic field, magnetic particles are absorbed to the inner wall of reaction tubes and the unbound materials are washed away from the solid phase in a magnetic field.

Second incubation: Acridinium-labelled anti-human IgM, conjugate is added for further reaction to form a new complex, the pre- Trigger and Trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units, a direct relationship exists between the amount of SARS -COV-2 IgM antibody in the sample and the RULs detected by iFlash optical system.

Results are determined via a calibration curve, which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent QR code.

Statistical analysis

Data was analyzed using Statistical Program for Social Science (SPSS) version 20.0 Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. We used the following tests: A one-way analysis of variance (ANOVA) when comparing between more than two means. Chi-square (X2) test of significance was used in order to compare proportions between two qualitative parameters. Parametric scale variables were analysed by independent sample t test, and nonparametric scale variables were analysed by Mann-Whitney U test. A two tailed P value < 0.05 was considered significant.

Results:

Age, sex and history of confirmed COVID exposure were insignificantly different in among the three groups. Temperature, O2 Saturation and SARS-COV2 IgM were significantly different among three groups (P value = <0.001). Temperature was insignificantly different between group II and group III. O2 Saturation was significantly higher in group I than group II and group III and was significantly lower in group II than group III. Patients with positive

IgM were significantly higher in group II compared to other groups, significantly higher in group III compared to group I (P value <0.001) Table 1

Table 1: Patients' characteristics, examinations SARS-COV2 IgM in the studied groups

		Groups			P value		
		Group I	Group II	Group III			
Age (years)		8.250 ± 4.876	5.950 ± 3.486	5.900 ± 2.845	0.095		
Sex	**Female**	5 (25.00%)	6 (30.00%)	11 (55.00%)	0.108		
	Male	15 (75.00%)	14 (70.00%)	9 (45.00%)			
History of Confirmed COVID Exposure	**Negative**	16 (80.00%)	10 (50.00%)	13 (65.00%)	0.138		
	Positive	4 (20.00%)	10 (50.00%)	7 (35.00%)			
Temperature		37.315±0.203	38.760±0.664	38.740±0.763	<0.001*	I&II	<0.001*
						I&III	<0.001*
						II&III	0.994
O2 Saturation on room air		96.850±1.631	88.950±4.883	92.550±5.735	<0.001*	I&II	<0.001*
						I&III	0.009*
						II&III	0.035*
Positive IgM		0 (0.00%)	12 (60.00%)	6 (30.00%)	<0.001*		

Data are presented as mean ± SD or frequency (%), *: significant as P value ≤ 0.05,

Table 2: Respiratory distress at time of presentation and chest examination in group II and non-respiratory symptoms in group III

Respiratory distress at time of presentation in group II	
I	6(30.00%)
II	4(20.00%)
III	6(30.00%)
IV	2(10.00%)
Chest examination in group II	
Free	8(40.00%)
Crepitation	6(30.00%)
Diminished air entry	2(10.00%)
Rhonchi	1(5.00%)
Mixed	3(15.00%)
Non-Respiratory Symptoms in group III	
GIT	11(55.00%)
CNS	2(10.00%)
Skin	1(5.00%)
Fever without focus	6(30.00%)

Data are presented as frequency (%), GIT: Gastrointestinal tract, CNS: Central nervous system.

CRP was insignificantly different between group II and group III. Patients with positive CXR at time of presentation were significantly higher in group II compared to other groups. (P value 0.005) Table 3

Table 3: CRP, Chest X-ray at time of presentation in group II and III

	Groups		P value
	Group II	Group III	
Positive CRP	15 (75.00%)	16 (80.00%)	0.705
Chest X-ray at time of presentation	17 (85.00%)	8(40.00%)	0.003*

Data are presented as frequency (%), *: significant as P value ≤ 0.05, CRP: C-reactive protein, CXR, Chest X-ray

CT (CO-RADS degree) was significantly different among three groups (P value <0.001). CO-RADS 2,4 and 5 were significantly higher in group II compared to other groups, CO-RADS 3 was insignificantly different between groups II and III. Table 4

Table 4: CT (CO-RADS degree) in the studied groups

CT (CORAD degree)	Groups			P-value
	Group I	Group II	Group III	<0.001*
Not done	20(100.00%)	0(0.00%)	10(50.00%)	
CO-RADS 2	0(0.00%)	4(20.00%)	1(5.00%)	
CO-RADS 3	0(0.00%)	6(30.00%)	9(45.00%)	
CO-RADS 4	0(0.00%)	6(30.00%)	0(0.00%)	
CO-RADS 5	0(0.00%)	4(20.00%)	0(0.00%)	

Data are presented as frequency (%), CT: Computed tomography, *: significant as P value ≤ 0.05.

Regarding CT (CO-RADS degree), IgM was negative in 10 (45.45%) who not done CT, 2 (9%) CO-RADS 2, 7 (31.82%) CO-RADS 3, 1 (4.55%) CO-RADS 4, 2 (9%) CO-RADS 5. While IgM was positive in 0 (0%) who not done CT, 3 (16.67%) CO-RADS 2, 8 (44.44%) CO-RADS 3, 5 (27.78%) CO-RADS 4 and 2 (11.11%) CO-RADS 5 with a significant difference among them with P-value (0.013). Regarding CXR, IgM was negative in 11 (50%) negative CXR and 11 (50%) positive CXR. While positive IgM was found in 4 (22.2%) negative CXR and 14 (77.8%) positive CXR without significant difference among them with P-value (0.071). Regarding CRP, IgM was negative in 6 (27.2%) negative CRP and 16 (72.7%) positive CRP. While positive IgM was found in 3 (16.6%) negative CRP and 15 (83.3%) positive CRP without significant difference among them with P-value (0.424). Table 5

Table 5:SARS-COV2 IgM relation with CT (CO-RADS degree), CXR at time of presentation and CRP

	IgM	P-value

		Negative	Positive	
CT (CO-RADS degree)	**Not done**	10(45.45%)	0(0.00%)	0.013*
	CO-RADS 2	2(9.09%)	3(16.67%)	
	CO-RADS 3	7(31.82%)	8(44.44%)	
	CO-RADS 4	1(4.55%)	5(27.78%)	
	CO-RADS 5	2(9.09%)	2(11.11%)	
Chest X-ray at time of presentation	**Negative**	11(50.00%)	4(22.22%)	0.071
	Positive	11(50.00%)	14(77.78%)	
CRP	**Negative**	6(27.27%)	3(16.67%)	0.424
	Positive	16(72.73%)	15(83.33%)	

Data are presented as frequency (%), CT: Computed tomography, CRP: C-reactive protein, *: significant as P value ≤ 0.05.

Discussion

"The serological window period for COVID-19 lasts for 2 to 3 weeks, making anti-SARS-CoV-2 antibody testing inapplicable for an early diagnosis of acute infection" [11].

We found in our study that O2 Saturation ranged from 95 - 99 % with a mean of 96.850 ± 1.631 % in group I, from 75 – 96 % with a mean of 88.950 ± 4.883 % in group II and from 80 - 97 % with a mean of 92.550 ± 5.735 % in group III. O2 Saturation was significantly different among groups (P value = 0.001). O2 Saturation was significantly higher in group I than group II and group III and was significantly lower in group II than group III.

Similarly, Perk et al., [12] demonstrated that hypoxia was defined as oxygen saturation values <92% within the first 48 hours of admission. Hypoxia was common in patients in COVID-19 patients (n = 15; 21.0%). Low oxygen saturation was noted mainly in arterial blood (SaO2).

In the present study, IgM was positive in no patients in group I, 12 (60%) in group II, 6 (30%) in group III. Patients with positive IgM were significantly higher in group II compared to other groups, significantly higher in group III compared to group I (P value <0.001).

Our results are in harmony with those reported by Hou et al., [13] who assessed "IgM and IgG antibody levels via chemiluminescence immunoassay. This study included a total of 338 hospitalized patients with confirmed COVID-19; among them, 171 (50.6%) patients were males and 167 (49.4%) were females. The patients were divided into three groups: mild (64 cases, 18.9%), severe (199 cases, 58.9%) and critical (75 cases, 22.2%). Their results showed

that in the mild, severe, and critical groups, IgM was detected in 81.3%, 82.9% and 82.7% of cases".

Nonetheless, Tang et al., [14] reported that "among the 99 cases, 52 (53%) were initially diagnosed with SARS-CoV-2 infection by positive NAT; 47 (47%) were identified later by positive immunoglobulin G (IgG) or IgM antibodies against SARS-CoV-2. There was a spectrum of antibody profiles in these 47 patients: IgM antibodies in 5 (11%)". Larger included sample size in their study and ethnic consideration could explain this difference between both studies.

In our study, CRP was positive in no patients in group I, 15 (75%) in group II, 16 (80%) in group III. CRP was significantly different in among three groups (P value <0.001). Patients was insignificantly different between group II and group III (P value <0.001).

In agreement with our study, Lomoro et al., [15] enrolled consecutive patients, with laboratory-confirmed SARS-CoV-2. The multi-modality imaging findings were assessed and compared. In the study, fifty-eight patients (36 men, 22 women; 18-98 years old) were included. Among these tests, chest X-ray, computed tomography (CT) and electrocardiogram (ECG) were performed in 22, 32 and 42 patients respectively. In 56 patients (96.5%), the levels of C-reactive protein were elevated.

Regarding chest examination in group II, 8 (40%) patients were free, 6 (30%) patients showed crepitation, 2 (10%) showed diminished air entry, 1 (5%) patient showed rhonchi, 3 (14%) patients showed crepitation, rhonchi, and diminished air entry.

In their study, Wang et al., [16] pointed of this study was to investigate "the highlights and clinical importance of respiratory auscultation in COVID-19 pneumonia utilizing an electronic stethoscope in isolation wards. This cross sectional, observational study was conducted among patients with laboratory-confirmed COVID-19. Standard auscultation with an electronic stethoscope was performed and electronic recordings of breath sounds were

analyzed. High-quality auscultation recordings (98.8%) were obtained, and coarse breath sounds, wheezes, fine crepitations, coarse crepitation and Velcro crackles were detected".

In the current study, chest X-ray at time of presentation was positive in 17 (85%) patients in group II, and 8 (40%) patients in group III. Patients with positive CXR at time of presentation were significantly higher in group II compared to other groups. (P value 0.003).

Coping with the present study, Pascual et al., [17] described "the clinical, laboratory, and chest X-ray findings in children with clinical picture of respiratory infection. To analyze the frequency of COVID-19 compared to other respiratory infections, and to describe the radiologic manifestations of COVID-19 in pediatric patients. A total of 231 children (90 (39%) girls and 141 (61%) boys; mean age, 4 y, range 1 month–16 years) underwent chest X-rays for suspected respiratory infections. They described that 73.2% (169/231) of the patients had abnormal chest X-ray".

According to our findings, CT (CO-RADS degree) was significantly different among three groups (P value <0.001). CO-RADS 2,4 and 5 were significantly higher in group II compared to other groups, CO-RADS 3 was insignificantly different between groups II and III

Furthermore, Zayed et al., [18] conducted "comparative study included 142 confirmed COVID-19 patients by RT-PCR test, with variable degrees of disease (mild to severe), the collection of data was from medical records, and patients with their first CT chest read for calculating CO-RADS and severity scoring system (CT-SS) score. The patients with severe COVID-19 disease were significantly older and had different comorbidities. They noted that CO-RAD score was significantly higher in severe case than in mild/moderate one; thus, the mean CO-RAD was 5 as opposed to 2 in other groups, $P < 0.001$".

In our study, regarding CT (CO-RADS degree), IgM was negative in 10 (45.45%) who not done CT, 2 (9%) CO-RADS 2, 7 (31.82%) CO-RADS 3, 1 (4.55%) CO-RADS 4, 2 (9%) CO-RADS 5. While IgM was positive in 0 (0%) who not done CT, 3 (16.67%) CO-RADS 2, 8

(44.44%) CO-RADS 3, 5 (27.78%) CO-RADS 4 and 2 (11.11%) CO-RADS 5 with a significant difference among them with P-value (0.013). Further, CXR, IgM was negative in 11 (50%) negative CXR and 11 (50%) positive CXR. While positive IgM was found in 4 (22.2%) negative CXR and 14 (77.8%) positive CXR without significant difference among them with P-value (0.071).

Based on our forementioned results that demonstrated that IgM could be a representative of COVID-19 severity, we can theorize that CO-RADS degree would concurrently increase in patients presented with IgM elevation.

Regarding CRP, IgM was negative in 6 (27.2%) negative CRP and 16 (72.7%) positive CRP. While positive IgM was found in 3 (16.6%) negative CRP and 15 (83.3%) positive CRP without significant difference among them with P-value (0.424).

Since COVID-19 outbreak, elevated level of CRP played as a valuable early marker in predicting the possibility of disease progression in COVID-19 patients, combining this with our hypothesis regarding the possibility of IgM to exhibit COVID-19 progression course can provide a suitable explanation for our current study findings [19].

Limitations: The relatively small number of patients enrolled in the study because relatively small number of febrile children coming to Tanta university because we are not febrile hospital, short follow-up period and financial limitation.

Conclusions:

In children with COVID-19, Serum IgM to SARS-COV-2 was significantly higher in febrile children in Tanta university during the period from March 2021 to February 2022. According to CT findings, CO-RADS 2,4 and 5 were significantly higher in febrile patients with positive SARS-Cov-2 serum Igm Ab.

Ethical Approval and Consent:

This study was approved by the Ethics Committee of the Tanta University School of Medicine. Written informed consent was obtained from a parent or guardian.

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List of abbreviation

2019-nCoV	Novel coronavirus
ARDS	Acute respiratory distress syndrome
COVID-19	Coronavirus disease-2019
CRP	C-reactive protein
CT	Computed tomography
CXR	Chest X-ray
ELISA	Enzyme-linked immune sorbent assay
ER	Emergency room
GIT	Gastrointestinal tract
Ig	Immunoglobulin
LFAs	Lateral flow assays
PCR	Polymerase chain reaction
RUS	relative light units
RD	Respiratory distress
RT-PCR	Reverse transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2