

Research Article

Prognostic value of complement 4 in covid-19 pediatric patients



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Abstract

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-caused pandemic of COVID-19, which has killed 2 million people worldwide and infected over 97 million more, is now the most catastrophic public health disaster in a century. **Objectives:** to assess the prognostic value of complement 4 with disease severity in covid-19 pediatric patients. **Patients and methods:** our patients were selected from Pediatrics inpatient department and outpatient pediatric clinic, faculty of medicine, Minia University. It was conducted during the period March 2021 till June 2021. One hundred and fifty patients were included in our study. **Result:** Out of 300 children were diagnosed as Covid-19, One hundred and fifty patients were included in our study according to the inclusion criteria divided into four groups (mild, moderate, severe and critical). C4 was significantly lower in both severe and critical groups than the other 2 groups (mild & moderate). **Conclusion:** Complement 4, indicating excessive complement activation and product consumption is significantly associated with presence of severe disease and increased mortality in patients with COVID-19. So, complement 4 can be a prognostic factor for mortality in covid -19 pediatric patients.

Keywords: COVID-19, pediatric patients, Complement 4, Prognostic value and severity.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-caused pandemic of Coronavirus Disease 2019 (COVID-19), which has resulted in over 97 million people infections and 2 million fatalities worldwide, has become the most serious public health catastrophe in the last century (Y. Dong et al., 2020)

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes the recent described coronavirus illness (COVID-19) has set a strain on healthcare systems worldwide (Suhas Gondi et al., 2020).

The significant rise of COVID19-infected patients in numerous hospitals emphasizes the requirement to understand the clinical, radiographic, and laboratory results related

to increased disease severity and mortality (Zhang X et al., 2020).

Human complement component 4 is a protein that comes from the human leukocyte antigen (HLA) system and is a part of the complex complement system. In conjunction with different components, it performs a variety of important functions in immunity, tolerance, and autoimmunity. Additionally, it plays a critical role in establishing the recognition pathways of the whole system initiated by antibody-antigen (Ab-Ag) complexes to the other effector proteins of the natural immune response (Wang H et al., 2021).

Complement 4 concentrations in the serum can be used to identify and assess immunological complex disorders and blood-associated diseases. The evaluation

of the complement system through SARS-CoV-2 got a lot of attention due to the possible negative effects of the system's uncontrolled activation on the structural and functional integrity of many tissues and organs. Results of studies showing the beneficial effects of corticosteroid therapy in COVID-19 cases confirm this suggestion, revealing that organ and tissue damage is in fact caused by an overactive host immune response manifested by complement system activation, that eventually promotes the release of pro-inflammatory cytokines and, as a consequence, a process of intravascular coagulation and cell necrosis (Zinellu A and Mangoni AA, 2021).

Aim of the work

The goal of this study is to evaluate complement 4's significance in predicting the degree of severity of the disease in pediatric patients with COVID-19 disease.

Material and methods

This is a retrospective case-control study. Our cases have been selected from the pediatric inpatient department as well as outpatient clinic, faculty of medicine, Minia University. It was carried out between March 2021 and June 2021.

In our study, 150 patients were included. All patients who were considered to be at a risk to have COVID-19 based on a chest CT scan had a positive PCR nasal swab for SARS-CoV-2. (Kim D et al., 2020). All children's parents provided both oral and written consent. The Minia College of Medicine Ethical Committee approved the study according to the Helsinki Declaration and its updates. Ethical approval number 35: 2021.

Our cases were classified into four groups as follows: (Kim D et al., 2020):

Group I (Mild): In 67 of the cases, COVID-19 symptoms (such as fever, coughing, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, and loss of taste and smell) were suspected, but instead shortness of breath, or abnormal chest imaging were not detected

Group II (Moderate): included 52 patients who were hospitalized to the

quarantine area of our intensive care unit with mild symptoms, tachypnea, and/or positive COVID-19 specific imaging but normal oxygen saturation (SpO₂ 92%).

Group III (Severe): comprised 17 patients that had tachypnea and other respiratory distress symptoms, a SpO₂ below 92%, a PaO₂/FiO₂ below 300, radiological lung infiltrates greater than 50% of the lung picture, or a worsening damage within 24 to 48 hours. They were also admitted to our intensive care unit's quarantine sector.

Group IV (Critical): included 14 patients manifested by:

- Acute Respiratory Distress Syndrome (e.g. tachypnea, SpO₂ <92 %, or PaO₂/FiO₂ ratio < 200 despite oxygen therapy)
- Multi-organ dysfunction and/or Septic shock or disturbed conscious level.

They were admitted to our intensive care unit's quarantine sector.

Inclusion Criteria

Our study included all pediatric COVID patients who had been proven to have the disease, whose ages ranged from one month to 18 years.

Exclusion Criteria

Any case with chronic disease was excluded from our study as:

- Chronic liver diseases.
- Endocrine disorders; diabetes mellitus, hypo, hyperthyroidism, adrenal diseases, ect.
- Crohn's disease associated growth failure.
- Hematological diseases; thalassemia, sickle cell disease.
- Cardiac disease; congenital heart disease, coronary artery disease, hypertension, ect.
- Chronic chest disease; bronchial asthma, other obstructive or restrictive lung disease.
- Any case refused to participate.
- Chronic drug intake.
- Ages less than 1 month and more than 18 years.

All patients were subjected to:

- Complete clinical history: Age, sex, and any associated diseases or drug use

were taken consideration when obtaining a complete medical history.

- Complete clinical assessment: All cases in the current study had a comprehensive clinical examination, which included taking their anthropometric measurements (weight, height, body mass index), as well as their blood pressure, pulse, temperature, and respiratory rate. Examinations of the heart, Chest, and abdomen.

Laboratory investigations:

Routine laboratory investigations including:

- Complete Blood Count (CBC)
- Serum ferritin level
- Serum D-dimer

Special laboratory investigations:

Complement 4: **fully automated chemistry analyser (MindrayBS-800, China).**

Statistical analysis

Data was analyzed using SPSS version 22 (Statistical Software package version 22). Descriptive analysis was performed. Quantitative data was represented as mean, standard deviation and range. Qualitative data were reported as frequencies and percentages and compared using **Chi square test** for qualitative data between groups.

- Graphs were produced by using Excel or SPSS version 22. P value was considered significant if it was ≤ 0.05 .

- **Kruskal Wallis test** for non-parametric quantitative data between the four groups followed by **Mann Whitney test** between each two groups.

- **One Way ANOVA test** for parametric quantitative data between the four groups followed by **post Hoc Bonferroni analysis** between each two groups.

Results

The clinical pathology lab of EL- Minia university hospital performed this retrospective study from March 2021 to June 2021.

150 cases—out of the 300 cases that were diagnosed with COVID-19—met the inclusion criteria and were included in the study.

Table (1) shows there was no statistically significant difference between the four groups as regard demographic data.

Table (2) shows that there were high statistically significant differences between the four groups as regard grades of respiratory distress, MIS –C, chest CT findings, need for mechanical ventilation and outcome which reveals higher mortality in both severe and critical groups than the other 2 groups (**p value <0.001**).

Table (3) shows that HB and Lymphocytes were significantly lower in both severe and critical groups. On the other hand, both TLC and PLT count were significantly affected in severe group only.

Table (4) shows that serum ferritin, D-dimer was significantly higher in both severe and critical groups more than the other 2 groups. While, C4 were significantly lower in both severe and critical groups than the other 2 groups.

Table (1): Demographic data of the studied groups

Item	Value	Mild	Moderate	Severe	Critical	P value
		N=67	N=52	N=17	N=14	
Age	Median IQR	16 (6-25)	13 (3.3-42)	12 (14-16)	12 (5.5-43.5)	0.953
Sex	Male Female	35(52.2%) 32(47.8%)	17(32.7%) 35(67.3%)	6(35.3%) 11(64.7%)	3(21.4%) 11(78.6%)	0.059

Table (2): Chest findings in the studied groups as regard examination

Item		Mild	Moderate	Severe	Critical	P value
		N=67	N=52	N=17	N=14	
Grades of RD	G I	33(49.3%)	0 (0%)	0(0%)	0(0%)	<0.001*
	G II	34(50.7%)	32(61.5%)	0(0%)	0(0%)	
	G III	0(0%)	20(38.5%)	7(41.2%)	8(57.1%)	
	G IV	0(0%)	0(0%)	10(58.8%)	6(42.9%)	
MIS-C	No	67(100%)	52(100%)	17(100%)	0(0%)	<0.001*
	Yes	0(0%)	0(0%)	0(0%)	14(100%)	
Chest CT findings	CORAD I	0(0%)	0(0%)	0(0%)	0(0%)	<0.001*
	CORAD II	22(32.8%)	0(0%)	0(0%)	0(0%)	
	CORAD III	45(67.2%)	28(53.8%)	0 (0%)	0 (0%)	
	CORAD IV	0 (0%)	24(46.2%)	5 (29.4%)	5 (35.7%)	
	CORAD V	0 (0%)	0 (0%)	12(70.6%)	9 (64.3%)	
Need for MV	No	67(100%)	52(100%)	0(0%)	0(0%)	<0.001*
	Yes	0(0%)	0(0%)	17(100%)	14(100%)	
Outcome	Survived	67(100%)	52(100%)	2(11.8%)	2(14.3%)	<0.001*
	Died	0(0%)	0(0%)	15(88.2%)	12(85.7%)	

Table (3): CBC parameter in the studied groups

Item		Mild	Moderate	Severe	Critical	P value
		N=67	N=52	N=17	N=14	
HB	Range	(9-16.3)	(6.5-12.8)	(6.1-12.5)	(6.5-12.7)	<0.001*
	Mean $\pm$ SD	12.3 $\pm$ 2.4	10.3 $\pm$ 1.9	9.1 $\pm$ 1.9	9.4 $\pm$ 1.8	
TLC	Range	(8.8-15.2)	(4.5-80.7)	(5.6-300)	(5.7-25)	0.003*
	Mean $\pm$ SD	12.5 $\pm$ 1.6	14.1 $\pm$ 16.9	45.5 $\pm$ 96	13.2 $\pm$ 4.9	
PLT	Median	371	295	200	298	0.001*
	IQR	(246-496)	(240-354)	(63.5-300)	(202-380)	
Lymphocytes	Lymphopenia	33(49.3%)	36(69.2%)	15(88.2%)	14(100%)	<0.001*
	Lymphocytosis	34(50.7%)	16(30.8%)	2(11.8%)	0(0%)	
Neutrophilia	No	51(76.1%)	36(69.2%)	8(47.1%)	7(50%)	0.056
	Yes	16(23.9%)	16(30.8%)	9(52.9%)	7(50%)	

Table (4): Covid laboratory markers in the studied groups

Item		Mild	Moderate	Severe	Critical	P value
		N=67	N=52	N=17	N=14	
Ferritin	Median	291	275	850	1136	<0.001*
	IQR	(219-350)	(217-324.5)	(604-925)	(937.5-1267)	
D-dimer	Median	0.8	0.7	8.9	8.9	<0.001*
	IQR	(0.5-1)	(0.4-2.3)	(4.6-11.5)	(6.3-9.8)	
C4	Range	(29-169)	(20-33)	(8-9)	(4-7)	<0.001*
	Mean $\pm$ SD	131.1 $\pm$ 28.6	25.1 $\pm$ 3.8	8.4 $\pm$ 0.4	5.1 $\pm$ 1	

Statistical analysis

- Data was analyzed using SPSS version 22 (Statistical Software package version 22). Descriptive analysis was performed.
- Quantitative data was represented as mean, standard deviation and range.
- Qualitative data were reported as frequencies and percentages and compared using **Chi square test** for qualitative data between groups.
- Graphs were produced by using Excel or SPSS version 22. P value was considered significant if it was ≤ 0.05 .
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Discussion

The complement system is a crucial part of innate immunity that causes pro-inflammatory reactions in response to viral infections. It has been suggested that the classical method of immune complex activation comes first, and then direct lectin activation of the complement system, and then the alternate approach of Toll-like receptor activation (Merle et al., 2015).

Both ICU and non-ICU patients have higher plasma levels of C4. Additionally, compared to non-ICU patients, ICU patients had greater plasma levels of the complement parameters (Bagherimoghaddam et al., 2022).

Regarding age and sex, we observed no statistically significant difference between the four groups in the current study. This agrees with Koichi Yuki et al., 2020 who found that all people are susceptible to SARS-CoV-2 without significant differences in sex or age. SARS-CoV-2 infects children under 18-year-old at a similar rate as adults (T. C. Jones et al., 2020).

In our study, we found significant difference between the four groups as regard grades respiratory distress, MIS- C, chest CT findings, need for mechanical

ventilation and outcome which reveals higher mortality in both severe and critical groups than the other 2 groups.

This can be explained by Kim D et al., who found more severe manifestations and more respiratory distress in both severe and critical patients (Kim D et al., 2020).

Also, Chung M et al., 2020 detected more advanced chest CT changes in severe and critical.

Feldstein et al., 2021 demonstrated features of MIS-C that develop with the worsening of the general condition of patients and thus the need for mechanical ventilation increases in these 2 groups (severe and critical).

In the current study, we found statistically significant difference between the four groups as regard hemoglobin and Lymphocytes which were significantly lower in both severe and critical groups.

This is in agreement with Wahlster L et al., 2020 who found autoimmune hemolytic anemia which commonly happens in severe and critical cases.

Khandait H et al., 2020 who found that angiotensin-converting enzyme 2 (ACE) is expressed on lymphocytes; the virus uses this to cause a direct cytotoxic effect leading to lymphopenia. BM-pancytopenia (which depletes all types of blood cells) is caused by ACE2 receptors in hematopoietic stem cells, and TNF-alpha activation during the cytokine storm promotes cell apoptosis.

Lymphopenia and thrombocytopenia can be also explained by De Souza TH et al., 2020 who found Changes in peripheral blood count including lymphopenia, thrombocytopenia, and elevated D-dimer levels are among the most reported results.

The breakdown of hematopoietic precursors in the bone marrow, damage brought on by virus-induced autoantibodies that form immune complexes and they are excreted from the body, consumption of platelets during the coagulation cascade,

and the development of thrombi are the causes of thrombocytopenia (Khandait H et al., 2020).

Neutrophilia is preceded by a cytokine storm, superimposed bacterial infection and is also explained by Khandait H et al., 2020 who found these hematological changes in covid 19 patients.

In our study, we found significant difference between the four groups as regard serum ferritin and were significantly higher in both severe and critical groups more than the other 2 groups.

This agrees with Deng F et al., 2021 who found that high-ferritin level at admission was associated with higher incidence of mortality.

This can be clarified by the fact that ferritin, an acute protein, rises in response to a variety of inflammatory disorders, such as cancer, an iron overload, and liver or kidney diseases (Melo et al., 2021).

In the current study, we found significant difference between the four groups as regard D-dimer and were significantly higher in both severe and critical groups more than the other 2 groups.

This agrees with He X et al., 2020 who found that D-dimer is product of fibrinolytic degradation of fibrin and elevated levels indicate that there is hypercoagulable state and secondary fibrinolysis in the body which is very useful in diagnosis of thrombotic diseases.

Patients with COVID-19 were reported to have hypercoagulable state with 71% of patients who died from COVID-19 were found to meet the DIC standard, this ratio among surviving patients was only 0.6% (Panigada M et al., 2020).

In the current study, we found significant difference between the four groups as regard complement 4 with significantly lower levels in both severe and critical groups than the other 2 groups.

Our result was in agreement with Zhao Y et al., 2020 that studied predictors for mortality in non survivors COVID-19 patients and found complement C4

concentrations remained significantly lower in patients with high severity or non-survivors.

Also, Zinellu and Mangoni, 2021 found that lower serum concentrations of C4 indicating excessive complement activation and product consumption are significantly associated with presence of severe disease and increased mortality in patients with COVID-19.

Excessive and unrestrained complement activation which might favor the development of systemic pro-inflammatory, pro-oxidant and pro-coagulant state with multi-organ dysfunction and increased risk of adverse clinical outcome (Santesteban-Lores et al., 2021).

Conclusion

Complement 4, indicating excessive complement activation and product consumption is significantly associated with the severity of the disease and higher mortality in patients with COVID-19.

So, complement 4 could be a prognostic indicator for mortality in COVID -19 pediatric patients

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