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D-dimer as a Potential Biomarker of Severity in Children Confirmed with COVID-19

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Background: Coronavirus disease 2019 (COVID-19) in children spreads easily and has a relatively high incidence. Severe complications in children confirmed with COVID-19 are thought to be related to the multisystem inflammatory syndrome, which is associated with coagulation disorders. D-dimer is a fibrin degradation end product which is easy to examine, affordable, fast and reliable. This study investigated the potency of D-dimer levels as a biomarker and assessed optimal cut-off value of D-dimer on severity of COVID-19 in children.

Materials and methods: An analytical observational study with a cross-sectional design was conducted in children aged 1-18 years confirmed to have mild, moderate or severe COVID-19 who were treated in the isolation room of Dr. Moewardi Hospital, Surakarta, Indonesia from September 2021 to February 2022. Statistical analysis was conducted using Mann-Whitney test and $p<0.05$ was considered as statistically significant. The cut-off value of D-dimer was determined with the receiver operating characteristic (ROC) curve.

Results: There were 39 children with COVID-19. They were in mild (n=14; 35.9%), moderate (n=19; 48.7%) and severe (n= 6; 15.4%) stages. There were significant differences in D-dimer levels between mild and moderate stages ($p=0.001$), and mild and severe stages ($p=0.001$). No significant difference in D-dimer levels between moderate and severe stages ($p=0.162$). The cut-off value of D-dimer was 485 µg/mL with 92% sensitivity and 71.4% specificity.

Conclusion: D-dimer can be used as a potential biomarker of severity in children with COVID-19.

Keywords: *D-dimer, COVID-19, severity, children*

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This virus attacks humans and includes a new variant that has never been previously identified. The types of coronaviruses that have been identified to cause respiratory diseases with severe symptoms are Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS).[1] The incubation period of COVID-19 infection is around 5-6 days, but in some cases, it can take more than 14 days.[2]

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Symptoms that often appear due to COVID-19 infection in humans are symptoms of acute respiratory disorders, such as fever, cough, and shortness of breath.[3] However, this viral infection can cause serious illness, such as pneumonia, acute respiratory syndrome, kidney failure, and even death in patients with comorbidities/risk factors or if not treated properly and promptly.[4]

The first case of COVID-19 infection was discovered in China on December 31, 2019 as a case of pneumonia whose cause could not be identified.[5,6] On February 11, 2020, the World Health Organization (WHO) called the new virus SARS-CoV-2 and the disease as COVID-19. There was a massive outbreak of respiratory disease throughout the world.[7-9] SARS-CoV-2 is a single-stranded RNA virus, isolated from some animals, which is then transmitted to humans.[10] With the increasing number of cases and research, it was finally discovered that this virus can be transmitted by human intermediary, so the number of cases increasing per day is very fast and significant. Finally, on March 11, 2020, WHO announced that COVID-19 had officially become a world pandemic.[11]

The latest case report from China, from 2,143 cases of children suspected with COVID-19, there are quite a number of cases with severe symptoms which are divided into several categories. The proportion of severe and critical cases in children aged <1 year is 10.6%, aged 1-5 years is 7.3%, 6-10 years is 4.2%, aged 11-15 years is 4.1% and 16 years is 3.0%.[12] Since COVID-19 has been discovered and reported, most of the research only focused on symptomatic adult patients. Meanwhile, with the increasingly rapid spread of the virus and the large number of new variants of the coronavirus, clinical identification and characteristics of the laboratory examinations results of the pediatric population are needed as a guide for clinical management, predicting disease severity, and determining prognosis.

Coagulopathies are severe complications of COVID-19. The pathophysiology of coagulopathy in COVID-19 is the result of interactions between proinflammatory or cytokine secretion, platelet hyperactivity, and endothelial cell wall damage.[13] Viral infections increase the production of cytokines by activating an inflammatory response to the nonspecific immune system. After the inflammatory process appears, the immune response increases and then platelet activation occurs. Endothelial cell disorders are also a risk factor for coagulopathy in COVID-19. In addition, an increase in secretion of von Willebrand factor due to endothelial damage[14], toll-like receptor (TLR) activation and complement also plays a role in coagulopathy in COVID-19.[15,16] D-dimer is part of the coagulation end product, commonly used as a biomarker for coagulopathy. The latest study finds that there is an increase in D-dimer levels and prothrombin time in cases with severe symptoms.[17] Another study finds that children with multisystem inflammatory syndrome associated with COVID-19 have higher levels of D-dimer.[18] D-dimer is the end product of fibrin degradation which is easy to examine, affordable, fast and reliable. Since there are few studies related to D-dimer levels in children, researchers are interested to conduct further research on the effect of D-dimer on the severity of pediatric patients with COVID-19 infection. This study investigated the potency of D-dimer levels as a biomarker and assessed optimal cut-off value of D-dimer on severity of COVID-19 in children.

Materials and methods

Study Design and Research Subjects

This study was an analytical observational study using a cross-sectional design with qualitative approach. The study was conducted in the COVID-19 isolation ward of Dr. Moewardi General Hospital, Surakarta. The target population in this study were pediatric COVID-19 patients aged 1-18 years, with mild, moderate, severe, and critical severity. Mild severity patients had signs and symptoms of COVID-19 *e.g.*, fever, headache, malaise, loss of taste and smell without dyspnea or abnormal chest imaging. Moderate severity experienced respiratory diseases with oxygen saturation (SpO_2) ≥94%. Severe COVID-19 patients had SpO_2<94%, respiration rate >30 bpm or lung infiltrates >50%. Patients with critical severity experienced loss of consciousness, respiratory failure, shock and/or multiple organ dysfunction.[19] This research has been registered and approved by the ethical clearance team of Dr. Moewardi General Hospital, Surakarta (No. S591908005-1471).

Children aged 1-18 years, whose parents or caregivers agreed to participate in the study, confirmed to have mild, moderate, severe, or critical COVID-19, and were treated in the isolation room of Dr. Moewardi Hospital, Surakarta, Indonesia from September 2021 to February 2022 were included in this study. We excluded pediatrics patients with a history of bleeding and coagulation disorders prior to COVID-19 and whose parents or caregivers did not agree to participate in this study. According to the calculation, the minimum sample size obtained was 39 samples.

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Statistical Analysis

The statistical analysis was performed with SPSS version 22 (IBM, Armonk, NY, USA). The optimal value of D-dimer as a predictor of COVID-19 severity was determined with the receiver operating characteristic (ROC) curve.

Results

Thirty-nine pediatric patients aged between 1-18 years met our inclusion criteria. The characteristics of the subjects were shown in Table 1. We classified the severity of COVID-19 symptoms into three categories, namely mild, moderate, and severe. Patients with mild symptoms tended to have the lowest D-dimer levels with a median of 356 µg/mL (220–1,510 µg/mL). The D-dimer levels of patients with moderate and severe symptoms were 1,138 µg/mL (312–8,130 µg/mL) and 1,915 µg/mL (602- 8,960 µg/mL), respectively. We found a significant difference in D-dimer levels among the three categories of severity. Patients with severe symptoms had the highest D-dimer level (p<0.001). There were significant differences in D-dimer levels between mild and moderate symptoms (p=0.001), as well as between mild and severe symptoms (p=0.001). In contrast, the D-dimer levels of subjects with either moderate or severe symptoms did not differ significantly (p=0.162). There were no significant differences in platelet count (p=0.089) (Table 2).

The level of D-dimer was statistically significant (p<0.001) in the categories of COVID-19 symptoms severity. We continued the analysis to obtain the threshold value of D-dimer as a predictor of COVID-19 symptoms in children. D-dimer levels in subjects with mild symptoms tended to be low, with a median of 356 µg/mL (220-1,150 µg/mL). D-dimer levels of patients with moderate to severe symptoms were high with a median of 1,497 µg/mL (312-8,960 µg/mL). Severity of COVID-19 symptoms were positively related with the levels of D-dimer.

The highest sensitivity (1-lowest specificity) determined the cut-off point of the D-dimer level. Based on the ROC curve (Figure 1), the area under the curve (AUC) was 0.869 with a cut-off point of 485 µg/mL, sensitivity of 0.920 and specificity of 0.714. This indicated that the D-dimer cut-off point of 485 µg/mL had a sensitivity of 92%. Therefore, moderate to severe COVID-19 symptoms in children can be detected with D-Dimer levels of 485 µg/mL. The specificity of the D-dimer level was 71.4%, indicating that the probability of COVID-19 patients with mild symptoms to have D-dimer level <485 µg/mL was 71.4% (p<0.001).

Discussion

In this study, pediatric subjects with COVID-19 had elevated D-Dimer levels and this elevation was correlated with the severity of COVID-19 symptoms. Abnormal levels of coagulation parameters and inflammatory biomarkers in COVID-19 patients have been reported in a recent study. Elevated levels of D-dimer, interleukin (IL)-6, and fibrinogen among COVID-19 patients are common in those with acute respiratory distress syndrome (ARDS). The pathogenesis of coagulation changes in COVID-19 involves alveolar damage, desquamation of pneumocytes, production of hyaline membranes, and interstitial inflammation which predominates in lymphocyte infiltration. Elevated levels of D-dimer mark the evidence of hyperfibrinolysis and increase the burden of inflammation induced by SARS-CoV-2.[19]

It has been reported that the coagulation parameters among children with COVID-19 subjects are abnormal. Only one of the 22 pediatric patients (5%) has a D-dimer level below 500 µg/mL. The mean level of D-dimer among subjects is 641 µg/mL. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) are extended with a mean peak of 17.8 s (13.8-43.3 s). The platelet count is normal, with a mean of 416,000/µL.[20] However, there are 11 patients (41%) with low platelet counts (<150,000/µL).[21] In this study, the increase in D-Dimer levels occurred significantly in subjects with mild, moderate and severe symptoms (p=0.002). In addition, the platelet counts among the subjects were normal, with a mean of 284,000/µL (74,000-821,000/µL). Platelet counts did not correlate with the severity of COVID-19 symptoms. However, measurement of fibrinogen and PT/aPTT has not become a routine examination in our centre.

In a previous study involving 82 subjects, the mortality rate of the subjects reaches 18.7%. In addition, the mean of D-dimer level among recovered subjects is 1.067 g/mL.[22,23] Another study also reveals similar findings that abnormal D-dimer levels are more likely to be observed in subjects with critical condition of severe symptoms compared to those with mild symptoms. D-dimer levels are also higher in deceased subjects than in recovered subjects.[24] The threshold level of D-dimer for prognosis of death among COVID-19 patients is 2.03 mg/dL. Death caused by multiorgan failure happens after there is an increase in the production of pro-inflammatory cytokines and chemokines by immune cells and infected cells. Procalcitonin is an indicator of inflammation and D-dimer levels >500 g/ml

Table 1. Characteristics of subjects.

Characteristics	Values
Sex, n (%)	
Male	25 (64.1)
Female	14 (35.9)
Age, n (%)	
1-5 years old	10 (25.6)
>5 years old	29 (74.4)
Comorbidity, n (%)	
With comorbidity	21 (53.8)
Malignancies (ALL, AML, CML, NHL)	8
Systemic lupus erythematosus (SLE)	2
Chronic kidney disease (CKD)	2
Epilepsy	2
Congenital heart disesae	4
Central nervous system infection	3
Without comorbidity	18 (46.2)
Ward, n (%)	
Regular ward	27 (69.2)
High-care unit (HCU)	8 (20.5)
Intensive-care unit (ICU)	4 (10.3)
Thorax X-ray, n (%)	
Pneumonia	20 (51.3)
Normal	9 (23.0)
Not inspected	10 (25.7)
Severity of COVID-19, n (%)	
Mild	14 (35.9)
Moderate	19 (48.7)
Severe	6 (15.4)
Laboratory findings, median (min-max)	
D-dimer (μg/mL)	719 (220-8,960)
Thrombocyte (/μL)	284,000 (74,000-821,000)
Leucocyte (/μL)	8,800 (2,200-259,400)
Lymphocyte (%)	21.8 (7.3-80.0)

ALL: Acute lymphocytic leukemia; AML: Acute myeloblastic leukemia; CML: Chronic myelocytic leukemia; NHL: Non-Hodgkin lymphoma.

are correlated with mortality. Elevated level of D-dimer is a sign of the coagulation process activation and known as continuity of the inflammatory process, since COVID-19 deaths are likely caused by a cytokine storm. This condition is considered as one of the indicators in the scoring system to predict the death risk.[25]

A study at Leishenshan Hospital, Wuhan, China, measures the D-dimer levels among hospitalized patients to be used as the first indicator or marker of poor prognosis in COVID-19 cases. From 1,643 subjects with COVID-19, 691 subjects are reported to have elevated D-Dimer levels. About 45% of these subjects have comorbidities. Subjects with elevated D-dimer levels in the first treatment tend to require follow-up therapy, such as high-flow oxygen, anticoagulants, antibiotics, and follow-up care in the intensive care unit (ICU). This study also find an increase

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Table 2. Laboratory findings in COVID-19 patients with different severities.

Profile	Median (Min-Max)			2 Mean Test			3 Mean Test *p* -value
	Mild (n=14)	Moderate (n=19)	Severe (n=6)	*p* -value[a]	*p* -value[b]	*p* -value[c]	
D-dimer (µg/mL)	356 (220-1,510)	1,138 (312-8,130)	1,915 (602-8,960)	0.001**	0.001**	0.162	<0.001**
Platelet (/µL)	282,500 (80,000-497,000)	322,000 (163,000-821,000)	215,500 (74,000-300,000)	0.259	0.173	0.039*	0.089
Leukocyte (/µL)	7,550 (3,700-68,000)	9,500 (2,700-259,400)	8,800 (2,200-25,600)	0.299	0.741	0.874	0.615
Lymphocyte (%)	25.20 (10.50-80.00)	21.50 (7.30-63.00)	20.00 (8.00-33.80)	0.362	0.564	0.924	0.644

[a]Mild vs. Moderate; [b]Mild vs. Severe; [c]Moderate vs Severe; *p<0.05; **p<0.01.

in IL-6 level, as well as monocytes and lymphocytes number. The increased in mortality among COVID-19 patients is significantly correlated with an increase in D-dimer levels (>1 g/mL) compared to subjects with low or normal D-dimer levels. This study supports our finding that elevated D-dimer levels was significantly correlated with the severity of COVID-19 symptoms. We observed a significant difference in D-dimer level between subjects with mild symptoms and those with severe symptoms. We found that severity of COVID-19 symptoms were positively related with the levels of D-dimer.[26]

A cohort study also suggests that an increase in the immature platelet fraction (IPF%) is thought to be associated with worsening patient outcomes, such as increasing need for intensive care and mechanical ventilation, as well as prolongation of care and increased mortality.[27] The percentage of IPF represents an increase in platelet production and is associated with acute and severe inflammatory states. COVID-19 patients, who have a low platelet count, have a higher risk of death during treatment.[27,28] Based on a meta-analysis of 19 studies in 2020, non-severe cases have high platelet counts. The condition of low platelet count is also found to be more common in severe cases.[29] In our study, platelets (p=0.089), leukocyte (p=0.615) and lymphocyte count (p=0.644) did not differ significantly among groups. This could be due to the prevalence of comorbidities or underlying diseases among subjects which may influence the underlying condition.

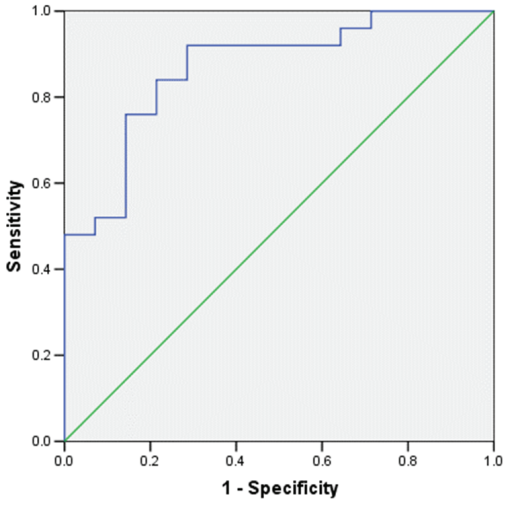

Table 2. ROC curve of D-dimer levels in COVID-19 patients with moderate-severe symptoms.

COVID-19 is often associated with hypercoagulation, which is manifested as venous thromboembolism and/or microthrombosis. The risk of venous thromboembolism is relatively high in COVID-19 patients with severe symptoms and critical illness. Therefore, thromboembolic prophylaxis can be considered. There is a robust protocol for anticoagulant therapy among adult patients with COVID-19. Ironically, data on pediatric patients are still limited. Presently, anticoagulant therapy with low-molecular-weight-heparin (LMWH) is associated with a good prognosis for COVID-19 patients with coagulopathy characterized by elevated D-dimer level. In severe or critical COVID-19 patients, the incidence of venous thromboembolism and arterial thrombosis is 25-27% and 4%, respectively. Disseminated intravascular coagulation is usually observed in severe or critical cases (2% of hospitalized patients), suggesting a poorer prognosis.

However, our study has several limitations. The study population with severe and critical symptoms was limited. Therefore, determination of the D-dimer cut-off point was

only ideal for mild to moderate/severe symptoms. The clinical advantage of D-dimer levels in mild to moderate/severe symptoms is uncertain. Therefore, further study is required.

Conclusion

D-dimer can be used as a potential biomarker of severity in children with COVID-19.

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