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RETROSPECTIVE EVALUATION OF LABORATORY FINDINGS OF SUSPECTED PAEDIATRIC COVID-19 PATIENTS WITH POSITIVE AND NEGATIVE RT-PCR

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ABSTRACT

INTRODUCTION: It was observed that the new coronavirus disease had a different clinical course in children compared to adults. Fewer cases and deaths have been reported in children.

MATERIAL AND METHODS: All children with suspected COVID-19 who applied to a secondary health care centre were included in this study and blood parameters were compared according to Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) results. This study is a retrospective, cross-sectional study and was conducted by accessing the hospital records of 40 paediatric patients aged 0–16 years. RT-PCR test was performed by isolating Viral RNA with RNA Isolation System after oropharyngeal and nasopharyngeal samples were taken from patients with appropriate sterile swabs and transferred to tubes containing SNP Viral Inactivation Solution. All statistical calculations were done with SPSS 23.0 (SPSS for Windows, Chicago, IL, SA). Mean values of continuous variables were compared between groups using the Mann-Whitney U test.

RESULTS: The results were evaluated as 95% confidence interval and p-value < 0.05 and were considered statistically significant. In the light of the blood results of a small number of paediatric cases who applied, the authors tried to determine the parameters that could increase the success in diagnosis in asymptomatic paediatric cases. C-Reactive Protein, ID-dimer (quantitative), leukocytes, monocytes, sodium, mean platelet volume levels were found to be significantly different between the patients with positive and negative COVID test results.

CONCLUSIONS: The results of this study reveal that the use of leukocytopenia, monocytopenia and mean platelet volume elevation as diagnostic markers may increase diagnostic success, especially in asymptomatic paediatric COVID-19 patients.

KEY WORDS: novel coronavirus, COVID-19, paediatrics, children, diagnosis, laboratory tests

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INTRODUCTION

The new coronavirus disease is a highly contagious respiratory disease that occurs in the Wuhan Province of China. On March 11, 2020, 119 thousand case reports and 4 thousand death notifications were made for this disease, which spread to all continents and more than 190 countries in the World [1, 2]. The World Health Organization (WHO) declared an emergency public health situation and pandemic on March 11, 2020 [3]. Fewer

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COVID-19 cases and deaths have been reported in children and teenagers than in adults [4–7]. The first case in children was reported in Shenzhen on 20 January 2020 [8, 9]. Later, many paediatric case series have been published. This study aims to compare blood parameters according to Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) results in paediatric COVID-19 patients.

MATERIAL AND METHODS

This is a retrospective and cross-sectional study, with data collected in a secondary health centre between March and June 2020. The study was conducted by reaching the hospital records of 40 paediatric patients aged 0–16 years who applied to the emergency department with suspicion of COVID-19 between these dates. All paediatric patients with suspected COVID-19 presenting to the emergency department were included in the study. 5 ml of blood from the patients was taken into a yellow capped tube containing polymer gel and clot activator silica particles for biochemistry analysis. The sample, which was centrifuged at 4100 rpm for 15 minutes and the serum part separated, was analysed. (C8000-Architect U.S.A). For complete blood count analysis, 1 ml of blood was taken into a purple capped tube containing ethylenediaminetetraacetic acid and anticoagulant. 2 ml of blood serum separated from the sample, which was centrifuged at 2500 rpm for 10–15 minutes, was taken into a yellow tube (BS-200 Chemistry Analyzer, Mindray, China). For coagulation analysis, 3 ml of blood was collected in a blue cap tube containing anticoagulant and citrate. (MTI Diagnostic Analyzers Germany). The sample was centrifuged at 1500 rpm for 15 minutes, the plasma was separated and transferred to a plastic tube. For blood gas analysis, 2 ml of blood sample was taken into the syringe containing lyophilized heparin and analysed. (ABL 800 FLEX Blood Gas Analyzer). For the RT-PCR test, oropharyngeal and nasopharyngeal swab samples were taken from the patients with appropriate sterile swabs and transferred to tubes containing SNP Viral Inactivation Solution. Viral RNA was isolated with the RNA Isolation System after 15 minutes at room temperature (ABI Prism ® 7000/7300/7500/7900).

Statistical reviews

All statistical calculations were performed with SPSS 23.0 (SPSS for Windows, Chicago, IL, SA). The continuous variables were expressed as mean ± standard deviation; categoric variables were defined as percentages (%). The normal distribution was determined by histogram and Kolmogorov-Smirnov test. Mean values of continuous variables were compared between the groups using the Mann-Whitney U test. The results were evaluated as 95% confidence interval and p-value < 0.05, which was considered statistically significant.

RESULTS

Table 1 lists the analysis of biochemistry blood parameters by the Mann-Whitney U test between RT-PCR positive and negative groups. Accordingly, according to the positive and negative results of the COVID-19 test of patients under 16 years of age, albumin, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Bilirubin (direct), Bilirubin (total), Calcium, Chlorine, Pro-Brain Natriuretic Peptide (Pro-BNP), procalcitonin, sedimentation, magnesium, urea, Troponin T, Lactate Dehydrogenase (LDH), ferritin, phosphorus, Gamma Glutamyl Transferase (GGT), glucose, creatine kinase, creatinine, mass Creatine kinase-MB (CK-MB) laboratory parameters measurement values are $p > 0.05$. There is no statistically significant difference between the measurement values.

The C-Reactive Protein (CRP) measurement values of the patients under the age of 16 who were positive as a result of the COVID-19 test were statistically significantly lower than the CRP measurement values of the ones that were negative as a result of the COVID-19 test ($z = -3.018$; $p < 0.01$).

The sodium measurement values of those who are positive after the COVID-19 test of patients under the age of 16 are statistically significantly higher than the sodium measurement values of those who are negative after the COVID-19 test ($z = 2.334$; $p < 0.05$).

Table 2 lists the analysis of hemogram blood parameters by the Mann-Whitney U test between RT-PCR positive and negative groups. Accordingly, according to the positive and negative results of the COVID-19 test of patients under 16 years of age, Red Blood Cell (RBC), Lymphocyte, MCH, Mean Erythrocyte Volume (MCV), Neutrophil, Basophil, Eosinophil, Platelet, Haematocrit, Haemoglobin, Neutrophil Lymphocyte Ratio (NLR), Procalcitonin (PCT), Platelet, Platelet Distribution Width (PDW), Pro-BNP, Red Cell Distribution Width (RDW-CV) laboratory parameters measurement values are $p > 0.05$. There

Table 1. Comparison of biochemistry blood parameters in patients under the age of 16 according to positive and negative COVID-19 test results

Parameters	RT-PCR Results	Mean rank	Sum of rank	U	z	p
Albumin	(+)	17.87	339.50	116.500	-0.601	0.548
	(-)	15.82	221.50			
ALP	(+)	14.88	253.00	100.000	-0.089	0.948
	(-)	15.17	182.00			
ALT	(+)	19.73	434.00	127.000	-0.876	0.381
	(-)	16.57	232.00			
AST	(+)	16.11	354.50	101.500	-1.704	0.088
	(-)	22.25	311.50			
Bilirubin (direct)	(+)	14.69	264.50	93.500	-1.293	0.196
	(-)	18.82	263.50			
Bilirubin (total)	(+)	11.61	162.50	57.500	-1.876	0.061
	(-)	17.39	243.50			
Calcium	(+)	12.77	191.50	71.500	-0.573	0.566
	(-)	14.50	159.50			
Cl-	(+)	14.06	225.00	87.000	-0.050	0.960
	(-)	13.91	153.00			
CRP	(+)	14.05	309.00	56.000	-3.018	0.003*
	(-)	24.69	321.00			
Ferritin	(+)	5.00	35.00	7.000	-0.798	0.425
	(-)	6.67	20.00			
Phosphor	(+)	4.50	27.00	6.000	0.000	1.000
	(-)	4.50	9.00			
GGT	(+)	13.44	242.00	71.000	-1.856	0.063
	(-)	19.54	254.00			
Glucose	(+)	16.13	322.50	112.500	-0.645	0.519
	(-)	18.35	238.50			
Creatine kinase	(+)	9.29	130.00	17.000	-0.504	0.614
	(-)	7.67	23.00			
Creatinine	(+)	18.66	410.50	128.500	-0.496	0.620
	(-)	16.88	219.50			
CK-MB mass	(+)	5.60	28.00	2.000	-1.640	0.101
	(-)	2.67	8.00			
Magnesium	(+)	5.33	32.00	7.000	-0.516	0.606
	(-)	4.33	13.00			
Pro-BNP	(+)	1.00	1.00	0.000	-1.000	0.317
	(-)	2.00	2.00			
Procalcitonin	(+)	6.91	76.00	10.000	-1.012	0.312
	(-)	9.67	29.00			
Sedimentation 60 minute	(+)	3.70	18.50	1.500	-0.594	0.552
	(-)	2.50	2.50			
Sodium	(+)	21.73	478.00	83.000	2.334	0.020**
	(-)	13.43	188.00			
Troponin T	(+)	6.00	48.00	12.000	-0.808	0.419
	(-)	7.50	30.00			
Urea	(+)	20.13	402.50	87.500	-1.838	0.066
	(-)	13.75	192.50			
LDH	(+)	2.00	4.00	1.000	-1.389	0.165
	(-)	4.25	17.00			

*p < 0.01; **p < 0.05; ALP — Alkaline Phosphatase; ALT — Alanine Aminotransferase; AST — Aspartate Aminotransferase; Cl — Chlorine; CRP — C-Reactive Protein; GGT — Gamma Glutamyl Transferase; CK-MB — Creatine kinase-MB; Pro-BNP — Pro-Brain Natriuretic Peptide; LDH — Lactate Dehydrogenase

Table 2. Comparison of hemogram blood parameters in patients under the age of 16 according to positive and negative COVID-19 test results

Parameters	RT-PCR results	Mean rank	Sum of rank	U	z	p
WBC	(+)	12.45	274.00	21.000	-4.165	**0.000***
	(-)	27.38	356.00			
RBC	(+)	17.64	388.00	135.000	-0.273	0.785
	(-)	18.62	242.00			
Basophil	(+)	16.75	368.50	115.500	-0.989	0.323
	(-)	20.12	261.50			
Eosinophil	(+)	18.70	411.50	127.500	-0.530	0.596
	(-)	16.81	218.50			
Lymphocyte	(+)	16.27	358.00	105.000	-1.297	0.194
	(-)	20.92	272.00			
Monocyte	(+)	13.41	295.00	42.000	-3.450	**0.001***
	(-)	25.77	335.00			
Neutrophil	(+)	15.91	350.00	97.000	-1.571	0.116
	(-)	21.54	280.00			
NLR	(+)	16.50	363.00	110.000	-1.127	0.260
	(-)	20.54	267.00			
PCT	(+)	17.20	378.50	125.500	-0.598	0.550
	(-)	19.35	251.50			
PDW	(+)	18.91	416.00	123.000	-0.689	0.491
	(-)	16.46	214.00			
Platelet	(+)	16.09	354.00	101.000	-1.434	0.151
	(-)	21.23	276.00			
Haematocrit	(+)	19.25	423.50	137.500	-0.536	0.592
	(-)	17.32	242.50			
Haemoglobin	(+)	19.02	418.50	120.500	-0.769	0.442
	(-)	16.27	211.50			
MCV	(+)	19.91	438.00	101.000	-1.434	0.152
	(-)	14.77	192.00			
MPV	(+)	21.20	466.50	72.500	-2.412	**0.016****
	(-)	12.58	163.50			
RDW-CV	(+)	15.64	344.00	91.000	-1.778	0.075
	(-)	22.00	286.00			

*$p < 0.01$; **$p < 0.05$; WBC — White Blood Cell; RBC — Red Blood Cell; NLR — Neutrophil Lymphocyte Ratio; PCT — Procalcitonin; PDW — Platelet Distribution Width; MCV — Mean Erythrocyte Volume; MPV — Mean Platelet Volume; RDW-CV — Red Cell Distribution Width

is no statistically significant difference between the measurement values.

The Monocyte measurement values of those who were positive after the COVID-19 test of patients under 16 years of age were statistically significantly lower than the Monocyte measurement values of those who were negative because of the COVID-19 test ($z = -3{,}450$; $p < 0.01$).

The Mean Platelet Volume (MPV) measurement values of the patients under the age of 16 who were positive because of the COVID-19 test were statistically significantly higher than the MPV measurement values of the ones that were negative as a result of the COVID-19 test ($z = -2.412$; $p < 0.05$).

The White Blood Cell (WBC) measurement values of the patients under the age of 16 who were posi-

Table 3. Comparison of coagulation blood parameters according to positive and negative COVID-19 test results in patients under 16

Parameters	RT-PCR Results	Mean rank	Sum of rank	U	z	p
aPTT	(+)	6.50	52.00	8.000	-0.818	0.413
	(-)	4.67	14.00			
D-dimer	(+)	6.10	61.00	6.000	-1.989	0.047**
	(-)	11.00	44.00			
INR	(+)	5.25	42.00	6.000	-0.522	0.602
	(-)	6.50	13.00			
Prothrombin time	(+)	5.25	42.00	6.000	-0.522	0.602
	(-)	6.50	13.00			

*$p < 0.01$; **$p < 0.05$; aPTT — Activated Partial Thromboplastin Time; INR — International Normalized Ratio

tive as a result of the COVID-19 test were statistically significantly lower than the WBC measurement values of the ones that were negative as a result of the COVID-19 test ($z = -4{,}165$; $p < 0.01$).

Table 3 lists the analysis of coagulation blood parameters by the Mann Whitney U test between RT-PCR positive and negative groups. Accordingly, according to the positive and negative results of the COVID-19 test of patients under 16 years of age, Activated Partial Thromboplastin Time (aPTT), International Normalized Ratio (INR), prothrombin time laboratory parameters measurement values are $p > 0.05$. There is no statistically significant difference between the measurement values.

The ID-dimer (quantitative) measurement values of the patients under the age of 16 who were positive as a result of the COVID-19 test were statistically significantly lower than the ID-dimer (quantitative) measurement values of the ones that were negative as a result of the COVID-19 test ($z = -1.989$; $p < 0.05$).

Table 4 lists the analysis of venous blood gas blood parameters by the Mann-Whitney U test between RT-PCR positive and negative groups. Accordingly, according to the positive and negative results of the COVID-19 test of patients under 16 years of age, BASE, Bicarbonate (HCO_3), Partial Carbon Dioxide Pressure (PCO_2), pH, Partial Oxygen Pressure (PO_2), Lactate laboratory parameters measurement values are $p > 0.05$. There is no statistically significant difference between the measurement values.

DISCUSSION

COVID-19 is an infection with a milder clinical course or an asymptomatic course in paediatric patients compared to adult patients. This is probably the reason why there were no paediatric case reports before 20 January 2020. In this study, blood parameters were compared according to RT-PCR results in 40 paediatric cases under 16 years of age. In the present study, the CRP level was found to be statistically significantly lower in cases with positive RT-PCR results. In the study of Cai J. et al., the CRP level was found to be high in 30% of paediatric positive cases [10]. The CRP level was found to be normal in paediatric positive cases in the studies of Chan et al., Zeng et al., Zhang Y.H. et al., Liu et al., Zhao et al [8, 11–14]. In the studies of Cai J.H. et al., Chen et al., CRP level was found to be high in all paediatric positive cases [15, 16]. In the study of Zhang G. et al., 50% of paediatric positive cases had a high CRP level [17]. In the study of Wang et al., the CRP level was found to be high in 9.7% of paediatric positive cases [18]. The reason why the CRP level was found to be lower in positive cases in this study may be that blood tests are performed in the late period of the disease.

In the study of Cai J. et al., the WBC level was found to be high in 30% of paediatric positive cases, and low in 10% [10]. In the study of Cai J.H. et al., Chen et al., Zhang G. et al., the level of WBC was high in all paediatric cases [15–17]. The WBC level was found to be normal in all paediatric cases in the studies of Chan et al., Zeng et al., Zhang Y.H. et al., Liu et al., Zhao et al [8, 11–14]. The WBC level was found to be low in 6.5% of the cases in the study of Wang et al. and 46.7% of the paediatric cases in the study of Feng et al [18, 19]. The WBC level was found to be low in the study of Kam et al [20]. In the present study, the WBC level was found to be statistically significantly lower in cases with positive RT-PCR results. Viral infections are the most common cause of low WBC (leukocyte) levels. Therefore,

Table 4. Comparison of venous blood gas blood parameters in patients under the age of 16 according to positive and negative COVID-19 test results

Parameters	RT-PCR Results	Mean rank	Sum of rank	U	z	p
BASE	(+)	15.72	251.50	60.500	-1.358	0.174
	(-)	11.50	126.50			
HCO_3	(+)	16.28	260.50	51.500	-1.803	0.071
	(-)	10.68	117.50			
pCO_2	(+)	15.50	248.00	64.000	-1.184	0.236
	(-)	11.82	130.00			
pH	(+)	14.25	228.00	92.000	-0.186	0.853
	(-)	14.83	178.00			
pO_2	(+)	14.56	233.00	79.000	-0.444	0.657
	(-)	13.18	145.00			
Lactate	(+)	12.27	184.00	64.000	-0.965	0.334
	(-)	15.18	167.00			

*$p < 0.01$; **$p < 0.05$; HCO_3 — Bicarbonate; pCO_2 — Partial Carbon Dioxide Pressure; pO_2 — Partial Oxygen Pressure

in this study, it was thought that the WBC level was low in COVID-19 positive cases. In this study, the monocyte level was found to be statistically significantly lower in paediatric positive cases than in negative cases. Monocyte cells are one of the types of leukocyte cells. In this study, the level of leukocyte cells was also found to be low. This may be the reason for the low monocyte level in the subtype. In addition, after monocyte cells are produced in the bone marrow, they reach the tissues and turn into macrophages. In the presence of viral infection, if the rate of production of monocytes in the bone marrow cannot match the rate at which monocytes turn into macrophages in tissues, there may be a relative decrease in the number of monocytes in the blood. This may be the reason for the low number of monocytes in this study.

In the study of Cai J et al., D-Dimer level was found to be high in 20% of paediatric positive cases [10]. In the study of Zhao et al., and the study of Chen et al., the D-Dimer level of paediatric cases was found to be normal [14, 16]. In the study of Wang et al., D-Dimer level was found to be high in 6.5% of paediatric cases [18]. In the present study, D-Dimer levels of positive paediatric cases were found to be statistically significantly lower than negative cases. D-Dimer may increase in many cases where fibrin destruction occurs such as malignancies, consumption coagulopathy, infections, trauma, surgical operations, chronic obstructive pulmonary diseases. The reason for the low D-Dimer level in positive cases in the present study may be that blood tests are performed in the late period of the disease.

MPV refers to the mean platelet volume. Platelet counts function and mean platelet volume vary during infective processes. In the present study, the MPV level of positive paediatric cases was found to be statistically significantly higher than negative cases. In the study of Gümüs et al., MPV level was found to be significantly higher in paediatric positive cases, and they predicted that this was a predictive value to help diagnosis, especially in asymptomatic paediatric cases [21]. The findings of the present study also support this result.

In this study, the sodium level in the blood was found to be statistically significantly higher in paediatric positive cases compared to negative cases. In the study of Kari et al., sodium level was found to be high in paediatric positive cases, and this was associated with the presence of underlying comorbid diseases [22].

CONCLUSIONS

COVID-19 shows an asymptomatic or milder clinical course in paediatric patients compared to adult patients. It is thought that this affects the admission process of paediatric patients to the hospital. The authors compared RT-PCR and blood results with data obtained from retrospective hospital records of 40 paediatric patients who presented with suspected COVID-19. The results of this study reveal that using leukocytopenia, monocytopenia, and MPV el-

evation as diagnostic markers, especially in asymptomatic paediatric COVID-19 patients, may increase diagnostic success.

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