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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

The Relationship Between ABO-Rh Blood Types and Disease Severity in Children with COVID-19 Infection

COVID-19 Tanılı Çocuklarda ABO-Rh Kan Grupları ile Hastalık Şiddeti Arasındaki İlişki

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Abstract

Aim: The purpose of this study was to evaluate correlation between ABO, Rhesus (Rh) blood type and the disease severity status, pneumonia status in children with COVID-19.

Material and Method: The retrospective multicenter study reviewed electronic medical files of all children younger than 18 years old with COVID-19 infection. Patients were divided into three groupas asymptomatic, mild illness and radiologically proven pneumonia. The differences in the ABO and Rh blood group distribution between COVID-19 patients and also the control group were analyzed.

Results: A total of 1026 patients, with a median age of 12 (1-18) years old from 5 different hospitals were included in the study. Of the patients, 32% (n=323), were asymptomatic, 59%(n=607) were mildly symptomatic, and 9%(n=96) were all cases of radiologically proven pneumonia. A total of 1600 children included as the control group. There was no statistically significant difference between the control blood groups and the COVID-19 patients' blood group distribution (p=0.062). When the laboratory characteristics were evaluated, it was determined that as the clinical severity of the patients increased; when age (p=0.012), leukocyte count (p=0.013), CRP (p=0.002), ferritin (p=0.0001) and D-dimer (p=0.049) had increased; and the lymphocyte counts had decreased (p=0.027). There were no istatistically significant difference between blood groups (ABO and Rh), just ABO status and clinical severity condition (respectively p=0.126, p=0.630). When clinical and laboratory data were evaluated according to Rh status, no statistically significant difference was found (p>0.05).

Conclusions: In our study with pediatric population, no difference was detected between blood types and/or Rhesus condition and COVID-19 severity.

Keywords: ABO blood group, COVID-19, risk factors

Öz

Amaç: Bu çalışmanın amacı, COVID-19'lu çocuklarda ABO, Rhesus (Rh) kan grubu ile hastalık şiddet durumu, pnömoni durumu arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntem: Çok merkezli çalışmada COVID-19 enfeksiyonu olan 18 yaşından küçük tüm çocukların elektronik tıbbi dosyalarından retrospektif olarak incelendi. Hastalar asemptomatik, hafif hastalık ve radyolojik olarak kanıtlanmış pnömonisi olanlar olarak üç gruba ayrıldı. COVID-19 hastaları ve kontrol grubu arasındaki ABO ve Rh kan grubu dağılımındaki farklılıklar analiz edildi.

Bulgular: Çalışmaya 5 farklı hastaneden medyan yaşı 12 (1-18) olan toplam 1026 hasta dahil edildi. Asemptomatik hastalar %32 (n=323), hafif semptomatik %59 (n=607) ve radyolojik olarak kanıtlanmış pnömoni tüm vakaların %9'u (n=96) idi. Kontrol grubu olarak toplam 1600 çocuk dahil edildi. Kontrol kan grupları ile COVID-19 hastalarının kan grubu dağılımı arasında istatistiksel olarak anlamlı fark yoktu (p=0,062). Laboratuvar özellikleri değerlendirildiğinde hastaların klinik şiddeti arttıkça; yaş (p=0,012), lökosit sayısı (p=0,013), CRP (p=0,002), ferritin (p=0,0001) ve D-dimer (p=0,049) değerlerinin yüksek olduğuve lenfosit sayılarının azaldığı saptandı. (p=0,027). Klinik şiddet durumu ile kan grupları (ABO ve Rh) ve sadece ABO durumu arasında istatistiksel olarak anlamlı fark yoktu (sırasıyla p=0.126, p=0.630). Klinik ve laboratuvar verileri Rh durumuna göre değerlendirildiğinde istatistiksel olarak anlamlı fark bulunmadı (p>0,05).

Sonuç: Pediatrik popülasyon ile yaptığımız çalışmamızda kan grupları ve/veya Rhesus durumu ile COVID-19 klinik şiddeti arasında fark saptanmadı.

Anahtar Kelimeler: ABO kan grubu, COVID-19, risk faktörleri

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INTRODUCTION

Since the outbreak of coronavirus disease 2019 (COVID-19) started in Wuhan, China in December 2019, the new novel infectious disease has caused serious pandemia infecting thousands of people worldwide (1). The range of disease may vary from asymptomatic to severe acute respiratory syndrome. The severe COVID-19 disease mainly affects adult population with certain risk factors (ie; older age, cardiovascular disease, diabetes mellitus, immune deficiency syndromes, etc) (2). The COVID-19 symptoms appear to be less severe in children than in adults (3,4). Most children may be asymptomatic carriers. Clinical manifestations in children with COVID-19 include fever and cough with some accompanied by fatigue, myalgia, nasal congestion, sneezing, sore throat, headache, dizziness, vomiting and abdominal pain. A few children exhibit pulmonary involvement. Shock, multi-organ failure, encephalopathy, heart failure, abnormal coagulation and acute renal failure have been rarely reported in children with COVID-19. The obvious question why COVID-19 infection in children has a milder course than in adults is not fully understood. It is speculated that repeated viral exposure in early life supports the immune system when it responds to COVID-19 infection. There is also speculation that the COVID-19 protein binds to the angiotensin-converting enzyme (ACE) 2, and that children may be protected against COVID-19 because this enzyme is less mature at a younger age (5,6).

Blood groups have been previously proposed in host susceptibility to infectious diseases (7). Many blood groups are receptors for toxins, parasites and bacteria, where they can facilitate colonization or invasion or evade host clearance mechanisms. Additionally, ABO antibodies can be considered part of the innate immune system against some bacterial pathogens and enveloped viruses that carry ABO-active antigens. Most recently, it is speculated that in adult patients with COVID-19 blood type A is associated with the worst outcome, while blood type O is associated with mild symptoms (8). To our knowledge, there have not been so much data to investigate ABO and Rhesus (Rh) blood group types in children with COVID-19 infection especially on pneumoniae. Therefore, the study aimed to examine if such a correlation exists in children infected with COVID-19.

MATERIAL AND METHOD

We conducted a retrospective multicenter trial in five major hospitals in Turkey to determine whether ABO and Rh blood types carry any risk/beneficial factor among children with COVID-19 infection. The study period consisted between March 2020 and December 2020. Demographic information, clinical symptoms and laboratory results were obtained from each patient's electronic medical files. All children with a documented positive COVID-19 nasal smear real-time reverse-transcriptase polymerase chain reaction (PCR) assay were included. In order to provide a homogeneous study subjects for the aim of trial, patients who had a past medical history of any chronic illness (related to respiratory, cardiology, immunology, neurology, metabolic, etc) were excluded from the study. The children with COVID-19 were classified into 3 groups which include asymptomatic, mild disease (ie; subfebrile fever, fatigue, myalgia, nasal congestion, cough etc), and patients with radiologically proven pneumonia. Control group consisted with children in whom ABO and Rh blood type was available in hospital health files. Those with suspected history for COVID-19 infection were not included in the control group. The study was approved by the Ethics Committee of the Mersin University (2021/53), and the institutional ethics review boards of all participating centers, and also from the government's medical research comittee for COVID-19.

Statistical Analysis

Data were collected from electronic health files and recorded via Statistical Package for Social Science (SPSS). Descriptive statistics were given as mean, standard deviation, median, minimum and maximum. In comparison of the variables of dependent groups, the "Paired Samples T Test" was used for the normally distributed variables, and the "Non-parametric Wilcoxon test" was used for the variables that did not show normal distribution. Comparisons for variables in independent groups, "Independent Samples T Test" was used for normal distributed data, and the"Mann-Whitney U test"in data that did not show normal distribution."Kruskal Wallis Analysis" was used in the analysis of data with more than two not normally distributed groups. "One-way Anova" was used for normally distrubuted more than two groups. In statistical comparisons, the level of significance was determined as $p<0.05$.

RESULTS

A total of 1026 patients from 5 different hospital included in the study. Fifty-one percent of the patients were male and their median age were 12 (1-18) years old. Patients were classified into three groups according to their clinical severity. Accordingly, asymptomatic patients comprised 32% (n=323) of all cases, mildly symptomatic were 59% (n=607), and radiologically proven pneumonia were 9% (n=96).

A total of 1600 children, 60% of whom were male, with a median age of 6.6 (1-18) years were included as the control group. When blood groups were evaluated, 35% (n=566) of the cases were A(+), 30% (n=474) were 0(+), 17% (n=270) were B(+), 8% (n=132) AB(+), 4% (n=65) A(-), 3% (n=54) 0(-), 2% (n=22) were B(-) and 1% (n=17) were

AB(-) (**Figure 1**). When the blood groups of the COVID-19 cases were evaluated, similar to the control group, the most common blood groups were A (+) 41%(n=422), 0(+) 29%(n=298), B (+) 13%(n=139) and AB (+) 7%(n=71). Other blood groups were 0(-) 4% (n=45), A (-) 3% (n=30), B(-) 2% (n=14) and AB(-) 1% (n=7) respectively (**Figure 2**). There was no statistically significant difference between the control blood groups (ABO and Rh) and the blood groups of cases with COVID-19 distribution; the both group were similar (p=0.062). There was no statistical difference between the groups in terms of ABO blood group (p=0.076) and Rh status (p=0.3).

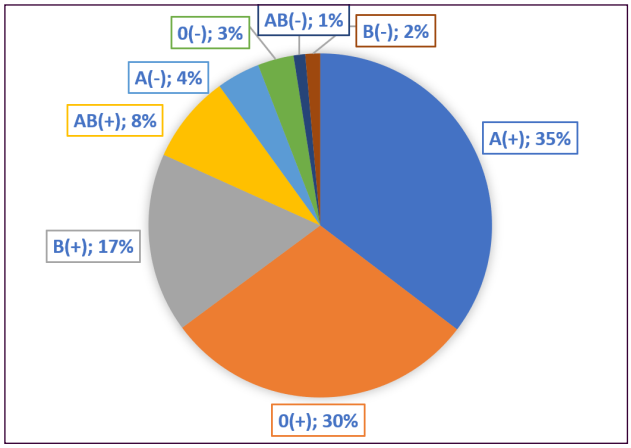

Figure 1: Distribution of the control blood groups.

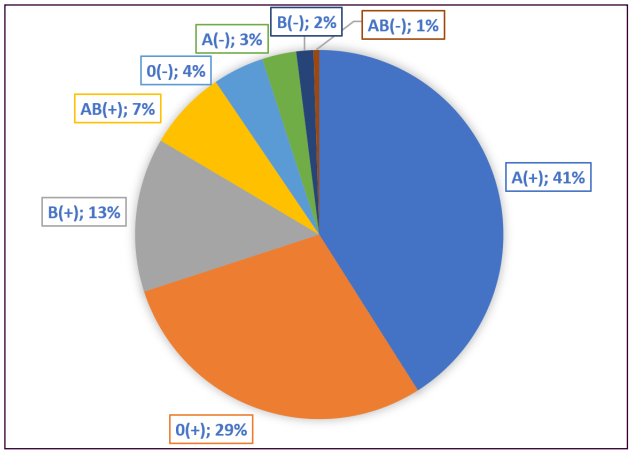

Figure 2: Distribution of the COVID-19 patients' blood groups.

Laboratory and demographic parameters according to the clinical severity of the cases were given in **Table 1**. When we look at the age and clinical severity status, a statistical difference was found between the groups, and it was determined that this difference was between mild symptomatic and asymptomatic groups in the post Hoc evaluation (p=0.007). Age was higher in mildly symptomatic group from asymptomatic ones (p=0.012). No statistical difference was found between the other clinical severity groups (p>0.05). When the laboratory characteristics are evaluated, as the clinical severity of the patients increases; age (p=0.012), leukocyte count (p=0.013), C-reactive protein (CRP) (p=0.002), ferritin (p=0.0001) and D-dimer (p=0.049) values were increased, and the lymphocyte counts were found to decrease (p=0.027) (**Table 1**).

Clinical severity statuses according to blood groups (ABO and Rh) are given in **Table 2**. According to the study, there were no statistically significant difference between blood groups and clinical severity condition (p=0.126).

Table 2: Distribution of blood groups according to COVID-19 clinical severity status

Blood groups	Asymptomatic 32% (n=323) % (n)	Mildly symptomatic 59% (n=607) % (n)	Pneumoniae 9% (n=96) % (n)	p
A(+)	41.8 (135)	40.2 (244)	44.8 (43)	0.126
A(-)	2.2 (7)	3.1 (19)	4.2 (4)	
B(+)	14.9 (48)	13.8 (84)	7.3 (7)	
B(-)	1.2 (4)	1.5 (9)	1 (1)	
0(+)	31.6 (102)	27.8 (169)	28.1 (27)	
0(-)	3.1 (10)	5.1 (31)	4.2 (4)	
AB(+)	5 (16)	7.9 (48)	7.3 (7)	
AB(-)	0.3 (1)	0.5 (3)	3.1 (3)	

* Chi-square test was used for statistical analysis.

Clinical severity statuses according to A, B, O and AB blood groups are given in **Table 3**. In the study, there were no statistically significant difference between ABO blood groups and clinical severity condition (p=0.630).

Table 1: Clinical features of the COVID-19 cases according to their clinical severity condition

	Asymptomatic n=323 (32%)	Mildly symptomatic n=607 (59%)	Pneumoniae n=96 (9%)	p
Age (months)	114 ± 67.1	129 ± 71.5	121 ± 75	0.012
Sex (F/M)	46% F, 54% M	51% F, 49% M	49% F, 51% M	0.251
Leukocyte (/mm3)	6301 ± 3431	6900 ± 3189	7662 ± 5623	0.013
Lymphocyte (/mm3)	2200 (110-12520)	2140 (90-17520)	1770 (10-21200)	0.027
Hemoglobin (gr/dL)	11.8 ± 1.6	11.9 ± 1.3	12.1 ± 1.9	0.197
C-reactive protein (mg/dl)	2±3.6 ; 1(0.3-15)	2.5±4.3 ; 1(0.3-19)	8.5±9.3 ; 5.5(1-31)	0.002
Ferritin (ug/dl)	12 (1-1026)	31 (1-4563)	54 (5-2383)	0.0001
D-dimer (µg/ml)	0.3 (0.1-24)	0.4 (0.1-31)	0.5 (0.2-30)	0.049

F: female, M: male.

Table 3: Distribution of A, B, O and AB blood groups according to COVID-19 clinical severity status						
% (n)	A % (n)	B % (n)	0 % (n)	AB % (n)	Total % (n)	p
Asymptomatic	31.4(142)	34 (52)	32.5 (112)	22.8 (17)	31.5 (323)	
Mildly symptomatic	58.2 (263)	60.8 (93)	58.5 (200)	64.6 (51)	59.2 (607)	0,630
Pneumoniae	10.4 (47)	5.2 (8)	9.1 (31)	12.7 (10)	9.4 (96)	
Total	100 (452)	100 (153)	100 (343)	100 (78)	1026	

When the blood groups of the COVID-19 cases are evaluated, no difference was found between the groups in terms of clinical data, except for D-dimer (p=0.021). D-dimer was highest at AB(-), followed by B(+), A(+), 0(+), A(-), AB(+), B(-), and lowest at 0(-).

When the laboratory data of the cases are evaluated according to the ABO blood groups, the leukocyte count was found to be the highest in the B group, and followed by 0, A and the lowest in the AB groups, respectively; and this difference was found to be statistically significant (p=0.016). No statistical difference was found in other parameters (**Table 4**).

When demographic features and laboratory data are evaluated according to Rh status, no statistically significant difference was found (p>0.05), **Table 5**.

DISCUSSION

The first description with ABO blood type and severe acute respiratory distress syndrome (SARS-1) was an observation of reduced likelihood of infection in patients with blood type O (9). Later, this interesting finding supported with more evidence by the discovery of virion particles replicating in epithelial cells of the respiratory tract in blood type A or B individuals were covered with A or B antigens (10). This provided the shed viral particles easily recognized by type O individuals harboring both anti-A and anti B antibodies in their sera. In addition, similar configuration found between the A antigen and parts of the ACE2 receptor which is the primary site of entry for the virus into the body. Thus, anti-A antibodies circulating in type O individuals might able to prevent the binding and subsequent cellular entry of the virion into the cells. This observation would fulfill the same biologic effect preventing cellular entry of novel COVID-19 (also named SARS-CoV-2). There is also an assumption of increased prevalence of hypercoagulability in individuals carrying blood type A which is linked to the severity of COVID-19 particularly in adults (11,12). In our study, there were not any hypercoagulability condition, but on D-dimer values AB(-) type blood group had the highest value according to the other blood groups.

Most recently, there is growing evidence of ABO and Rh blood groups are associated with risk for COVID-19 illness in adults (13–16). Most studies have concluded a relation between ABO blood groups and COVID-19 infection with respect to blood type O induviduals were less infected than other blood types. In contrast, blood types A and AB found to be a high risk for pneumonia, mechanical ventilation requirement, prolonged intensive care unit admission and death. Additionally, few studies suggested

Table 4: Demographic features and laboratory data of the COVID-19 cases according to ABO blood groups.					
	A	B	O	AB	p
Age (months)	124 ± 71	120 ± 67	125 ± 71	125 ± 72	0.953
Sex (F/M)	46% F, 54% M	55% F, 45% M	46% F, 54% M	47% F, 53% M	0.107
Leukocyte (/mm3)	6254 ± 3391	6900 ± 3189	6865 ± 3287	5661±2825	0.016
Lymphocyte (/mm3)	2000 (10-21000)	2140 (90-17520)	2300 (10-12000)	1737 (10-10170)	0.109
Hemoglobin (gr/dL)	12.3± 1.5	11.9 ± 1.3	12.0 ± 1.4	11.7 ± 2.3	0.117
C- reactive protein (mg/dL)	3.1±5.6 ; 11(0.3-33)	2.5±4.3 ; 1(0,3-19)	3.8±7.6 ; 11 (0.3-55)	4.5±7.2;1.1 (0.2-32)	0.222
Ferritin (ug/dl)	25 (1-2380)	31 (1-4563)	26 (1-4563)	23,5 (12-504)	0.303
D-dimer (µg/ml)	0.3 (0.1-28)	0.4 (0.1-31)	0.34 (0.03-24)	0,3 (0,12-29)	0.332
Abbrevations: F: female, M: male.					

Table 5: Demographic features and laboratory data of COVID-19 cases according to Rh status			
	Rh pozitive	Rh negative	p
Age (months)	114 ± 67.1	129 ± 71.5	0.545
Sex (F/M)	46% F, 54% M	51% F, 49% M	0.872
Leukocyte (/mm3)	6301 ± 3431	6900 ± 3189	0.317
Lymphocyte (/mm3)	2200(110-12520)	2140 (90-17520)	0.387
Hemoglobin (gr/dL)	11.8 ± 1.6	11.9 ± 1.3	0.265
C- reactive protein (mg/dL)	2±3.6;1(0.3-15)	2.5±4.3;1(0.3-19)	0.624
Ferritin (ug/dl)	12 (1-1026)	31 (1-4563)	0.716
D-dimer (µg/ml)	0.3 (0.1-24)	0.4 (0.1-31)	0.076
Abbrevations: F: female, M: male.			

that Rh negatif blood type had more protective effect than Rh positive type in above mentioned morbidity and mortality (17,18). In our study there were not any clinical significant difference between blood types, ABO blood groups or Rh status and clinical severity condition.

Another interesting observation is the proportion of O blood group individuals to non-O blood group individuals may vary in different countries (19,20). It is well known that some countries heavily struck by the morbidity and mortality of COVID-19. Such countries are the United States, Italy, Spain and Brazil which all shared a percentage of group O individuals lower than 40% of the population. While countries showing relatively less COVID-19 mortality such as Saudi Arabia, Egypt, and Singapore all had a percentage of O blood group individuals greater than 40%. Our country appears to be in the lower percentage of blood type O countries with a distrubition of blood type A 39.99%, blood type O 28.26%, blood type B 17.09% and blood type AB 14.66%, respectively (21).

To our knowledge, the current study is the pioneer multicenter trial in investigating the risk ABO and Rh bloods groups in children with COVID-19 with a considerable number of study participants. Importantly, our study control group reflects similar findings of the national blood type research results (22).

In this study, higher age, leucocyte count, CRP, ferritin, D-dimer values were associated with cinical severity. In another studies or meta-analyzes these findings were similar with our study (23–25).

In similar studies, the proportion of blood group A in patients infected with SARS-CoV-2 was significantly higher than that in healthy controls (39.3% vs. 32.3 %, p=0.017), while the proportion of blood group O in patients infected with SARS-CoV-2 was significantly lower than that in healthy controls (13). In our study, especially in pediatric population, there was no any significant difference between blood groups. Both of control and COVID-19 group were similar (p=0.062).

According to population-based cohort study to determine whether ABO and Rh blood groups are associated with risk for SARS-CoV-2 infection and severe coronavirus disease 2019 (COVID-19) illness; there was also a lower risk for severe COVID-19 illness or death associated with type O blood group versus all others (adjusted relative risk-aRR-, 0.87 [CI, 0.78 to 0.97]; Absolute risk differecence (ARD),-0.8 per 1000 [CI, -1.4 to -0.2]). Also with Rh negative versus Rh positive (aRR, 0.82 [CI, 0.68 to 0.96]; ARD, -1.1 per 1000 [CI, -2.0 to -0.2]) status (17). So the O and Rh blood groups may be associated with a slightly lower risk for SARS-CoV-2 infection and severe COVID-19 illness. In our study both of control and COVID-19 blood group distribution were similar (p=0.062). Also there was no statistical difference between the groups distribution in terms of blood group (ABO; p=0.076) and Rh status (p=0.3).

In another study; COVID-19 patients with blood group A or AB required mechanical ventilation (p= 0.02) compared with patients with blood group O or B (15). Also total leucocyte counts, and D-dimer values were higher in A or AB group compared to group O or B (15). In our study, acoording to clinical laboratory results of blood groups, leucocyte count were higher in O or B group contrastly to the similar study (p=0.016). Also D-dimer values were not statistically different between blood groups (p=0.332)

CONCLUSION

In our study with pediatric population, there was no difference between blood types or Rhesus condition and COVID-19 severity. There may be more meaningful results that can be obtained in groups with more participants.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocols were approved by Mersin University Clinical Researches Ethics Committee (Decision No: 2021/53, Date: 01/20/2021).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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