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Effects of EDTA and Sodium Citrate on Platelet Indices: Should we use MPV or MPV/PC Ratio?

EDTA ve Sodyum Sitratın Trombosit İndeksleri Üzerindeki Etkileri: MPV'yi mi Yoksa MPV/PC Oranını mı Kullanmalıyız?

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ABSTRACT

Objective: This study was performed to compare the effectiveness of anti-coagulation with ethylenediamine tetraacetic acid (EDTA) and citrate on mean platelet volume (MPV) measurement.

Methods: EDTA and citrate-based anti-coagulant blood samples from the same patients were read in the auto-analyzer at 0, 30, 60, and 120 minutes after sampling and the results were compared.

Results: A total of 54 patients, 29 of whom were women, over the age of 18 were included in the study. Baseline blood MPV values were found to be 0.524 fL greater in the EDTA-based group ($p<0.001$). When the difference between the time periods in the EDTA group was examined, it was observed that there was a significant increase in deltaMPV values in each 30-minute time period. When the difference between the time periods in the citrate-based group was examined, there was a significant difference at the 30^{th} and 60^{th} minutes ($p<0.001$), however the difference disappeared at the second hour ($p>0.05$). When the deltaMPV values of the EDTA and Citrate groups were compared, it was found that there was no difference at 30 and 60 minutes ($p=0.531$ and $p=0.566$, respectively). In addition, it was found that there was no significant difference between deltaMPV/platelet count ratios (deltaMPV/PC) in all time periods in the EDTA group ($p>0.05$) and there was a significant difference between all time periods in the citrate group ($p<0.001$).

Conclusion: Results in the first hour were similar in both anti-coagulation groups. However, additional increases were observed in each half-hour period in both groups. Making measurements

ÖZ

Amaç: Bu çalışma, etilendiamin tetraasetik asit (EDTA) ve sitrat ile yapılan anti-koagülasyonun ortalama trombosit hacmi (MPV) ölçümü üzerindeki etkinliğini karşılaştırmak için yapılmıştır.

Yöntemler: Aynı hastalardan alınan EDTA ve sitrat bazlı anti-koagülan kan örnekleri, örneklemeden 0, 30, 60 ve 120 dakika sonra oto-analizörde okundu ve sonuçlar karşılaştırıldı.

Bulgular: Çalışmaya 29'u kadın olan, 18 yaş üstü toplam 54 hasta dahil edildi. Başlangıç kan MPV değerlerinin, sitrat grubuna karşın EDTA bazlı grupta 0,524 fL daha yüksek olduğu bulundu ($p<0,001$). EDTA grubundaki zaman periyotları arasındaki fark incelendiğinde her bir 30 dakikalık zaman diliminde deltaMPV değerlerinde anlamlı bir artış olduğu gözlemlendi. Sitratlı grupta zaman periyotları arasındaki fark incelendiğinde, 30. ve 60. dakikalarda anlamlı fark bulunurken ($p<0,001$), 2. saatte bu fark ortadan kalktı ($p>0,05$). EDTA ve Sitrat gruplarının deltaMPV değerleri karşılaştırıldığında 30. ve 60. dakikalarda fark olmadığı görüldü (sırasıyla; $p=0,531$; $p=0,566$). Ayrıca EDTA grubunda tüm zaman periyotlarında deltaMPV/platelet sayı oranları (deltaMPV/PC) arasında anlamlı fark olmadığı ($p>0,05$), buna karşın sitrat grubunda tüm zaman periyotları arasında anlamlı fark olduğu bulundu ($p<0,001$).

Sonuç: İlk saatteki sonuçlar her iki anti-koagülasyon grubunda da benzerdi. Ancak her iki grupta her bir yarım saatlik periyotta ek anlamlı artışlar gözlendi. MPV ölçümlerinin ilk bir saat içinde ve aynı yarım saatlik zaman diliminde yapılması oluşabilecek ölçüm hatalarını ortadan kaldırır. Anti-koagülan olarak EDTA

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ABSTRACT

of MPV within the first hour and in the same half-hour period minimizes the measurement errors that may occur. When EDTA is used as an anti-coagulant, it performs better than MPV due to the fact that there is no change in the MPV/PC ratio during the first two hours.

Keywords: Anti-coagulation method, EDTA, MPV, MPV/PC ratio, platelet indices, sodium citrate

ÖZ

kullanıldığında, ilk iki saat içinde MPV/PC oranında bir değişiklik olmaması nedeniyle MPV'den daha iyi bir performans gösterir.

Anahtar Sözcükler: Anti-koagülasyon yöntemi, EDTA, MPV, MPV/PC oranı, trombosit indeksleri, sodyum sitrat

Introduction

Platelets have a critical role in immune reactions, fibrosis, and normal hemostasis. Platelets are activated by classical agonists such as platelet activating factor, thromboxane A2, ADP and proinflammatory cytokines and accumulate in the damaged area and initiate fibrosis and inflammation by releasing their contents (1). Platelets are primarily involved in the inflammation cascade in many rheumatological diseases with neutrophils and lymphocytes (2). Mean platelet volume (MPV), a measure of the mean size of platelets, is often used as a platelet activation marker. Large platelets secrete more proinflammatory cytokines and prothrombotic factors than small platelets. Thus, MPV is used both as an indicator of the severity of inflammation and as a platelet activation marker (3). It has been shown that MPV can be used as a biomarker to predict increased activity or disease severity in some diseases (4-6).

The blood samples should be anticoagulated to enable electronic cell counters to count blood cells. For this purpose, sodium citrate or ethylenediamine tetraacetic acid (EDTA) is used, generally. In previous studies, it has been shown that EDTA causes swelling in the platelet by increasing intracellular cAMP and increasing the permeability of the plasma membrane, which results in an increase in MPV (7). In a study conducted by Bath and Butterworth (8), MPV would increase as the time elapsed, until the blood was counted, when EDTA was used as an anticoagulant, and they suggested that tests could be done more safely by using citrated blood. In the literature, whether the MPV level is lower or higher in patients with Familial Mediterranean fever (FMF) compared to healthy controls is still controversial. Sakallı and Kal (4) found a significant correlation between proteinuria and MPV in their study on patients with FMF-induced amyloidosis. However, in a similar study by Bakan et al. (9), such a correlation was not found.

It is known that varying results can be obtained due to the lack of a standardized method of MPV measure, technical differences in electronic cell counters, different anticoagulants used, and the variability of the time elapsed between the contact of the blood with the anticoagulant and it is put into the counter. In 2022, considering the factors such as the use of advanced devices that we use in the modern era and the prolongation of the time between the anticoagulant contact and the reading of the blood after the construction of large-scale hospitals, it is not known exactly how far we advance in this regard. In the present study, we aimed to investigate the effectiveness of anti-coagulation with EDTA and citrate on platelet indices.

Materials and Methods

Study Design and Study Population

This study was designed as a single-center, prospective study. The patients who were admitted to internal medicine department between April 15th, 2022 and May 15th, 2022 and over the age of 18 were included in the study. Patients with Wiskott-Aldrich syndrome, essential thrombocythemia, splenectomy, hereditary macrothrombocytopenia, hematological or solid organ malignancies, those using anti-aggregant or anti-coagulants, alcohol users, pregnant women, and patients receiving immunosuppressive therapy and chemotherapy were excluded from the study. A written informed consent was obtained from each patient for all diagnostic procedures. The study protocol was approved by the institutional ethics committee (date: 05.04.2022/no: E-10840098-772.02-2203). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Collection

Blood samples were collected from the patients who were admitted to the internal medicine outpatient clinic and met conditions for the study and the results were recorded in digital media. Data including age, sex, height, weight, blood pressure, comorbidities such as coronary artery disease, diabetes, or hypertension and complete blood count results were recorded. Blood samples of the patients (n=54) were collected from the brachial vein in the antecubital region and stored in two tubes, one with sodium citrate and one with EDTA. To collect samples in a citrate tube, 0.5 mL of sodium citrate and 4.5 mL of blood were added and mixed gently by inverting the stoppered tube. Tripotassium ethylenediaminetetraacetic acid, also known as K3 EDTA, was used to prepare the tube with EDTA. The collected blood samples were stored at room temperature (20-22 °C). Sysmex XN-550 (Sysmex, Japan) autoanalyzer device was used for blood count. MPV was measured by impedance technology with Sysmex XN-550 hematological analyzer. The duraiton between the collection of the blood samples and reading on the automatic blood cell counter was 10 min on average and this initial count was defined as the zero min (baseline blood). Then, at the next 30, 60 and 120 min, the same samples were read again in the auto-analyzer.

At the time of admission, complete blood count analysis including hemoglobin level, leukocyte count, platelet count (PC), absolute neutrophil count, absolute lymphocyte count, and MPV was performed for each patient. Relevant ratios for MPV/PC ratio, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio were calculated and included in the analysis.

Statistical Analysis

Statistical analyses were performed by using the SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation, median (minimum-maximum) or number and frequency, where applicable. The Student's t-test was used to compare normally distributed variables between the groups and the Mann-Whitney U test was performed to compare non-normally distributed variables between the groups. Categorical variables were analyzed by using the chi-square test. Pearson correlation coefficient was used to analyze the correlation between the EDTA group and citrate group. A value of $p<0.05$ was considered statistically significant.

Results

After applying the exclusion and inclusion criteria, 54 volunteer patients over 18 years of age, 29 of whom were female, were included in the study. The mean age of the patients was 43.1±13.9 years and the mean age of the male group (49.2±11.7) was significantly higher compared to the females (37.7±13.7) (F=1.08, p=0.002). The distribution of the sexes was similar ($\chi 2=0.296$, p=0.586). The mean body mass index (BMI) was 26.6±4.7, and there was no difference between the sexes in terms of BMI values (F=2.86, p=0.073). The rate of smokers was 16.7% (n=9). Systolic, diastolic and mean arterial blood pressures and comorbidities of the patients are shown in Table 1.

When baseline blood (zero-min blood) values were compared between the two groups, it was observed that white blood cell, neutrophil, lymphocyte, platelet, hemoglobin, and hematocrit values were significantly higher in the EDTA group (Table 2). The MPV values obtained in the baseline blood count were found to be 0.524 fL greater in the EDTA group compared to the citrate group (t=14.2; p<0.001). When the deltaMPV values were compared according to the time intervals including 0-30, 30-60, 60-120 min, a significant increase was observed (deltaMPV30-0-EDTA: 0.285±0.2 fL; t=9.7; p<0.001, deltaMPV60-30-EDTA: 0.194±0.2 fL; t=8.5; p<0.001, and deltaMPV120-60-EDTA: 0.130±0.2 fL; t=4.7; p<0.001, respectively). When the deltaMPV values were compared according to the time intervals including 0-30, 0-60 and 0-120 min, again it was observed that deltaMPV values increased significantly (deltaMPV30-0-EDTA: 0.285±0.2 fL; t=9.7; p<0.001, deltaMPV60-0-EDTA: 0.480±0.2 fL; t=16.9; p<0.001, and deltaMPV-120-0-EDTA: 0.609±0.3 fL; t=14.4; p<0.001, respectively) (Table 3).

In the citrated blood group, when the MPV values were compared according to the time intervals including 0-30, 30-60 and 60-120 min, it was found that there were significant differences between the baseline value and 30th and 60th min (deltaMPVCit-30-0: 0.320±0.3 fL t=7.2, p<0.001; deltaMPVCit-60-30: 0.130±0.3 fL, t=2.9, p=0.005 respectively), while there was no significant difference at the 2nd hour (deltaMPVCit-120- 60; -0.056±0.3 fL, t=-1.4, p=0.172) (Table 3).

Table 1. Demographic and clinical characteristics of patients

		N	Percent %	Mean ± SD	Min	Max	t	p
Age (year)	Total	54	100	43.1±13.9	18.0	68.0		
	Female	29	53.7	37.7±13.7	18.0	67.0	-3.3	**0.002**
	Male	25	46.3	49.2±11.7	25.0	68.0		
BMI (kg/m^2)	Total	54	100	26.6±4.7	15.4	40.3		
	Female	29	53.7	25.5±4.9			-1.8	0.073
	Male	25	46.3	27.8±4.2				
Systolic BP (mmHg)		54	100	114.6±7.9	100.0	145.0		
Diastolic BP (mmHg)		54	100	78.2±4.5	64.0	95.0		
MAP (mmHg)		54	100	90.4±5.0	79.3	112.0		
Smoker	Smoker	9	16.7					
	Non-smoker	45	83.3					
Diabetes		10	18.5					
Hypertension		6	11.1					
CAD		2	3.7					
Hyperlipidemia		6	11.1					

BMI: Body mass index, CAD: Coronary arterial disease, MAP: Mean arterial pressure, SD: Standard deviation

When the deltaMPV values of the groups were compared, no significant differences were found at 30 and 60 min (respectively delta-deltaMPV30-0: -0.035 fL, t=-0.6, p=0.531; delta-deltaMPV60-0: 0.030 fL, t=0.6, p=0.566; delta-deltaMPV60-30: 0.065 fL, t=1.4, p=0.182), however we found that deltaMPV values were significantly higher in the EDTA group at 120 min (delta-deltaMPV120-60: 0.185 fL, t=3.9, p<0.001) (Table 3).

When the MPV/PC ratios were compared in the groups, no significant differences were found between the baseline, 30, 60, and 120 min in the EDTA group (p>0.05), while significant differences were found between the MPV/PC ratios at 30, 60 and 120 min in the citrate group (p<0.001) (Table 4).

There was no significant difference in MPV, delta MPV, and MPV/PC harvested with EDTA or Citrate between patients with or without diabetes, coronary artery disease, and hyperlipidemia (p>0.05). In addition, there was no significant differences of MPV values according to the presence of hypertension in both groups (p>0.05). It was found that, MPV/PC ratio at 30, 60 and 120 min in the EDTA group were higher in patients with hypertension (U=231, p<0.01; U=222, p<0.05; U=214, p<0.05 respectively), however no significant relationship was found in the citrate group (p>0.05).

Discussion

In this study, we examined the changes in MPV values as the time elapsed between the contact of blood in hemogram tubes containing EDTA and citrate as anticoagulants and loading into the automatic blood count device. The baseline, 30th, 60th and 120th min MPV values of the blood samples in EDTA group were found to be significantly higher compared to the citrated blood (0.524 fL, t=14.2, p<0.001; 0.489 fL, t=9.6, p<0.001; 0.554 fL,

Table 2. Baseline (0 min) complete blood count analysis

		N	Mean ± SD	Min-max	t	p
WBC (x10^6/L)	EDTA	54	6743.3±1539.6	Min =3,510; max =10,530	14.6	**<0.001**
	Citrate	54	6050.7±1363.1	Min =33.70; max =9,580		
Neutrophil (x10^6/L)	EDTA	54	3806.3±1058.4	Min =1,760; max =6,710	16.7	**<0.001**
	Citrate	54	3392.2±951.6	Min =1,650; max =6,140		
Lymphocyte (x10^6/L)	EDTA	54	2227.1±721.6	Min =990; max =4,420	9.9	**<0.001**
	Citrate	54	2007.4±638.9	Min =890; max =4,090		
Hgb (g/dL)	EDTA	54	13.5±1.7	Min =9.2; max =17.0	28.2	**<0.001**
	Citrate	54	12.1±1.5	Min =8.3; max =15.1		
Hct (%)	EDTA	54	40.5±4.7	Min =28.8; max =50.1	25.0	**<0.001**
	Citrate	54	36.7±4.2	Min =26.0; max=45.0		
PC (x10^9/L)	EDTA	54	244.5±58.8	Min =63.0; max =369.0	12.3	**<0.001**
	Citrate	54	185.5±41.6	Min =82.0; max =275.0		
MPV (fL)	EDTA	54	10.2±0.8	Min =8.7; max =12.4	14.2	**<0.001**
	Citrate	54	9.6±0.8	Min =8.3; max =12.1		
MPV/PC	EDTA	54	0.045±0.02	Min =0.03; max =0.18	-3.4	**<0.001**
	Citrate	54	0.056±0.02	Min =0.03; max =0.15		
NLR	EDTA	54	1.87±0.8	Min =0.77; max =5.37	1.1	0.295
	Citrate	54	1.85±0.9	Min =0.74; max=5.45		
PLR	EDTA	54	0.12±0.05	Min =0.02; max=0.28	8.9	**<0.001**
	Citrate	54	0.10±0.04	Min =0.04; max =0.23		

EDTA: Etilendiamin tetraasetik asit, Hb: Haemoglobin, Hct: Haematocrit, MPV: Mean platelet volume, MPV/PC: Mean platelet volume to platelet count ratio, NLR: Neutrophil-to-lymphocyte ratio, PC: Platelets count, PLR: Platelet-to-lymphocyte ratio, SD: Standard deviation, WBC: White blood cell

Table 3. Differences between time frames and anti-coagulants for MPV

Paired-Samples t-test	Mean (fl)	SD ±	95% CI		t	Sig (2-tailed)
			Lower	Upper		
deltaMPV-30-0-EDTA	0.285	0.22	0.23	0.34	9.7	**0.000**
deltaMPV-60-0-EDTA	0.480	0.21	0.42	0.54	16.9	**0.000**
deltaMPV-120-0-EDTA	0.609	0.31	0.52	0.69	14.4	**0.000**
deltaMPV-60-30-EDTA	0.194	0.17	0.15	0.24	8.5	**0.000**
deltaMPV-120-60-EDTA	0.130	0.20	0.07	0.19	4.7	**0.000**
deltaMPV-30-0-Citrate	0.320	0.33	0.23	0.41	7.2	**0.000**
deltaMPV-60-0-Citrate	0.450	0.35	0.36	0.54	9.6	**0.000**
deltaMPV-120-0-Citrate	0.394	0.34	0.30	0.49	8.5	**0.000**
deltaMPV-60-30-Citrate	0.130	0.33	0.04	0.22	2.9	**0.005**
deltaMPV-120-60-Citrate	-0.056	0.30	-0.14	0.02	-1.4	0.172
delta-deltaMPV-30-0 (E-C)	-0.035	0.41	-0.15	0.08	-0.60	0.531
delta-deltaMPV-60-0 (E-C)	0.030	0.37	-0.07	0.13	0.60	0.556
delta-deltaMPV-60-30 (E-C)	0.065	0.35	-0.03	0.16	1.4	0.182
delta-deltaMPV-120-0 (E-C)	0.215	0.44	0.10	0.33	3.6	**0.001**
delta-deltaMPV-120-60 (E-C)	0.185	0.35	0.09	0.28	3.9	**0.000**

EDTA: Etilendiamin tetraasetik asit, MPV: Mean platelet volume, SD: Standard deviation

Table 4. Differences between time frames and anti-coagulants for MPV/PC

Paired-Samples t-test	Mean (fl)	SD ±	95% CI		t	Sig (2-tailed)
			Lower	Upper		
deltaMPV/PC-30-0-EDTA	0.004	0.02	-0.00019	0.0077	2.0	0.062
deltaMPV/PC-60-0-EDTA	0.003	0.02	-0.0008	0.0066	1.54	0.130
deltaMPV/PC-60-30-EDTA	0.003	0.01	-0.001	0.0066	1.5	0.130
deltaMPV/PC-120-0-EDTA	0.004	0.02	-0.001	0.0083	1.8	0.071
deltaMPV/PC-30-0-Citrate	0.009	0.01	0.005	0.0129	4.7	**0.000**
deltaMPV/PC-60-30-Citrate	0.009	0.02	0.003	0.0159	3.2	**0.003**
deltaMPV/PC-60-0-Citrate	0.019	0.03	0.011	0.0265	4.8	**0.000**
deltaMPV/PC-120-0-Citrate	0.025	0.03	0.0162	0.0331	5.8	**0.000**

EDTA: Etilendiamin tetraasetik asid, MPV/PC: Mean platelet volume to platelet count ratio, SD: Standard deviation

t=13.1, p<0.001; and 0.739 fL, t=15.4, p<0.001, respectively) (Table 3). As the time elapsed until the EDTA blood was read in the automatic blood count device, significant increases were observed in MPV values in every half an hour (deltaMPV30-0: 0.285 fL, p<0.001; deltaMPV60-30: 0.194 fL, p<0.001; and deltaMPV120-60: 0.130 fL, p<0.001) (Table 3). Similarly, significant increases were observed in MPV values in the 30th and 60th min until the citrated blood was read in the automatic blood count device, however the MPV values remained stable at the second hour and no significant change was observed (deltaMPV30-0: 0.320 fL, t=7.2, p <0.001; deltaMPV60-30: 0.130 fL, t=2.9, p=0,005; deltaMPV120-60: 0.056 fL, t=-1.4, p=0.172, respectively) (Table 3).

When the deltaMPV values at 0-30 and 30-60 min were compared between the groups, no significant differences were found (delta-deltaMPV0-30: -0.035 fL, t=-0.6, p=0.531; delta-deltaMPV60-30: 0.065 fL, t=1.4, p=0.812; delta-deltaMPV 60-0: 0.030 fL, t=0,60, p=0.556, respectively) (Table 3). However the differences between the groups were significant in terms of the deltaMPV values at 60-120 min (delta-deltaMPV60-120: 0.22 fL, t=3.6, p<0.001). Similar results were obtained in both anticoagulation groups, provided the blood was read in the auto-analyzer within the first hour.

When we searched Pubmed for MPV, we found that 2,183 articles were written in the last five years. In a study by Dastjerdi et al. (10), consistent with this study, the results were similar in both anticoagulation types, if the collected blood samples were loaded into the autoanalyzer and the differences were not significant. However, they did not examin the differences for each half-hour period of the first hour. In a study by Lancé et al. (11), MPV was measured every half hour for four hours. Similar to our study, they emphasized that platelets swelled until the first 120 min in EDTA-based blood, whereas platelet swelling in citrate-based blood occured only in the first hour. However, in this study, it was not emphasized that the measurements should be made, particularly in the same half-hour time period of the

first one-hour. In another study, it was reported that MPV was not affected when low concentrations of citrate were used, and therefore anti-coagulation with citrate was safe (7). Otherwise, in our study, we observed that significant increases in MPV continued with citrated blood as the incubation period extended in the first hour. The differences between the half-hours in the first hour were even significant.

The MPV value has been widely used in many studies to determine disease progression and disease activity, as well as to detect the need for intensive care earlier in some patients, and to predict mortality. However, the results of many studies are frequently contradictory. Khan et al. (12), found no change in MPV in patients with psoriasis in their study. Jiang et al. (13) reported that mean platelet volume is a strong, independent prognostic factor in patients with DM and stable coronary artery disease treated with elective percutaneous coronary intervention. In the study conducted by Akbaş (14) in patients with pediatric migraine, PC increased, whereas MPV values decreased compared to the control group and it was concluded that inflammation played an important role in the pathophysiology of migraine. Vélez-Páez et al. (6) reported that MPV and MPV/PC ratio could be used as indicators of disease severity and mortality in patients with sepsis. There were publications stating that measurement of MPV might be useful in cardiovascular disease risk assessment (15,16), whereas it was emphasized that larger platelets were not associated with the incidence and severity of coronary artery disease in a study conducted on patients with metabolic syndrome (17). In another study, MPV was found to be a predictor of early prognosis in ischemic stroke, whereas PC was found to be a predictor of early prognosis in hemorrhagic stroke (18).

The conflicting results related to MPV in the literature has caused these results to be questioned. In most studies, it has been determined that MPV values increase more significantly when EDTA is used as an anti-coagulant. Similarly, in our study, the increase in MPV values was found to be significantly higher in the EDTA group. However, we found that MPV was also significantly increased in the citrate group, although not as much as in the EDTA group. When the deltaMPV values of both groups at 0-30, 0-60 and 30-60 min were compared, we found that there was no significant difference between both groups (p>0.05). However, while a significant increase in MPV value continued in EDTA blood, it remained stable in citrated blood, after the first hour. As a result, deltaMPV values at the 2nd hour were found to be significantly higher in the EDTA group compared to the citrate group (p<0.001). Therefore, if the samples are loaded into the analyzer within the first hour, one of the two anticoagulated blood may be preferred. However, we concluded that whether the studies should be performed in the first or second half of the first hour should be standardized to increase the power of the studies and to minimize the variability.

The relationship between PC and MPV/PC ratio and diseases has been investigated in many studies. Similar to previous studies, we also found an inverse correlation between MPV and PC in the measurements made with both anticoagulants (r=-0.35 for EDTA, p=0.009; r=-0.29 for citrate, p=0.031) (19). Zhong and Peng (20) concluded in their study on patients with coronavirus disease 2019 that MPV/PC ratio could be used as a useful marker predicting severe pneumonia. In a study including patients with glioblastoma multiforme, the MPV/PC ratio was found to be an independent predictor of survival without progression (21). Lin et al. (22) concluded in their study that the MPV/PC ratio could be used as an independent risk factor associated with disease progression in various cancer types. In our study, while there was no significant difference between deltaPC30, deltaPC60 and deltaPC120 in the EDTA group (p>0.05), a significant decrease in PC was observed in the citrate group as the time elapsed, resulting in a significant difference between deltaPC30, deltaPC60 and deltaPC120 (p<0.001). Comparing the MPV/PC ratio, there was no significant difference between the 30th, 60th and 120th min in the EDTA group (p>0.05), whereas MPV/PC at the 30th, 60th and 120th min differed significantly in the citrate group, due to the decrease in PC over time (p<0.001) (Table 3). We concluded that EDTA should be preferred as an anti-coagulant, since there was no time limitation in studies related to MPV/PC ratio and there was no significant difference between time periods. Since the MPV/PC ratio obtained in EDTA blood did not change for 2 hours, we suggested that it might be preferred over MPV. Likewise, since there was no significant change in PC, we concluded that EDTA should be used as an anticoagulant without time constraints.

Icli et al. (23) showed that MPV was increased in patients with hypercholesterolemia. However, in our study, no correlation was found between MPV or MPV/PC ratio and hyperlipidemic patients (F=1.16, p>0.05, F=21.2, p>0.05, respectively). The fact that this was a small-scale study or the normalization of total cholesterol values with the hypolipidemic drugs used by the patients might affect it. In the previous studies it was shown that both gestational diabetes and uncontrolled diabetes were positively correlated with MPV (24,25). However, in our study, no correlation was found between MPV, PC and MPV/PC ratio and diabetes in both anti-coagulated groups (p>0.05). In a study conducted by Sansanayudh et al. (15), increased MPV was associated with coronary artery disease. However, we did not find such a relationship in our study (p>0.05). In addition, no correlation was found between the MPV values and hypertension in both groups (p>0.05). On the other hand, although there was a significant correlation between MPV/PC ratio and hypertension in the EDTA group at 30th, 60th, and 120th min (U=231, p=0.017; U=222, p=0.032; U=214, p=0.048, respectively), no relationship was detected in the citrate group (p>0.05).

Study Limitations

The limitations to our study include the small sample size, the limitation of the measure to 120 min, and not being able to verify MPV values with different measurement methods.

Conclusion

In conclusion, if the blood is loaded into automatic blood cell autoanalyzers in the first hour, there is no difference between the use of citrate or EDTA as an anti-coagulant, and any of them can be preferred. However, since the increase in MPV values differs significantly between each 30-min time frame of the first hour, in both groups, we suggest that the measurements should be performed in the same half-hour time frames. We concluded that EDTA should be preferred as an anti-coagulant because there was no time limitation and no significant difference between time periods in studies related to MPV/PC ratio. We concluded that it would be more appropriate to prefer MPV/PC ratio to MPV values, since there was no difference even between each 30-min time frame for the first 2 hours.

Ethics

Ethics Committee Approval: The study protocol was approved by the institutional ethics committee (date: 05.04.2022/no: E-10840098-772.02-2203).

Informed Consent: A written informed consent was obtained from each patient for all diagnostic procedures.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: T.K., İ.B., Concept: T.K., Design: T.K., Data Collection or Processing: T.K., İ.B., Analysis or Interpretation: T.K., İ.B., Literature Search: T.K., İ.B., Writing: T.K., İ.B.

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