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RESEARCH ARTICLE OPEN ACCESS

IS ACID CITRATE DEXTROSE SOLUTION (ACD) BETTER THAN OTHERS ANTICOAGULANTS FOR PSEUDOTHROMBOCYTOPENIA?

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ABSTRACT

Pseudothrombocytopenia is a worrying clinical phenomenon that can result in an error of diagnosis and inadequate therapeutic conduct, if the necessary knowledge to differentiate this condition from the real condition does not act. It is not known for certain what causes this condition. The mean platelet volume (MPV) and the normal bleeding time indicate that the platelet decrease is induced during the in vitro process. It is worth mentioning that the phenomenon of thrombocytopenia linked to the EDTA anticoagulant is not permanent, and may fluctuate periods when false thrombocytopenia is detected or not. This is due to the unknown pathophysiology of antibody production. Some researchers attribute the phenomenon of platelet IN VITRO agglutination only when the EDTA anticoagulant is present. Because of this, a clinical laboratory protocol will be developed when Pseudothrombocytopenia is suspected. The simple method consists of shaking the EDTA tube in the vortex for approximately one minute in order to dissolve the platelet aggregates, and to be reprocessed in the equipment if it is not efficient, a recollection using other tubes with alternative anticoagulants such as, for example, sodium citrate or ACD. These two tubes have shown extremely positive results in preserving the coagulation sample. The aim of this study is to evaluate the effectiveness of the ACD anticoagulant in relation to EDTA and sodium citrate anticoagulants in patients who present with pseudothrombocytopenia through a data survey. As a methodology, medical records will be used anonymously for patients from the Clinical Analysis Laboratory - Campinas / SP, who are laboratory-dependent EDTA. The medical records will be evaluated retroactively for a period of six months to assess laboratory data against the different anticoagulant effects. The data will be compiled and analyzed with statistical software and shows that ACD is better than the others anticoagulants.

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INTRODUCTION

Platelets are anucleated cytoplasmic fragments produced from the megakaryocyte, derived from the bone marrow, with erythrocytes and leukocytes constitute the elements that make up the blood (Mesa; Alfonso, 2000). In 1969, EDTA-dependent pseudothrombocytopenia was described by Gowland as a false thrombocytopenia indicated by the devices caused by the formation of platelet aggregates most commonly in the EDTA tube with blood. This phenomenon was not observed when the platelet count was performed on a smear immediately after puncture, or when other types of anticoagulants were used (van der Meer, 2002). The formation of platelet aggregates occurs when the blood comes in contact with EDTA (ethylenediamino tetraacetic acid)anticoagulant, making the differential diagnosis difficult when in fact the patient has thrombocytopenia. This condition can occur regardless of the existence of any disease or use of drugs. It is believed that it may be linked to autoantibodies that in the presence of EDTA bind to a specific glycoprotein promoting platelet agglutination. This phenomenon according to the literature represents among 0.09 to 0.11% of the cases of false EDTA-linked thrombocytopenia (Green, 2015). It is assumed that its highest incidence is in hospitalized patients, patients with liver, autoimmune and neoplastic diseases and patients who are under the use of medications for coronary intervention. However, it is important to highlight that pseudothrombocytopenia can occur autonomously in relation to the

use of drugs or the coexistence of any type of comorbidity (Lippi; Plebani. 2012). The pathophysiological nature of pseudothrombocytopenia is still unknow. However, it is believed that antibodies present in the plasma recognize and bind to an epitope of glycoprotein IIb (GPIIb), part of the GPIIb / IIIa complex of the platelet surface, causing platelet agglutination to occur. This epitope is only exposed in the presence of EDTA. The presence of antibodies in the plasma is fluctuating and may alternate periods when pseudothrombocytopenia is detected or not (Onder, 1980). The aim of this study was to evaluate the effectiveness of using the anticoagulant Citric Acid, Sodium Citrate and Dextrose (ACD) in relation to EDTA anticoagulants and Sodium Citrate in patients who had pseudothrombocytopenia through a survey of laboratory data.

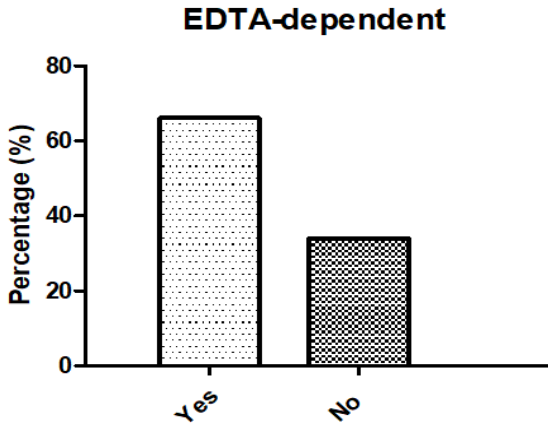

Figure 1. Percentage of EDTA-dependent and non-dependent patients

METHODOLOGY

This is a study with a quantitative, retrospective and cross-sectional approach. The study was approved by the Ethics and Research Committee of the University Center of Fundação Hermínio Ometto, Araras, SP, Brazil, CAAE: 39524820.7.0000.5385. The research was developed using data from a Clinical Analysis Laboratory located in the city of Campinas, state of São Paulo, Brazil, this laboratory being a reference for the Metropolitan region. Access to any additional information about the patients was not obtained, only the platelet values were assessed from the collection performed on EDTA, Sodium Citrate and ACD anticoagulants. After the suspicion of a case of pseudothrombocytopenia with microscopic visualization of platelet aggregates, the EDTA tube is subjected to agitation in a vortex tube homogenizer for one minute and automatically reprocessed. In situations where the platelet value is normal, the patient was not considered EDTA-dependent platelets and, therefore, was not submitted to a new collection in the laboratory. For the cases in which the agitation was not effective in dispersing the platelet aggregate, the patient was submitted to a new collection by vacuum venipuncture to obtain whole blood, these were placed in tubes containing Sodium Citrate, ACD and again in EDTA.Platelet counts were performed automatically to assess pseudothrombocytopenia. The data obtained were analyzed with ANOVA test and Tukey's post-test using the GraphPad Prism 5.0 program with a 95% significance level ($p < 0.05$).

RESULTS AND DISCUSSION

It was obtained 38 samples of patients suspected of pseudothrombocytopenia.

Table 1. Patients of the study, levels of EDTA, Citrate and ACD and dependence of the patients to EDTA Note the presence of ACD-Citrate relation

Patient	EDTA	CITRATE	ACD	%	DEPENDENT	ACD - CIT
1	47	161	184	14,29%	Y	Y
2	93	148	180	21,62%	Y	Y
3	101	33	112	239,39%	Y	Y
4	123	176	208	18,18%	Y	Y
5	57	123	154	25,20%	Y	Y
6	35	22	52	136,36%	Y	Y
7	94	23	142	517,39%	Y	Y
8	26	281	299	6,41%	Y	Y
9	122	135	154	14,07%	Y	Y
10	74	68	83	22,06%	Y	Y
11	13	175	180	2,86%	Y	Y
12	147	73	120	64,38%	N	Y
13	270	194	203	4,64%	N	Y
14	69	34	50	47,06%	N	Y
15	46	187	225	20,32%	Y	Y
16	6	181	221	22,10%	Y	Y
17	139	95	111	16,84%	N	Y
18	43	126	134	6,35%	Y	Y
19	114	144	133	-7,64%	Y	N
20	96	185	206	11,35%	Y	Y
21	61	43	51	18,60%	N	Y
22	125	64	97	51,56%	N	Y
23	49	24	41	70,83%	N	Y
24	81	52	64	23,08%	N	Y
25	121	152	157	3,29%	Y	Y
26	14	84	135	60,71%	Y	Y
27	179	40	160	300,00%	N	Y
28	181	95	133	40,00%	N	Y
29	152	136	224	64,71%	Y	Y
30	122	154	180	16,88%	Y	Y
31	94	146	188	28,77%	Y	Y
32	294	161	218	35,40%	N	Y
33	91	203	196	3,45%	Y	N
34	106	83	151	81,93%	Y	Y
35	74	32	61	90,63%	N	Y
36	74	226	238	5,31%	Y	Y
37	98	156	192	23,08%	Y	Y
38	730	603	627	3,98%	N	Y

From the total of 38 samples, 25 patients (66%) confirmed the EDTA-dependent condition and 13 patients (34%) underwent new EDTA sample collection and they showed results within the reference values. In Table I, below, the patients who did not have EDTA-dependent characteristics were kept in the study, because even in these cases, although the patient was not considered EDTA-dependent platelets, the ACD showed better results than Sodium Citrate. The figure 1, represents in percentage the proportion of EDTA-dependent patients and patients in which the suspicion was not confirmed. It can be observed that 34% of the patients, after a new collection, did not present characteristics of pseudothrombocytopenia. Platelets play a key role in thrombus formation and in tissue repair, in addiction in hematological parameters are crucial to understand patient homeostasis. Tripotassium EDTA (K3EDTA) is the anticoagulant currently recommended for making routine full blood cell counts. However, it causes isovolumetric platelet sphering that may be recorded as an apparent increase or decrease in mean platelet volume (MPV), we observed in our study that ACD shows results in platelets count more sensitive than others anticoagulants to pseudothrombocytopenia diagnosis (Braester 2003; Fozza 2014). The pathophysiology of EDTA-induced pseudothrombocytopenia is still uncertain. However, EDTA after chelating calcium can expose endogenous antibodies directed against the platelet glycoprotein IIb / IIIa complex. Thus, autoantibodies present in plasma recognize and bind to an epitope of glycoprotein IIb (GPIIb) that is part of the GPIIb / IIIa complex of the platelet surface, promoting platelet agglutination (Dusse et al. 2004; Farias et al. 2010). In our study, it is observed that the majority of the patients in the study were EDTA-dependent. In a study that evaluated 60 consecutive cases of thrombocytopenia in clinical hematology obtained over two years after which they concluded that pseudothrombocytopenia was the second leading cause of thrombocytopenia (incidence of 17%) (Ferreira, 2013). In the laboratory routine, when pseudothrombocytopenia is suspected, the tube containing EDTA must be incubated in a 37°C water bath, with subsequent homogenization to exclude the possibility of agglomeration, this procedure results in greater time demand by the technical team, as observed in our study, in cases where the collection is carried out in ACD there is no such need (Ahn, 2002). The EDTA-dependent syndrome, as EDTA pseudothrombocytopenia occurs "*in vitro*", that is, it occurs only when the patient's blood comes into contact with the EDTA anticoagulant, thus either aggregation or platelet satellitis can cause the hematological apparatus to erroneously read the platelets which results in a false platelet result.

CONCLUSION

Based on what was presented in the study, it is possible to observe the effectiveness of the ACD in 66% of the cases.

It is suggested that a change in the protocol for patients suspected of pseudothrombocytopenia incorporating the anticoagulant ACD in these cases specifically. There is a need for research on the cases of false thrombocytopenia given the uncertainty regarding the pathophysiological nature, and this study may direct a new view in relation to this phenomenon.

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