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Pseudothombocytopenia in Total Blood with Ethylenediaminetetraacetic Acid

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Abstract

Pseudothrombocytopenia is an in-vitro phenomenon caused by an interference in automated hematological equipment due to a false reading of platelets counts. Pseudothrombocytopenia's prevalence in hospitalized patients varies between 0.1 to 2%. In outpatient patients some studies report a prevalence as high as 17%. If pseudothrombocytopenia is not identified it can lead to incongruous clinical decisions making. A cross sectional study with 1401 sample was performed to demonstrate these interferences, these samples were tested by a reference laboratory in Quito, Ecuador. Samples were collected in an occupational health evaluation using anticoagulant EDTA tubes and processed by in an automated analyzer Sysmex XN-3000, equipment that actively participate within Quality Assurance programs established by a Quality Management System with a 3,9% total error, 3.9% imprecision and -3.8% inaccuracy [6 Sigma]. Thrombocytopenia in this study were 0.7% [IC 95% 0.3 - 1.1%] [10/1.401]. Based in qualitative estimation and post incubation counting, the real thrombocytopenia prevalence was 0.29% [IC 95% 0.01- 0.6%] [4/1.401] and pseudothombocytopenia was 0.4% [IC 95% 0.07- 0.7%] [6/1401]. Verification protocols must be implemented in clinical laboratory to its prompt and correct identification. More studies are necessary to correctly identify the prevalence of Pseudothrombocytopenia in hospitalized and outpatient population.

Keywords: EDTA; Outpatient; Prevalence; Pseudothrombocytopenia

Introduction

Pseudothrombocytopenia is an in-vitro phenomenon caused by an interference in automated hematological equipment due to a false reading of platelets counts. It has different reasons such as use of anticoagulants [EDTA], platelet satellitism, giant platelets and platelets agglutination [1-3]. In the case of the pseudothrombocytopenia associated to EDTA´s use, this is due to cation quelation which generates an exposition of the GP IIb-IIIa complex of platelets which reacts with antiplatelets autoantibodies EDTA dependent IgG type, which exceptionally react in the cold [4-7]. This provokes a reversible platelet agglutination expressed with visible platelets accumulation in the blood smear performed in this samples [4,8]. Despite, that is a phenomenon has been described before, EDTA´s anticoagulant is commonly used for hematological analysis of routine within the laboratory due to is ability to preserve cellular morphology [7].

Pseudothrombocytopenia's prevalence in hospitalized patients varies between 0.1 to 2%. However, in outpatient patients some studies report a prevalence as high as 17%, which difficult the possibility of an adequate clinical correlation, this will allow to presume that this might be a false thrombocytopenia [9,10]. If a Pseudothrombocytopenia is not adequately identified it can lead to inappropriate clinical decisions making, some as critical as blood transfusion, splenectomy, intravenous immunoglobulin administration, bone biopsy, unnecessary specialist referral and delay of program surgeries or urgent ones; this is why is so important to identify this finding in the laboratory through protocols implementation that determined if the cell count performed is valid [1,3,6,11-13]. If a thrombocytopenia´s alarm

in the automated instrument is detected a blood smear should be performed, specially to search platelets agglutination in order to rule out pseudothrombocytopenia [14].

Additionally, it is recommended in these cases, to incubate the blood at 37˚C for 15 minutes and to perform a new read immediately [the majority of autoantibodies react a frigori], it should be considered that despite incubation aggregation could persist in 20% of cases [15]. Another form of verification is to analyze in a new blood sample, or a parallel sample collected with sodium citrate at 3,2%, even though there isn´t a producer´s declaration about the validation of this sample type in automated hematological equipment [16]. Despite, there are other methods that could rule out this in vitro phenomenon. They are not part of habitual practice in clinical laboratories which puts patients at risk to obtain inadequate results that could enhance unnecessary clinical decisions [17]. This study establishes Pseudothrombocytopenia´s prevalence associated with EDTA in outpatients samples derived to a reference laboratory.

Material and Methods

A cross sectional study with 1401 samples was performed to demonstrate these interferences, these samples were tested by a reference laboratory with International Accreditation ISO 15189:2012 in Quito, Ecuador [≈2800 msnm]. All samples were collected in an occupational health evaluation with anticoagulant EDTA tubes. Samples were subjected to a pre-analytical verification process, according to acceptance and rejection habitual protocols to be processed by the automated analyzer Sysmex XN-3000, equipment that actively participate within Quality Assurance Programs established by a Quality Management System parameters with a 3,9% total error, a 3.9% imprecision and a -3.8% inaccuracy [6 Sigma]. Samples were analyzed within the normal habitual procedures to process cell blood count. If analyzed samples had a read below < 60 x 10^3/µL platelets, the equipment will automatically set an alarm. Once the samples with an alarm were identified, they went under the reference center´s verification protocol for platelets which could be visualized under (Figure 1).

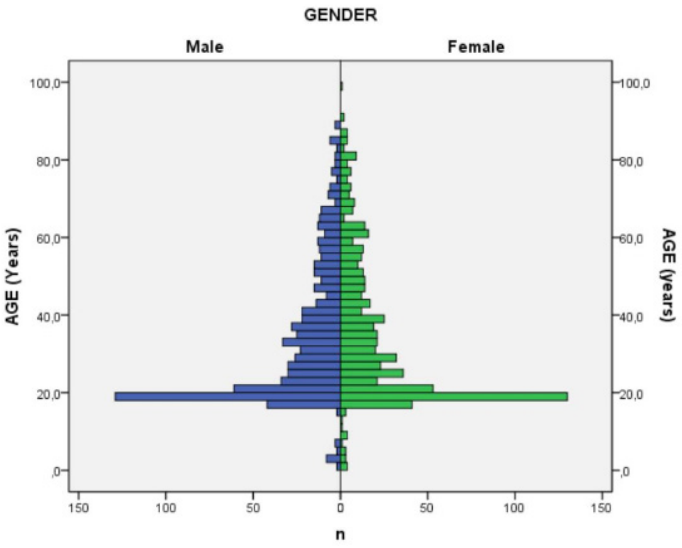

Figure 1: The demographic information, the cell blood count and platelets counts.

This protocol has two procedures: the first one is a blood smear visualization of peripheral blood sample to obtain an estimation of platelets, and the second one is carry out a new platelets counts using the same sample pre-heated at 37˚C and follow by vortex in the same equipment. The demographic information, the cell blood count and platelets counts were registered in a Microsoft excel sheet for cleaning up and then analyzed by program SPSS v14. Quantitative variables were expressed in mean and standards deviation and the qualitative variables were expressed by simple frequencies and percentages. The prevalence was presented in percentage [%] with its $IC_{95\%}$, for the quantitative comparison of the platelet count Wilcoxon´s test was used and for the comparison before and after of thrombocytopenia Chi2 of McNemmar was used, accepting as valid a significant value of 95% [α=0.05].

Results

All the samples sent to the reference laboratory were evaluated during a period of 30 days [n=1401]. The median

patients age was included in the study. They were 29 years [range: 1-98years] independent of patient's gender. A 51.5% [n=721] of the samples were male patients. The behavior of the main components of the Cell blood Count for the general sample and disaggregated for gender are shown in Table 1. According to the findings of CBC patients results, 5,6% [$IC_{95\%}$ 4.4 - 6.8%] of samples presented leukocytosis and 6.5% [$IC_{95\%}$ 5.2 - 7.8%] anemia. Taking as a reference for leukocytosis to all leukocytes value above 10.000 x 10^3 cells/mL, considering that value as the mean of leukocytes obtained in demographic studies in the age range found in our study [1-98 years]. On the other hand, anemia was considered when hemoglobin was below 12 g/dL for women and 13 g/dL for men. The anemia prevalence and leukocytes for both gender are shown in Table 2.

COMPONENT	X ± S	[Min-Max]
Leukocytes [x10^3/mL]	6866,5±2035,2	[1680-24620]
Male [n=721]	6796,7±1944,7	[2400-24620]
Female [n=680]	6940,5±2125,9	[1680-21030]
Eritrocytes x10^6/uL	5161,2±662,2	[2040-7670]
Male [n=721]	5476,2±612,8	[2930-7020]
Female [n=680]	4827,3±537,7	[2040-7670]
Hemoglobin [g/dL]	15,4±1,9	[5,7-20,4]
Male [n=721]	16,4±1,7	[9,1-20,4]
Female [n=680]	14,3±1,5	[5,7-18,5]
Hematocrite [%]	0,456±0,054	[0,201-0,594]
Male [n=721]	0,483±0,048	[0,272-0,594]
Female [n=680]	0,427±0,043	[0,201-0,564]
CMV [fL]	88,5±4,6	[64,3-114,6]
Male [n=721]	88,4±4,1	[73,9-114,6]
Female [n=680]	88,7±5	[64,3-102,7]
HCM [pg]	29,8±1,8	[17,5-39,9]
Male [n=721]	30±1,5	[23,8-39,9]
Female [n=680]	29,7±2	[17,5-34,7]
CHCM [g/dL]	33,7±0,9	[27,3-36,3]
Male [n=721]	34±0,8	[30,4-35,7]
Female [n=680]	33,4±0,9	[27,3-36,3]
RDWSD	42,8±3,7	[34,5-69,4]
Male [n=721]	42,2±3,5	[34,5-63,5]
Female [n=680]	43,5±3,8	[34,8-69,4]
RDWCV [%]	13,3±1,2	[11,4-22,7]
Male [n=721]	13,1±0,9	[11,5-18,8]
Female [n=680]	13,5±1,4	[11,4-22,7]
PLATELETS [x109/L]	279,7±67,1	[28-785]
Male [n=721]	265,8±63,5	[28-785]
Female [n=680]	294,4±67,6	[104-560]

Table 1: Distribution of Cell Blood Count components. General sample [n=1401].

	PREVALENCE % [$IC_{95\%}$]
Anemia	
Male [n=721]	6 [4.3 - 7.]
Female [n=680]	7.1 [5.2 - 9]
Leukocytosis	
Male [n=721]	4.4 [2.9 - 5.9]
Female [n=680]	6.9 [5.0 - 8.8]

Table 2: Leukocytosis and Anemia by gender.

The thrombocytopenia prevalence found in this study was of 0.7% [$IC_{95\%}$ 0.3 - 1.1%] [10/1.401]. Samples were categorized as thrombocytopenic when criteria under the verification protocol with the qualitative estimation counting and the hematological counting after incubation at 37°C was found (Figure 2). The individual behavior of the presented cases identified with thrombocytopenia are shown in Table 3. Also, the median behavior and percentile of thrombocytopenia's cases with their initial platelets counts, qualitative estimation and post- incubation of the samples. Based in qualitative estimation and post incubation counting, the real prevalence of thrombocytopenia was of 0.29% [$IC_{95\%}$ 0.01-0.6%] [4/1.401] and the pseudo thrombocytopenia was of 0.4% % [$IC_{95\%}$ 0.07-0.7%] [6/1401].

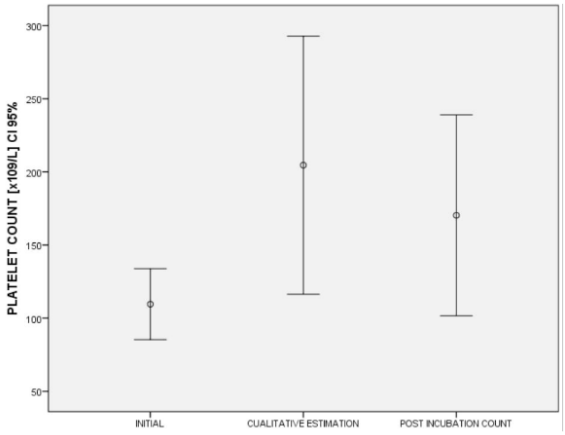

Figure 2: Qualitative estimation counting and the hematological counting after incubation at 37°C was found

PLT count methodology*	Median [2.5TH - 97.5TH]
Automatic Analyzer [Initial]	117 [28-144]
Qualitative Estimation	169 [42-448]
Automatic Analyzer [Post incubation]	152 [28-390]
*PLT Initial vs Estimation vs Post-Incubation p<0.05 [Wilcox Sign Test] / Estimation vs Post-Incubation p>0.05 [Wilcox Sign Test].	

Table 3: Distribution of platelets by counting methodology in Patients with thrombocytopenia [n=10].

Discussion

Pseudothombocytopenia is a phenomenon mediated by an immunological patron caused by the presence of EDTA and cold antiplatelet autoantibodies that cause aggregation [2]. The findings of thrombocytopenia in this study were 0.7% [$IC_{95\%}$ 0.3 - 1.1%] [10/1.401]. Based in qualitative estimation and post incubation counting, the prevalence of real thrombocytopenia was 0.29% [$IC_{95\%}$ 0.01- 0.6%] [4/1.401] and pseudothombocytopenia was 0.4% [$IC_{95\%}$ 0.07- 0.7%] [6/1401]. Pseudothombocytopenia´s prevalence is above the findings of Prates et al. of 0.196% [18]. On the other hand, the identified values in this study are lower than other studies performed in outpatient patients such as Silvestri´s et al. findings of 15.3% prevalence [19]. However, Silvestri´s population was about 111 patients compared to 1401 patients analyzed in the present study which may impact over the found prevalence. Moreover, Silvestri´s study was performed over a population that was referred by an isolated thrombocytopenia which means that the prevalence identified is from a population that presented thrombocytopenia, and they also identified psedothombocytopenia induced by EDTA [19].

Silvestri et al. initially collected samples with K3 EDTA tubes in patients with suspected of pseudothrombocytoepenia, a different collection of blood sample was performed using tubes with K3 EDTA, sodium citrate at 3,8% and heparin at different times 0, 20, 40, 60, 120, 180, 240 minutes after blood withdrawal [19]. Therefore, that study demonstrated the significant fall of platelets counting at 60 minutes and it also displayed a more profound fall in platelets number when more time pass by. This study, also obtained samples in other type of tubes such a sodium citrate and heparin, which evidenced the discordant values with EDTA, demonstrating EDTA´s pseudothombocytopenia. In our study, we did not develop a protocol identification of pseudothrombocytopenia related to the time of the sample´s collection, because the sample were collected outside of our facilities and only referred to us.

However, Silvestri´s findings showed important information that evidenced how the platelets values decay over time when this spurious related its EDTA dependent [19]. The Range of patients age included a population between 1-98 years, which is a broad range that allow us to make a study in an entire population instead of an age group. The information generated by this study allowed us to identify certain age groups in which is more frequent to identified pseudothombocytopenia and in future to perform studies that are specific to those age groups more frequently affected by this phenomenon. One of the major limitations by performing tests in a reference laboratory center is the lack of patients clinical information. This is the main reason why it is mandatory to implement protocols that assure that the reported determination are accurate and not spurious results avoiding propitiate inadequate clinical decisions making. However, these findings allow us to use this important evidence to be used as a reference in our setting.

Conclusion

Pseudo thrombocytopenia is a frequent alteration found in the laboratory setting and it must be identified due to its impact over clinical decisions making. Verification protocols must be implemented in clinical laboratory to its prompt and correct identification. More studies are necessary to correctly identify the prevalence of Pseudothrombocytopenia in hospitalized and outpatient population.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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