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Comparison between visual inspection of turbidity and quantitative determination of triglycerides in plasma bags

Comparação entre a inspeção visual da turbidez e a determinação quantitativa de triglicerídeos em bolsas de plasma

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ABSTRACT

Introduction: Blood donor screening is an important stage of quality control in blood banks. Turbidity caused by increased levels of triglycerides is detected by visual inspection, but there is no consensus on its threshold at which plasma should be considered inappropriate for donation.

Objective: Compare triglycerides dosage and visual turbidity in decision making for the disposal of plasma.

Material and methods: Plasma bags (n=205) were classified by visual inspection as clear, moderately turbid or turbid and triglyceride concentration were determined in serum and plasma with enzimatic-colorimetric methodology by automation.

Results: Our results show a positive correlation between serum and plasma triglycerides levels (r=0,94) but we observed a higher concentration in serum when compared to plasma samples (p<0,03). Most of the plasma were classified as moderately turbid or turbid (75%). Visual inspection and triglycerides levels were moderately correlated in serum (rb=0,57) and plasma (rb=0,52). However, moderately turbid samples showed discordance between serum or plasma triglyceride levels and the visual inspection.

Discussion: Our findings corroborate with the literature data, supporting the subjectivity of the visual inspection. We recommend further studies to determine which triglyceride threshold should be used for the disposal of plasma bags combined with automated methods to enhance visual classification accuracy.

Conclusion: Quality improvement actions are critical for standardization of the screening in order to avoid unnecessary disposal of the plasma bags.

Key words: triglycerides; blood banks; vision screening.

RESUMO

Introdução: *A triagem das bolsas de plasma é uma etapa importante do controle de qualidade nos hemocentros. A turbidez causada pelo aumento de triglicerídeos é detectada por inspeção visual, mas não há consenso acerca do limite no qual o plasma deve ser considerado inadequado para doação.*

Objetivo: *Comparar a dosagem de triglicerídeos e a turbidez visual na tomada de decisão para o descarte do plasma.*

Material e métodos: *As bolsas de plasma (n=205) foram classificadas pela inspeção visual como límpidas, moderadamente turvas ou turvas e as concentrações de triglicerídeos foram determinadas no soro e plasma com metodologia enzimática-colorimétrica por automação.*

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Resultados: *Nossos resultados mostraram uma correlação positiva entre soro e plasma (r=0,94) mas observamos que a concentração de triglicerídeos foi mais alta no soro do que no plasma (p<0,03). A maioria das bolsas foi classificada como moderadamente turva ou turva (75%). A inspeção visual e a concentração de triglicerídeos apresentaram uma correlação moderada para o soro (rb=0,57) e plasma (rb=0,52). Entretanto, no grupo moderadamente turvo, houve uma divergência significativa entre as concentrações de triglicerídeos no soro/plasma e a inspeção visual.*

Discussão: *Nossos achados corroboram com a literatura, reforçando a subjetividade da inspeção visual. Nós recomendamos a realização de estudos futuros para determinar o limite dos níveis de triglicerídeos para o descarte de plasma em conjunto com a combinação de métodos automatizados para aumentar a acurácia da classificação visual.*

Conclusão: *As ações voltadas para a melhoria da qualidade são críticas para a padronização da triagem e evitar o descarte desnecessário de plasma.t*

Palavras-chave: *Triglicerídeos; Bancos de Sangue; Triagem.*

INTRODUCTION

Due to the increase in life expectancy and the consequent multiplication of cases of chronic diseases related to aging, in the last decade an exponential growth in the demand for blood donations has been identified worldwide, including in Brazil[1,2]. In situations such as hemorrhages, some cases of cancer, cardiovascular surgery, transplants and traffic accidents, the most suitable therapeutic method is blood transfusion[1].

The whole blood bag is fractionated to obtain erythrocyte, plasma and platelet components. The plasma component can be divided into fresh frozen plasma (PFC), common plasma, cryoprecipitate free plasma and cryoprecipitate. Quality control is a set of activities used to monitor and evaluate production processes, being essential to guarantee the quality and standardize the conduct adopted in blood centers. Quality indicators demonstrate whether the critical practices of the process are being carried out properly and whether the results are within an acceptable range[3-5].

The first step in this process is the rigorous screening that blood bags undergo to ensure their viability. After fractionation, visual inspection of the plasma is performed to determine whether the bag will be accepted for donation, based on characteristics such as lipemia, jaundice, hemolysis, presence of fibrin or leakage[3,4,6]. Regarding lipemia, if any turbidity observed in the plasma, caused by the accumulation of triglycerides, of varied origin[7-10] and which can generate inconveniences such as analytical or clinical problems[11]. Despite this, there is no consensus on the level of triglycerides above which blood must be considered inadequate for the production of blood components, generating different approaches in many countries[11].

Considering that, currently, the literature provides little information on the subject and that the supply of donations in blood banks is often limited, it is essential to discard bags that actually pose some risk or cause harm to the patient. Thus, this study compared the visual inspection methodologies and the quantitative determination of triglycerides to detect turbidity in plasma bags.

MATERIAL AND METHOD

The study was approved by the Research Ethics Committee of the Paraná Hematology and Hemotherapy Center (HEMEPAR) under number 2,585,376. Blood bags with anticoagulant CPD (citrate, phosphate and dextrose) were obtained from 205 donors. The plasma bags (n=205) were submitted to a visual evaluation based on the turbidity of the samples against a dark background, containing the word "LIPEMIA" according to the classification criteria adopted by the authors: group 1 (clear) when the word LIPEMIA appears in a form transparent, group 2 (moderately cloudy) when the word appears opaque, and group 3 (cloudy) when the word does not appear. Regarding triglyceride dosage, the following values were considered (without fasting)[12]: desirable <175 mg/dL, high between 176-499 mg/dL and very high >500 mg/dL. For standardization purposes, the concentration of triglycerides or "degree of lipemia", when >175 mg/dL, the term "lipemic" was used, regardless of the aspect of the sample. The bags were photographed and filed. Aliquots were obtained from the bag of plasma resulting from the fractionation of the bag of whole blood, and serum, obtained from the same donors in tubes with gel and clot activator (collected to perform the serological tests). The plasma/serum samples were stored and preserved in a freezer at a temperature below -20ºC, at the School Laboratory of Clinical Analysis of the Federal University of Paraná (LEAC-UFPR) and

subsequently, the concentrations of triglycerides were determined in the serum/plasma by the method colorimetric enzyme (Trinder Method), commercial kit (Labtest®), in the Cobas Mira biochemical analyzer. The equipment was previously calibrated (Calibra Labtest®) and submitted to internal quality control at levels 1 (*Qualitrol 1H Labtest®*) and 2 (*Qualitrol 2H Labtest®*).

Regarding the statistical analysis, categorical variables were described by absolute (N) and relative (%) frequency. Correlation between dosages was verified by Pearson coefficient (r) and triglyceride concentrations were compared between different visual classifications by Kruskall-Wallis test. The classifications "moderately cloudy" and "cloudy" were grouped for subsequent analyses. The correlation between visual inspection and triglyceride concentration was investigated by the biserial coefficient (rb) while the relationship between the two variables was assessed by the simple linear regression model. The agreement between visual inspection and the classifications obtained by measuring serum and plasma triglycerides was determined using the chi-square test for adherence. McNemar's test was used to check whether there was a significant difference between serum and plasma triglyceride levels. Stata Program v.12 and R v.3.5.1 were used (significant differences for $p \leq 0.05$).

RESULTS

The bags that showed turbidity (groups 2 and 3) were predominantly from male donors ($p=1.13x10^{-12}$) and the levels of triglycerides among men were higher, for both serum and plasma ($p=1.24\ x10^{-10}$ and $p=6.2x10^{-08}$, respectively), and 68% of men had triglyceride levels ≥175 mg/dL. Triglyceride concentrations were determined in isolation in serum and plasma for purposes of comparison between samples. Triglyceride concentration was higher in serum than in plasma for the three groups and both female and male groups ($p<0.03$). However, the results showed a positive correlation between serum and plasma ($r=0.94$, $p<2.2\times10^{-16}$) - Figure 1.

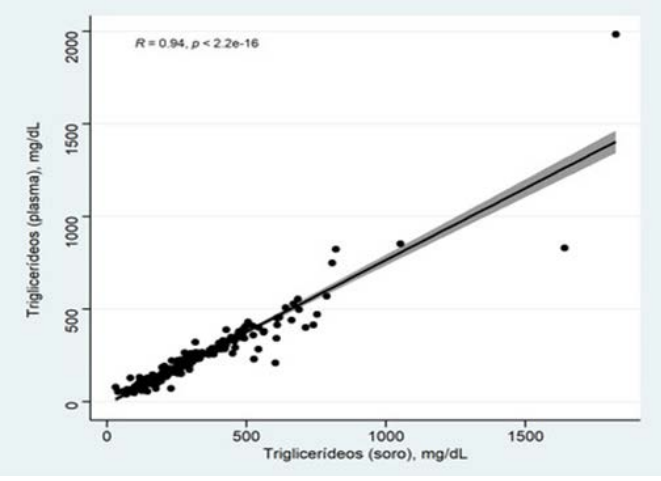

FIGURE 1 – *Correlation analysis for triglyceride quantifications between the serum and plasma samples (n=205).*

Of the total number of bags analyzed (n=205), 51 (25%) were classified as clear, 96 (47%) as moderately cloudy and 58 (28%) as cloudy. Triglyceride concentrations were determined considering lipemic (≥175 mg/dL) and non-lipemic (<175 mg/dL) serum. Visual inspection identified 25% of clear plasma bags (group 1), 47% with moderate turbidity (group 2) and 28% with cloudy plasma (group 3). Table 1 shows the variation in serum/plasma triglyceride levels (x±sd) for groups 1, 2 and 3. Divergences were observed in group 1 (p=0.008) and especially in group 2 (p=7, 56x10-6), while the classification remained similar for group 3 (p=0.13), that is, 98% (serum) and 91% (plasma) were lipemic (≥175 mg/dL) in the turbid samples. The correlation analysis comparing visual inspection and triglyceride concentration showed a moderate correlation for both serum (rb=0.57) and plasma (rb=0.52) Figure 2.

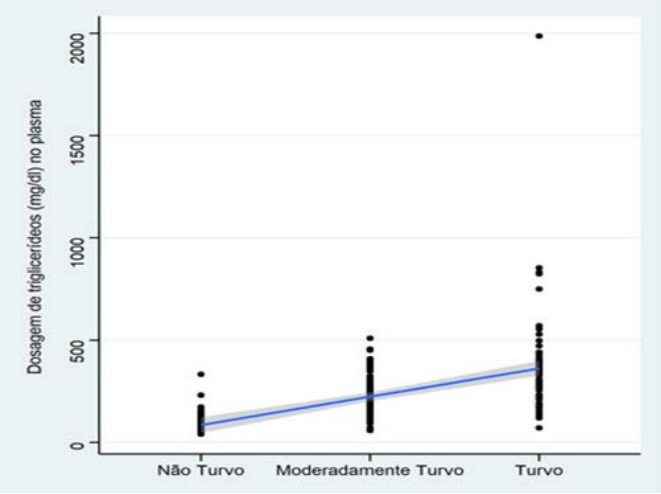

FIGURE 2 – *Scatter diagram of the relationship between visual inspection and dosages serum (2A) and plasma (2B) triglycerides.*

Footnote: *Figure 2A - Serum (r=0.57) and Figure 2B - Plasma (r=0.52). Source: The authors (2020).*

The difference in visual inspection for clear and cloudy groups, when compared to serum triglyceride concentrations (non-lipemic and lipemic) was not significant (p=0.07), but for plasma there was a significant difference ($p=4.98x10^{-11}$) in 24.9% of the samples. From this result, the disagreement between serum/plasma triglyceride concentrations and classification by visual inspection was evaluated and a significant difference was observed ($p= 9.08x10^{-9}$) - Table 2.

TABLE 1- Categories of visual inspection taking into account the concentrations of triglycerides obtained in serum and plasma (n=205)

	Group 1 (25%)		Group 2 (47%)		Group 3 (28%)	
	Serum	Plasma	Serum	Plasma	Serum	Plasma
Triglycerides*	140±72,7	103±50,9	280±137	206±97	502±298	379±271
Lipemic (%)						
No	40 (78 %)	49 (96%)	22 (23%)	44 (46%)	01 (02%)	05 (09%)
Yes	11 (22 %)	02 (04%)	74 (77%)	52 (54%)	57 (98%)	53 (91%)

Footnote: *Triglycerides in mg/dL (x±dp). Source: The Authors (2020).

TABLE 2- Proportion of lipemic and non-lipemic serum/plasma considering the degree of lipemia and visual inspection

Triglycerides mg/dL (serum)	Triglycerides mg/dL (plasma)	Total	Group 1 Clear	Group 2 Moderately cloudy	Group 3 Cloudy	P
< 175	< 175	63	40 (78,4%)	22 (22,9%)	1 (1,7%)	$9{,}08x10^{-9}$
≥ 175	< 175	35	9 (17,7%)	22 (22,9%)	4 (6,9%)	
≥ 175	≥ 175	107	2 (3,9%)	52 (54,2%)	53 (91,4%)	

Source: The Authors (2020).

DISCUSSION

When comparing the bags donated by men and women, it was noted that the most turbid samples came from male donors. This finding was corroborated by other studies, such as Peffer et al., which found that 87% of the cases of lipemic bags were also from male donors[15]. In a study by Knuth and Horowitz, these findings were justified by the fact of the plasma concentration of triglycerides in women, reach the maximum peak after 2 hours of ingestion of a fat meal, gradually decreasing until reaching the basal level after 6 hours.16 That is, although women have a higher body fat index, men have a peak of plasma triglyceride concentration 3-4 hours after ingestion, taking up to 9 hours to return to baseline levels[15,16].

As is known, the accumulation of triglycerides can lead to turbidity in the serum or plasma, which can have different origins, such as postprandial metabolism, metabolic disorders such as hypertriglyceridemia, intravenous administration of lipids, in addition to the use of ethanol and some medications[7-10]. The presence of lipids in high concentration in the donated bag can cause inconveniences such as analytical or clinical problems. In relation to analytical problems, in less sophisticated systems, turbidity causes interference since light transmission is part of the detection method, producing falsely high results. Currently, this situation can be avoided with the use of automated systems that are not interfered with by turbidity[10,11]. Considering clinical issues, there is no consensus on the level above which plasma should be considered inadequate for donation or for production of components, generating different approaches in many countries[11]. Some authors argue that it is unlikely that the transfusion of lipemic plasma free of the infectious agent has significant acute effects on receptors, in addition to those associated with the ingestion of a fatty food[9]. However, other authors state that some issues should be studied, such as the appropriate lipemia threshold for the use of blood in transfusion and whether hypertriglyceridemia is really an absolute contraindication for plasma donations due to the potential presence of diseases such as dyslipidemia, kidney disease or coronary heart disease in receivers[11].

Since blood donors are instructed to have a light meal before performing the donation, a clear visual appearance and triglyceride concentrations of up to 175 mg/dL were expected, a value considered adequate for non-fasting patients[12]. As a hypothesis, it was expected to find moderately turbid samples in cases of high concentration of triglycerides between 176-499 mg/dL, and intense turbidity when above 500 mg/dL. However, as observed, it is not always possible to correlate turbidity visual with the concentration of triglycerides. The higher concentration of triglycerides was detected in serum compared to plasma, and the lower concentration of triglycerides in plasma can be explained by the presence of anticoagulant CPD present in the bags. Despite the mechanisms not fully understood, in the literature there are data showing that the concentration of some analytes may be lower due to anticoagulants, which initiate the release of intracellular fluid promoting dilution[17]. This dilution can still occur due to the volume of anticoagulant itself employee, since Consolidation Ordinance No. 05 of September 28, 2017[3] recommends a volume of 60-65ml of anticoagulant to collect 450 ± 45ml of whole

blood. In addition, there are studies showing that plasma glucose, urea and creatinine dosages are also lower in plasma compared to serum dosages[18,19]. Despite this, there was an evident positive correlation between serum and plasma, that is, despite the literature[18,19] considering the serum as the most suitable sample for measurements, in those cases where the results are not used for diagnostic or treatment purposes. Of patients, both samples could be used as criteria in visual inspection.

Quality control and standardization are essential from the selection of grants. Hemotherapy services must have protocols that define the type of control required for each blood component and the minimum expected parameters. For example, PFC, used when specific factor concentrates are not available, must be stored for up to 24 hours to maintain adequate factor levels[4,5]. In the present study, triglyceride concentrations (x±dp) for serum and plasma obtained in the different categories of visual inspection, and variability can be observed within the same group. The low percentage of clear bags (25%) is noteworthy, considering that some blood centers adopt as a criterion the disposal of plasma with any degree of turbidity. Most bags were classified as group 2 (moderately turbid) and some samples of serum (23%) and plasma (46%) in this group had triglyceride values below 175mg/dL, thus considering that other causes of turbidity were excluded, plasmas were discarded unnecessarily if only lipemia was considered. In groups 1 (clear) and 3 (cloudy), the discrepancies were much smaller. The fact that triglyceride dosages just above 175mg/dL do not necessarily lead to turbidity, the concentration above which the sample is cloudy being variable, was observed in our study, with 22% of clear bags having a higher value. Of 175mg/dL. The Brazilian Society of Cardiology provides reference values for triglycerides based on expected values for diagnostic purposes or to adjust the treatment of patients at cardiovascular risk, but they are not values that take into account possible transfusion risks. Correlation analysis comparing visual inspection and triglyceride concentration showed a moderate correlation for serum/plasma and the dispersion of triglyceride concentration values also increased with increasing turbidity. This information shows the difficulty in correlating visual inspection with the concentration of triglycerides, and is in line with the opinion of Lippi et al (2013), who states that, although the practice of visual inspection of bags has been the only approach to establishing the presence of interfering substances (free hemoglobin, hyperbilirubinemia and lipemia) for decades, it has inherent limitations, as it is qualitative, poorly standardized, subjective and unreliable compared to the analytical assessment of turbidity[11].

Corroborating these results, a lower agreement was observed in group 2 - moderately cloudy. The difference in visual inspection compared to the degree of lipemia determined in serum was not significant (p=0.07), but for plasma there was a significant difference ($p=4.98x10^{-11}$) in 24.9% of the samples. When the disagreement between visual inspection and the degree of lipemia was evaluated using serum or plasma, a significant difference was observed ($p= 9.08x10^{-9}$). That is, when triglyceride concentrations are used to determine whether the pouch is cloudy or not, there is a discrepancy between visual inspection and the isolated use of the triglyceride value, both in serum and plasma, impacting the decision-making on the release of the grant for donation.

To meet the routine quality control needs of a blood bank, a quick and sensitive method of identifying any turbidity is needed. The study conducted by Simundic et al[20] compared the results of visual inspection with the automated spectrophotometry method in the detection of lipemia, jaundice and hemolysis, and found that from the total number of samples classified as lipemic by visual inspection, 61% of the samples were actually lipemic. In view of this, the subjectivity of visual inspection can be one of the justifications for the outliers found in our study. Regarding automated methods for quantifying interferences in the sample, a major advance was represented by the introduction of serum indices (SI), which imply the automatic detection of hemolysis, hyperbilirubinemia and lipemia. These new tools encompass systematic monitoring of serum or plasma absorbance at various wavelengths[21]. According to the study by Lippi et al., the routine use of IS seems to include a reduced response time, quantitative expression of results, minimizes the high degree of variability of the subjective assessment, mainly blood bags with turbidity in the intermediate zone[11,21], which refers to group 2 in our study. Within this context, the choice of method for determining turbidity as an excluding factor for the release of the blood component, whether by visual inspection, by automated methods or by the association of the two methods, is essential for the quality control of the production process of the banks of blood.

CONCLUSION

The subjectivity in the assessment of lipemia in blood bags by visual inspection was evidenced, especially with regard to moderately turbid samples. In order to reduce unnecessary plasma disposal, but on the other hand, ensure the quality of selected bags, our study suggests that turbidity disposal due to lipemia is better standardized.

Conflicts of Interests: The authors declare they have no competing interests.

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