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Procedures to minimize interference of hypertriglyceridemia in laboratory exams of lipemic samples in acute pancreatitis: a case report

Procedimentos para minimizar interferência da hipertrigliceridemia em determinações laboratoriais de amostras lipêmicas em pancreatite aguda: relato de caso

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ABSTRACT

Lipemia can be characterized by serum turbidity caused by accumulation of lipoproteins and is a major cause of interference in laboratory analyzes. Hypertriglyceridemia (HTG) is the third most common cause of acute pancreatitis (AP). Diagnosis and laboratory monitoring of AP associated with HTG are hampered by the intense lipemia associated. This case report proposes an approach to laboratory interference caused by HTG in patient diagnosed with AP and actions target at achieving reliable laboratory tests, free from interference and useful in clinical decision making.

Key words: hypertriglyceridemia; triglycerides; clinical laboratory techniques; pancreatitis.

INTRODUCTION

Lipemia is characterized by turbidity of serum or plasma caused by accumulation of lipoprotein particulates[1]. The different classes of lipoproteins vary in size and contribute differentially to serum turbidity. Chylomicrons are those that have the greatest potential in causing turbidity in sample[2]. Therefore, hypertriglyceridemia (HTG) is an important interference in laboratory analysis[3].

The most common cause of HTG is inadequate fasting[4]. However, several clinical conditions may culminate in HTG, such as diabetes *mellitus*, chronic renal failure, alcoholism and pancreatitis[5-7].

In the initial phase of acute pancreatitis (AP), HTG is a common finding in up to 53% of patients[8, 9]. High levels of triglycerides (TG) can increases the amount of free fatty acids toxic to the pancreatic tissue[10], producing inflammatory response and activation of enzymes, which may result in AP[1, 6].

HTG-induced AP corresponds to 38% of cases[10]. TG levels above 1,000 mg/dl have been proposed as sufficient to induce AP[11], however it is an arbitrary criterion, since AP may occur at higher or lower levels[1].

Understanding lipemia as an important interfering in laboratory tests[7], the diagnosis and monitoring of HTG-induced AP can be damaged or adversely affected due to analytical interferences in the laboratory.

Several laboratory methodologies may suffer lipemia interference[3]. The spectrophotometry is the most affected. Lipoprotein particles absorb the light inversely proportional to wavelength used in the analysis. Thus, methods using lower wavelengths are the most affected[12].

Concentrations above 500 mg/dl, of TG hinder the reading the endpoint of amylase reaction in colorimetric tests[13, 14]. In high concentrations, they may form a separate layer at the top of patients serum[15], accounting for up to 25% of the total volume[16, 17]. This physical phenomenon can result in false decrease in electrolytes and metabolites concentration, especially in equipment with a sample sensor that pipette the upper layer of serum[17]. This is due to the behavior of the hydrophilic analytes and ions which are distributed in the aqueous phase, in the lower portion of the tube[18].

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There are several case reports in literature concerning association between AP and HTG, but few intend to evaluate aspects of laboratory diagnosis, methodological limitations imposed by lipemia and management for resolution. This case report suggests an approach to laboratory interference caused by HTG in serum from a patient diagnosed with AP and presents actions aimed at achieving reliable and useful laboratory tests in clinical decision making. The study was approved by the Research Ethics Committee of the Instituto Multidisciplinar em Saúde, campus Anísio Teixeira – Universidade Federal da Bahia, protocolo nº 801.250/2014.

CASE REPORT

A male patient, 46 years old, brown, was admitted in the emergency room reporting pain on the umbilicus scar, in distress, high intensity and without worsening or relief factors. He was treated with scopolamine and simethicone and released after relieving symptoms. On the first day after discharge, the patient progressed with pain worsening and syncopal episode at his residence, and then forwarded to the Hospital. He reported smoking and alcoholism for 31 years, teetotaler for the last two years. Currently, he claims alcohol consumption over the weekends (sic). He referred high-fat diet and arterial hypertension for more than 10 years taking captopril. He denied diabetes *mellitus*. At clinical examination, the patient was lucid and oriented in time and space, good general condition, eutrophic, with severe dyspnea that required the introduction of nasal oxygen catheter, anicteric and pale mucous 1/4+. He presented distended abdomen painful at superficial and deep palpation. In the second day of hospital stay (DHS), computerized tomography was performed diagnosing AP with necrotic area in the cephalic region of the organ. He presented anemia (hemoglobin [Hb] 9.5 g%) and leukocytosis (11,600 leukocytes), with neutrophilia (7,308) and left shift (26%) in the third DHS.

Blood samples for laboratory tests showed a significant lipemia (**Figure**), with com TG concentration of 12,355 mg/dl.

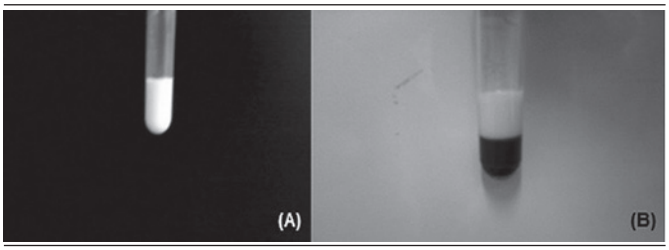

FIGURE – *Blood samples showing lipemia*
A) lipemic serum sample; B) whole blood sample after centrifugation, TG 12,355 mg/dl concentration.
TG: triglycerides.

Most of the laboratory tests suffered direct interference of the TG levels. The patient's serum was subjected to dilutions with NaCl 150 mmol/l (0.85%) solutions. Biochemical measurements were performed at LabMax 240® equipment by spectrophotometry. The results were corrected according to dilution factors used, guided by the limit of detection and the interference level of TG (**Table 1**).

Hb was determined in an automated hemocytometer (Celldyn 3700 - Abbott®), by cyanmethemoglobin method. The lipemia correction for Hb and RBC parameters was carried out by replacing the plasma volume by an equal volume of saline solution after centrifugation of the whole blood (with ethylenediaminetetraacetic acid [EDTA]). The pre and post-correction are shown in **Table 2**.

The measurement of electrolytes was performed in AVL 9180 Roche® equipment only in the fourth DHS, with a decrease in TG concentration to 3,149 mg/dl, hyponatremia was detected (Na: 127 mmol/l – reference: 136-145 mmol/l).

Patient progressed satisfactorily with the use of ceftriaxone, metronidazole, insulin, dipyrone, metoclorpramide and tramadol. He was discharged after 14 days of hospitalization.

TABLE 1 – Methodological details of the dilution strategy for resolution of lipemia

Test	Method	Wavelength (λ)	Detection limit	TG interference (mg/dl)[1]	Dilution required[2]	Dilution used[3]	Post dilution results
Urease UV	Enzimatic	340	0.94 mg/dl	> 1.800	1:6	1:15	4.26 mg/dl
Creatinine K	Colorimetric	510	0.12 mg/dl	> 900	1:14	1:15	0.027 mg/dl
Alkaline phosphatase	Enzimatic	405	2.4 U/l	> 1.800	1:6	1:5	626.6 U/l
Amylase	Enzimatic	405	2.4 U/l	> 1.800	1:6	1:5	150.6 U/l
Bili-T	Colorimetric	546	0.09 mg/dl	> 1.500	1:9	1:5	6.52 mg/dl
Bili-D	Colorimetric	546	0.02 mg/dl	> 1.000	1:13	1:15	4.5 mg/dl
Glucose	Enzimatic	505	0.62 mg/dl	> 1.000	1:13	1:15	57.5 mg/dl
Gamma GT	Kinetic	405	2.48 U/l	> 1.000	1:13	1:15	27.1 U/l
AST/SGOT	Kinetic	340	1.75 U/l	> 650	1:20	1:15	10 U/l
ALT/SGPT	Kinetic	340	1.75 U/l	> 650	1:20	1:20	10.5 U/l

[1]: TG concentration able to cause interference in analysis; [2]: dilution required to eliminate the interference of 12,355 mg/dl TG in the analysis; [3]: result obtained after dilution to multiply by the dilution factor; UV: ultraviolet; Bili-T: total bilirubin; Bili-D: direct bilirubin; Gamma GT: gamma-glutamyltransferase; AST: aspartate aminotransferase; SGOT: serum glutamic-oxaloacetic transaminase; ALT: aspartate alanine aminotransferase; SGPT: serum glutamic-pyruvic transaminase; TG: triglycerides.

TABLE 2 – Interference and correction of lipemia in erythrogram

Parameters	Before correction[1]	After correction[2]	Variation (%)[3]
RBC (10^6/mm^3)	4.71	4.7	0.002
Hb (g%)	19.3	13	32.64
Ht (%)	49.8	39.3	21.08
MCV (u3)	105.73	83.43	21.09
MCH (pg)	40.97	27.6	32.63
MCHC (%)	39.1	33.07	15.4

[1]: results obtained by lipemic blood analysis; [2]: results of lipemic plasma after saline (NaCl 0.85%) replacement; [3]: analytical error between the pre and post-procedure analysis; RBC: red blood cells; Hb: hemoglobin; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

DISCUSSION

AP cases with TG level so exacerbated (12,355 mg/dl) are not common. The literature reports concentrations between 1,000 and 1,800 mg/dl of TG causing AP[7, 10, 13]. This patient had history of HTG as triggering factor of AP due to the history of alcohol abuse, smoking and high-fat diet.

In cases where HTG is not related to inappropriate fasting and has direct association with the underlying disease, and the elimination of interfering factor is not possible, it is essential an appropriate management of samples and analysis for a reliable diagnosis.

There are not many techniques described for resolution of lipemia interference in samples for laboratory testing. In 2014, Nikolac proposed three methods of resolution: 1) sample centrifugation in ultracentrifuge for separation of lipoproteins by density – this method imposes great limitations because few laboratories have such equipment; 2) extraction using polar solvents – this method prevents the determination of lipophilic substances; 3) performing sample dilutions in saline solution for elimination of interference[19], used in this case.

As dilution requires sample manipulation, analysts should be aware when pipetting to prevent contamination of the sample. The knowledge of detection limit and the interference level of TG are essential for the dilutions be carried out accurately in order to eliminate interference in the analysis. After evaluation of the techniques, it was decided to perform two dilutions in the proportions 1:5 and 1:15, which included all analysis in this case.

Creatinine was the only parameter in which the dilution procedure did not resolve the interference. The creatinine reagent K suffers TG interference in levels higher than 900 mg/dl. The manufacturer suggests that samples with levels above 1,800 mg/dl are diluted in the ratio 1:2 and emphasizes that higher dilutions produce significant errors with underestimation of creatinine. This is due to inapropriated analitical sensitivity of assay, which has limited detection limit (0.12 mg/dl), about 17% of the lower reference limit. To resolve this situation, it is suggested using other markers of renal disease, such as urea, which enzymatic methodology assigns lower limit of detection, and cystatin C (in urine), immunological method.

For amylase assay, the manufacturer defines that TG levels between 1,800 mg/dl to 3,500 mg/dl are able to reduce by 10% the serum levels of the enzyme. Thus, even with sufficient dilution to eliminate interference, it is prudent to refer in the report the possibility of underestimation of the result by HTG interference. Furthermore, the use of lipase would be more feasible in these cases since it is more specific to pancreatic damage, and produce less interference of TG concentration (> 2,000 mg/dl)[13, 18, 20].

Regarding the analysis of electrolytes, lipemia does not interfere in the methodology, but there are physical changes in patient's serum, with formation of a lipid layer larger than those in non lipemic samples, avoiding ions measurements[12].

For Hb detection by spectrophotometry (540 nm), TG concentrations greater than 250 mg/dl can affect results; thus, since the TG levels found are 40 times greater than the interference limit, the replacement of lipemic plasma for NaCl 0.85% solution was needed[20]. Table 2 shows the variation between analyzes (before and after the correction) is approximately 33% to increase Hb, which directly impacts on RBC parameters calculated from Hb. These data show the relevant TG interference in blood count, and may impact in clinical decision, and the importance of correction.

The great technological apparatus in clinical laboratories has made the laboratory analytical phase increasingly reliable, and criticism to automated processes is the gap between analyst and procedure. In cases as reported, without the corrective actions proposed, the release of useful and reliable laboratory results for diagnosis and monitoring of patients would not be possible.

Based on the above, it is essential that the analyst holds the knowledge of the techniques used and acts properly to ovecome

the analytical interferences in the laboratory. These often require simple measures. The solutions proposed make the laboratory tests more reliable for diagnosis and clinical follow-up, allowing the laboratory to effectively fulfill the mission to assist qualified diagnosis.

RESUMO

A lipemia pode caracterizar-se por turvação no soro provocada por acúmulo de lipoproteínas e constitui importante causa de interferência em análises laboratoriais. A hipertrigliceridemia (HTG) é a terceira causa mais comum de pancreatite aguda (PA). O diagnóstico e o acompanhamento laboratorial de PA associada à HTG são dificultados pela intensa lipemia associada. Este relato de caso propõe uma abordagem sobre as interferências laboratoriais causadas pela HTG em paciente diagnosticado com PA e as ações direcionadas para realização de exames laboratoriais confiáveis, isentos de interferência e úteis na tomada de decisões clínicas.

Unitermos: hipertrigliceridemia; triglicerídeos; técnicas de laboratório clínico; pancreatite.

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