International Journal of Research in Medical Sciences
Saha B. Int J Res Med Sci. 2017 Oct;5(10):4610-4613
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20174186

Short Communication

Spurious hypertriglyceridaemia in unconscious patient

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Received: 06 September 2017
Accepted: 11 September 2017

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ABSTRACT

Background: Routine biochemical investigation of one patient admitted with a diagnosis of CVA as mentioned in the requisition revealed high hypertriglyceridaemia in a non-lipemic serum for the first time. Later, after scrutiny of the case sheet in the ward, it was found that apart from other management, blood was drawn about 90 minutes after administration of 4th dose of oral glycerol through nasogastric tube and sent to the laboratory. This was suspected to be the probable cause. In order to find out the degree of interference in blood sample, a small amount of glycerol was brought from the ward to experiment.
Methods: Glycerol was initially diluted to 1 in 100 in distilled water and then artificially mixed in various dilutions with pooled serum from indoor patients and pooled heparinized plasma from outdoor patients. These samples were subjected to triglycerides estimation while routine analyses were going on.
Results: A concentration of about 13,50,500 mg/dl falsely measured triglycerides was found in glycerol solution. This needed a dilution of 1 in 1500 for distilled water, serum and plasma which had 0, 190 and 113 mg/dl triglycerides respectively to bring the level to near the highest range of linearity. The recovery of added glycerol in distilled water was almost 100% but there was some positive bias more with plasma than serum.
Conclusions: The spurious hypertriglyceridaemia in serum resulted from positive interference due to temporary high glycerolaemia by the commonly employed GPO-PAP method for its measurement and would cause the same depending on glycerol level.

Keywords: False, Glycerol, Hypertriglyceridaemia, Plasma, Spurious, Serum

INTRODUCTION

Triglycerides in serum/plasma have been measured routinely by all laboratories using commercially available reagent kit from many sources which involve glycerol-3-phosphatase oxidase - paraaminoantipyrine (GPO-PAP) method. The calibration and evaluation of its performance by the auto-analyzers are accomplished by the commercially available system calibrator and quality control sera respectively as a part of good laboratory practice before allowing routine sample analyses. Normally, high level of triglycerides is found in lipemic serum which is almost directly proportional to the degree of lipemia. A case is reported here in which serum triglycerides level was found to have a concentration of 1574 mg/dl in nonlipemic serum for the first time. This blood sample was sent from a 58-year-old patient who was admitted in a medical ward with the diagnosis of cerebrovascular accident (CVA) as mentioned in the requisition. Later, on the same day after scrutiny of the case sheet, it was learnt that the patient was admitted for unconsciousness. Computerized tomography scan revealed intracerebral haemorrhage. Apart from others pertinent to the management of the case, oral glycerol was administered through nasogatric tube at a dose of 30 ml eight hourly. The sample was taken about 90 minutes

after the 4th dose. This was suspected to be the probable cause for false hypertriglycermia in the above case.

Review of literature subsequently revealed that oral administration of glycerol was required to reduce intracranial tension caused by intracerebral haemorrhage.[1,2] Pharmacokinetic study in healthy volunteers (n=10) after a single dose of 1.2 g/kg glycerol following 12 hour fasting revealed maximum serum glycerol level ranging from 1285 mg/dl to 2283 mg/dl after 1 to 2 hour of ingestion.[3] A similar study carried out for 30 minutes involving patients with head injury after administration of 50% glycerol (0.5 gm/kg) in one minute through nasogastric (n=4) and enterodudenal (n=5) route showed rapid elevation to reach the maximum concentration within 10 minutes with greater reduction in intracranial pressure in the latter but the Tmax and Cmax were not statistically significant between the two routes.[4] A case of pseudohytriglyceidaemia in a 74 year aged lady with a level ranging from 1815 mg/dl to 2693 mg/dl was reported due to oral glycerol therapy for glaucoma with a dose of 50 ml every 8 to 12 hours for two months but returned to her pre-treatment level of 72 mg/dl on 3rd day after withdrawal of glycerine treatment.[5]

Out of curiosity, about 1 ml of the glycerol administered to the patient was brought to the laboratory. Estimation of triglycerides was done after diluting the glycerol as an unknown sample and it was found to have an unbelievably high level. In order to ascertain the level of triglycerides which was falsely measured so in the glycerol solution itself and to investigate the degree of its interference in the triglycerides assay by deliberate artificial contamination in serum and plasma, about 5 ml of glycerol was taken from a new bottle of glycerol subsequently to carry out the following study.

METHODS

The oral glycerol [glycerine/glycerol-I.P. (Indian Pharmacopoeia), $C_3H_8O_3$, M.W. 92.1, specific gravity - 1.249%, glycerol content on anhydrous basis - >98% - <101%, Orient Pharmaceutical Laboratories, Kolkata, India] administered to the patient through nasogastric tube was brought from the ward for analysis to the Biochemistry laboratory on the day of the following experiment. The highly viscid solution was primarily diluted to 1 in 100 (Sample X) by dissolving 100 ul of the glycerine solution in 9.9 ml distilled water from which finally sample 1 (1 in 1000 dilution), sample 2 (1 in 1500 dilution) and sample 3 (1 in 2000 dilution) were made by adding 100 ul of sample X to 0.9 ml, 1.4 ml and 1.9 ml distilled water respectively.

On the preceding day, serum from indoor patients and heparinised plasma from outdoor patients were pooled after completion of analyses before discarding. These were mixed well, kept at room temperature for 30 minutes and then at 4°C in the refrigerator for about 18 hours after which the pooled samples were kept at room temperature for about two hours with intermittent mixing to allow auto-agglutination mainly and thereafter centrifuged. The pooled serum was denoted as sample Y while pooled plasma as sample Z after transferring to separate test tubes. Sample 1,2,3, Y and Z were first analyzed as unknown samples along with the samples from patients in the Olympus autoanalyzer, model AU640 (Olympus Pte Ltd., Japan) using triglycerides reagent kit by GPO-PAP method (REF 11488872 216, Cobas/Roche, Roche Diagnostics GmbH, Mannheim) after calibration with Beckman Coulter (BC) System Calibrator followed by ensuring quality performance by BC Control Serum 1 and BC Control Serum 2 [REF 66300, REF ODC 003 and REF ODC 004, Beckman Coulter Ireland Inc., Ireland] which were in vogue for more than a decade to note the levels of the above samples.[6]

Due to very high levels exceeding the linear range of measurement of triglycerides in sample 1, 4, 7 and 8, other experimental samples were prepared as follows. Sample 1A, 4A, 7A and 8A were prepared by diluting 1 in 2 in distilled water of sample 1, 4, 7 and 8 respectively. Sample 4, 5 and 6 were prepared by adding 100 ul of sample X to 900 ul, 1400 ul and 1900 ul respectively of sample Y and similarly sample 7, 8 and 9 were prepared by adding 100 ul of sample X to 900 ul, 1400 ul and 1900 ul respectively of Sample Z.

All these samples i.e. sample 1A,2,3,4, 4A,5,6,7, 7A, 8, 8A and 9 including sample Y and sample Z together were subjected to run as separate set of samples consecutively eleven times within about 40 minutes to check precision by temporarily keeping the routine analyses of patients' sample in abeyance.

RESULTS

The levels found in sample 1,4,7 and 8 exceeded the upper limit linear range (1000 mg/dl). These were displayed in the autoanalyzer as 1323F, 1365F, 1423F and 1020F mg/dl respectively for which sample 1A, 4A, 7A and 8A were made to ascertain the correct level. The results have been shown in Table 1.

A concentration of about 13,50,500 mg/dl triglycerides was found in glycerol solution by the above system of investigation. This was calculated by taking average of sample 1A, 2 and sample 3 due to very remarkably very high level of triglycerides in sample X for technical reasons with the variation of 0.84% from the mean in the setting of a maximum 0.48% CV error at the final measured level. The micropipette tip was required to be slightly cut to allow proper suction of the highly viscid glycerol solution. Despite this including meticulous efforts, slight problem was encountered in dispensing the actual volume of glycerine due to invariable removal of about more than 1ul by the capillary action while wiping out the outside of the micropipette tip to remove extra glycerol when preparing primary sample X (1 in 100).

Subsequent dilutions by the conventional micropipette to make 1 in 1000, 1 in 1500 and 1 in 2000 dilution could have magnified the error to a considerably significant extent. The initial level of pooled serum and plasma were 190 and 113 mg/dl respectively. Table 2 showed that the recovery of glycerol was nearly 100% in sample no 2,3 and 5 but somewhat higher up to a maximum 22% in others taking into account of triglycerides level of both in plasma and serum in the concerned samples.

Table 1: The levels of triglycerides measured by GPO-PAP method.

Sample no.	2	3	5	6	9	Y	Z	1A	4A	7A	8A
Mean±SD (mg/dl)	889±4.3	678±2.9	961±3.8	754±3.9	815±4.1	190±0.9	113±0.6	681±2.9	709±2.6	743±2.9	527±2.3
CV%	0.48	0.42	0.39	0.51	0.5	0.47	0.5	0.42	0.36	0.39	0.43

n=12 for sample 2,3,5,6,9, Y and Z ; n=11 for sample 1A, 4A, 7A and 8A

Table 2: Recovery as triglycerides by GPO-PAP method from artificial contamination of sample by glycerol.

Sample no.	2	3	5	6	9	1A	4A	7A	8A
Actual level (mg/dl)	902	675	900	675	675	675	676	676	451
Observed level (mg/dl)	885	674	966	762	821	681	709	754	527
% Recovery	98.11	99.85	107.3	112.9	121.6	100.88	104.88	111.53	116.85

DISCUSSION

The principle of estimation of triglycerides in serum/plasma involves measurement of glycerol essentially by cleaving triglycerides enzymatically first to release glycerol which is allowed to undergo subsequent chemical reactions for final estimation photometrically.[6]

Oral glycerol is rapidly absorbed from the gastrointestinal tract with a $t_{½}$ of 0.5-0.75 hours and 80% of which is metabolized while rest 20% is eliminated by unknown pathways.[7] The absorption of glycerol from the intestine by an individual unconscious patient with cerebral haemorrhage with or without other comorbid conditions is presumably not the same as that by a conscious and/or somewhat ambulatory patient including healthy individuals. In the setting of already three previous doses of glycerol (30 ml dissolved in about 70-80 ml water), the last dose with the same amount was given at about 5 AM and the sample was drawn at about 6:30 AM. Hence, the level found in the concerned patient was reflective of his blood glycerol at that point of time.

Glycerol blanking in triglycerides estimation has not been in vogue by almost all the clinical biochemistry laboratories where commonly available commercial triglycerides reagent kits from different companies are used. These are calibrated by the multi-parameter system calibrator followed by ensuring quality control using multi-parameter quality control (QC) sera. In our laboratory of 600 bed hospital, glycerol blanking was never adopted in last twenty-five years, even now although triglycerides kit from various sources was /has been used over the years in four autoanlyzers as follows. Reagents from Boehringer Mannheim (BM)/Roche, Ranbaxy and Pointe Scientific using BM/Roche make calibrator and QC sera in Hitachi 704 autoanalyzer (Germany); Roche, Randox, Olympus/Beckman Coulter (BC), Priman, Accurex and DiaSys in Olympus AU 400 and AU 640 autoanalyzers (Japan) and Roche, Dirui and Seikisui in Dirui CS 6400 (China) using BC calibrator and QCs. The method of estimation of triglycerides and glycerol in serum/plasma are different.

The serum from indoor patients and plasma from outdoor patients were deliberately pooled to observe the matrix effects of glycerine in the mixed sample. The former was indicative of heterogeneous mixture of administered drugs (oral and parenteral), intravenous fluids and the various biochemical changes in various stages of different severe illnesses while the latter represented heterogeneous mixture of oral medications mainly including biochemical alterations relevant to the diseases in relatively stable ambulatory patients.

In view of negligible pipetting and %CV error, the positive bias in recovery from plasma/serum was due to matrix effect of serum/plasma. The same seemed to rise with the increase in volume of matrix of both. The positive interference seemed to be due to interferences by some unknown agents which resulted from chemical reactions of glycerol with various constituents of serum and plasma. The degree of such interferences might be unpredictable in a given case. The level of glycerol being falsely measured as triglycerides by the original solution and its matrix effect had not been reported earlier.

In the present case, the straw coloured serum was not lipemic at all but the initial level of triglycerides exceeded the linear range of measurement for which a dilution of 1 in 2 and 1 in 5 in distilled water were made to observe the actual level to be 1574 mg/dl. Had the

level been around 200 mg/dl despite having considerably low actual value, this would have passed unnoticed. Without physical inspection, suspicion and thinking seriously before validation, this might have created great confusion about the credibility of the report particularly when repeat investigation after a considerable gap could have showed remarkably wide variation to the lower side, even within the normal reference interval. However, the real problem of false hypertriglyceridamia in nonlipemic serum due to hyperglycerolaemia would be encountered in the rare disease of glycerol kinase deficiency, even more bewilderingly without evidence for glycerol kinase deficiency.[8-10] Glycerol estimation is required in these cases including further investigations. Even if glycerol blanking was done in above concerned patient, false elevation of triglyceride would presumably had occurred due to very high level of glycerol in blood since there would be an upper limit for that assay also. Hence, this relatively rare problem of interference in a general hospital would continue to bother due to chemistry of principle of its estimation by the routine method.

Keeping the above in view, it is concluded that spurious elevation of triglycerides in serum resulted from positive interference due to temporary high level of glycerol in blood by the commonly employed GPO-PAP method for its measurement. Such spurious elevation would occur to a variable extent depending on glycerol level in the blood at the time of blood sampling for investigation. This should be kept in mind while interpreting triglycerides level in patients undergoing treatment for unconsciousness with increased intracranial tension.

ACKNOWLEDGEMENTS

Author would like to thank Durgapur Steel Plant hospital for utilizing its facilities to carry out the study as per his wishes.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

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Cite this article as: Saha B. Spurious hypertriglyceridaemia in unconscious patient. Int J Res Med Sci 2017;5:4610-3.