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TEACHING FILES (GRAND ROUNDS)

VARIATION IN HEPATITIS B VIRAL LOAD TITRES IN A SPAN OF ONE WEEK IN A PATIENT NOT ON ANTIVIRALS- HOW TO INTERPRET?

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Polymerase chain reaction, Sample processing, Haemolytic interference, Viral PCR, Antiviral agents, Hepatitis B diagnosis.

Clinical Problem:
A 28-year-old primigravida presented in December 2024 at 17 weeks of gestation with incidentally positive results for hepatitis B surface antigen (HBsAg), hepatitis B envelope antibodies (anti-HBeAg) and total hepatitis B capsule antibodies (anti-HBcAg). She was negative for IgM anti-HBcAg and anti-hepatitis C antibodies. She reported no history of jaundice. Her husband was negative for HBsAg and was advised hepatitis B vaccination. On presentation, her weight was 44.8kg. Her initial hepatitis B viral load (VL) was 3,67,125 IU/mL with a log value of 5.5. Abdominal ultrasound was normal. Hepatitis B VL was repeated after 6 days of the initial test and was found to be 80,725 IU/mL with a log value of 4.9. She was advised to repeat the hepatitis B VL and follow-up in 1 month.

How do you explain the variability in the hepatitis B viral load results of the patient?

Discussion:

The dramatic difference in the estimated hepatitis B VL of a patient not on hepatitis B treatment, measured 6 days apart in the same laboratory using the same assay is very strange and warrants investigation into the aberrancy. The explanation for these aberrant results may lie within the technical aspects of the hepatitis B VL assays. According to the National Laboratory Guidelines for the testing of Viral Hepatitis from the Ministry of Health and Family Welfare, India, strict quality control measures for sample storage, transport and processing must be followed, in order to ensure accuracy of the results.[1] For the testing of the hepatitis B viral load, prior to nucleic acid extraction, the patient's blood sample may be stored for up to 24 hours at room temperature and up to 72 hours at 2 to 8 degrees C. Deoxyribonucleotide (DNA) extraction should be done according to the assay's instructions. Adsorption of the extracted DNA can be done using polypropylene and polyethylene tubes. Storage of the extracted DNA prior to testing can be done in polyallomer and some special polypropylene tubes.[1] Blood samples, prior to DNA extraction, if stored at temperatures not conforming to the one recommended in the guidelines, may undergo hemolysis. Hemolytic products, particularly free hemoglobin, hematin, hemin, lactoferrin and immunoglobulin G, have been shown to interfere with the detection of nucleic acids in blood samples and thus, hemolyzed samples may falsely report a low VL or an absent VL.[2,3,4] Free hemoglobin reduces the activity of DNA polymerase and thus, reduces the rate of amplification. Free hemoglobin and hematin also cause fluorescence quenching.[4] Thus, the drastic fall in the patients VL within 6 days may be explained by a delay in sample processing and inadequate temperature regulation for storing the sample. On the other hand, repeated temperature changes in the sample, such as with frost-free refrigerators, may also interfere with the accurate detection of VL. Frost-free refrigerators work on a freeze-thaw cycle, in which the temperature is first increased and then it is dropped, resulting in the refreezing of the sample. This process has been shown to break down nucleic acids and can result in a drastic fall in the detected VL.[1,5] In our patient, the sudden drop in the hepatitis B VL could not be explained clinically, which prompted us to search for a possible technical reason for this result. We found that a delayed processing of the blood sample, more than 24-hours after collection and storage at room temperature, led to the aberrant hepatitis B VL.

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