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CASE REPORT

A Case of Falsely Elevated Total Bile Acids in a Pregnant Woman

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SUMMARY

Background: **Total bile acid (TBA) is commonly used in pregnant women as a diagnostic and monitoring marker for intrahepatic cholestasis of pregnancy (ICP). Over 90% of pregnant women suffering from ICP have an increase in serum TBA levels.**
Methods: **We present the case of a 27-year-old pregnant woman whose TBA level initially reached 152.5 µmol/L, far exceeding the threshold for concern in ICP. However, upon resampling and testing the following day, her TBA levels returned to within the normal reference range (< 10 µmol/L). To investigate this substantial fluctuation over a single day, we performed thorough maintenance and cleaning of the instrument before retesting the serum samples for TBA. Additionally, serum samples from both days were sent to two independent laboratories for reanalysis using different testing systems.**
Results: **Two samples were analyzed using different testing systems, yielding consistent results. The falsely elevated TBA levels were attributed to the patient's non-fasting state and the recent intake of a high-fat meal prior to blood collection.**
Conclusions: **When TBA levels are unusually elevated in a pregnant woman without symptoms of ICP, a repeat test should be performed under strict fasting conditions. The patient should also be educated on the importance of adhering to fasting protocols for accurate blood testing.**
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KEYWORDS

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INTRODUCTION

Total bile acids (TBA) are the final substances of cholesterol catabolism in the liver and are closely related to cholesterol absorption, metabolism, and regulation. Intrahepatic cholestasis of pregnancy (ICP) is primarily characterized by skin pruritus and increased levels of TBA in the blood serum of the pregnant women [1]. The levels of TBA are currently considered to be the most sensitive and specific biochemical marker for diagnosing and monitoring ICP. Over 90% of pregnant women suffering from ICP have an increase in serum TBA levels [2,3]. According to the Chinese Medical Association (ChMA), a fasting serum TBA level of

≥ 10 μmol/L is considered diagnostic for ICP [4]. Serum TBA is usually recommended to be measured in pregnant women in the fasting state due to the higher diagnostic specificity of the fasting TBA test [5].

CASE PRESENTATION

A 27-year-old woman, who is 39 weeks and 6 days of pregnancy, was admitted to the hospital with elevated blood glucose for over 2 months. On August 4th, the blood sample was taken for biochemical tests, and the TBA result was 152.5 μmol/L. The liver function indicators were also unusually elevated (Table 1), consistent with the laboratory indicators outlined in the Guidelines for the Diagnosis and Treatment of Intrahepatic Cholestasis in Pregnancy by the Chinese Society of Infectious Diseases [6]. However, the pregnant woman did not exhibit symptoms such as skin pruritus and yellowish discoloration of the sclera. Therefore, the patient's blood was collected again on the morning of August 5th and the TBA result was measured to be 6.1 μmol/L. The clinician reached out to our laboratory to query about the potential for an error in the test, given the significant difference in TBA results within a single day.

In response to clinical concerns, we retrieved the patient's serum sample collected on August 4th, which had been stored at 4℃, for reanalysis. As TBA levels in serum remain stable between 2℃ and 8℃ for up to one week, the sample was considered appropriate for retesting. Currently, the laboratory measures TBA using the enzyme cycling method where bile acids react with 3α-hydroxysteroid dehydrogenase (3α-HSDH) and oxidized β-thionicotinamide ureido purine dinucleotide to generate 3-keto steroids and β-thionicotinamide ureido purine dinucleotide (Thio-NADH). 3-Keto steroids undergo a reaction in the presence of 3α-HSDH and Thio-NADH to generate bile acids and Thio-NAD. The enzyme cycle amplifies the little quantity of bile acids present, allowing for the indirect determination of their concentration. This is achieved by measuring the change in absorbance at 405 nm of Thio-NADH generated during a specific period [7,8]. We re-evaluated the patient's serum specimens from August 4th and August 5th using two separate biochemistry analyzers in our laboratory, and two serum samples were divided and sent under cold chain conditions to two independent laboratories for analysis. Considering the potential carryover present in the biochemical analyzer, both samples had TBA measured individually after performing the cleaning procedure on the instrument without admixing it with other samples. The results indicated that the patient's serum samples over two days, tested across different systems and reagent kits from various manufacturers, were consistent with our reported findings (Table 2). This ruled out the possibility of instrument- or reagent-related issues, leading us to conclude that the reported TBA results for the patient over the two days are accurate and reliable.

What, then, could explain such a significant discrepancy in the TBA results of the pregnant woman, observed just one day apart?.

It has been reported that TBA testing is strongly influenced by food [9]. Upon reviewing the serum sample collection times, we found that the sample on August 4th was collected at 09:31 am, while the sample on August 5th was collected at 06:16 am. We compared the results of the two samples (Table 1) and observed that, in comparison to August 4th, the lipids, glucose and TBA results, which were more affected by the diet, exhibited a decline on August 5th. Conversely, the results of ALT, AST, GGT, and ALP, which were not influenced by diet, showed varying changes. Generally speaking, pregnant women, especially those nearing term, tend to follow a diet characterized by small, frequent meals. On August 4th, after the pregnant woman was admitted to the hospital following her outpatient visit, the blood sample was collected at 09:31 am. It was suspected that the patient may have consumed food earlier in the day. However, when asked by the nurse if she had had breakfast, the patient, despite having eaten, may have considered that she had not had a "proper" breakfast. Consequently, the nurse proceeded to collect the sample under the doctor's order for a “fasting biochemistry complete set”, despite the patient not being in a true fasting state. To verify our hypothesis, we contacted the patient who confirmed that she had indeed eaten two fried sausages and a carton of milk on August 4th while awaiting hospital admission, in addition to an excessive quantity of Japanese food the preceding night. Therefore, we concluded that the biochemical results from August 4th likely reflected the postprandial state, while those from August 5th represented a true fasting state.

In this case, the physician, confronted with elevated TBA levels, assessed the probability of ICP in the patient as quite high, necessitating potential immediate transfer to the operating room for a cesarean section. However, the patient's elevated TBA resulted from the patient's consumption of a high-fat diet before the test. The nurse did not strictly enforce the requirement for fasting blood collection in TBA testing. The definition of fasting blood collection requires that, for a specified period prior to blood collection the patient does not eat or drink any calorie-containing foods or beverages. Typically, the fasting period lasts 8 to 12 hours, depending on the specific requirements of the test. During this period, the patient may drink small amounts of water, but they should avoid coffee, tea, juice, alcohol, or any other beverages containing sugar or fat, as these can affect the blood tests [10,11]. In a fasting state, the levels of glucose and lipids in the blood remain relatively stable, as are TBA as highlighted in this case. Fasting minimizes the dietary impact on TBA testing, providing a clearer assessment of the patient's bile acid metabolism and liver function, which is crucial for diagnosing conditions such as ICP. This occurs because postprandially, particularly following the ingestion of high-fat foods,

Table 1. Comparison of biochemical results from patients' serum samples collected over two consecutive days.

Test item	Date	
	August 4th	August 5th
ALT (U/L)	83.1	101.9
AST (U/L)	142.7	103.9
ALP (U/L)	603.2	548.7
GGT (U/L)	86.6	98.1
CHOL (mmol/L)	7.63	6.55
TG (mmol/L)	4.99	2.63
HDL-C (mmol/L)	1.83	1.67
LDL-C (mmol/L)	4.48	3.87
APOA1 (g/L)	2.20	2.07
APOB (g/L)	1.42	1.21
GLU (mmol/L)	5.44	4.65
TBA (μmol/L)	152.5	6.1

ALT Alanine transaminase, AST Aspartate transferase, ALP alkaline phosphatase, GGT Gamma-glutamyl transferase, CHOL Cholesterol, TG Triglycerides, HDL-C High-density lipoprotein cholesterol, LDL-C Low-density lipoprotein cholesterol, ApoA apolipoprotein A, ApoB apolipoprotein B, GLU Glucose, TBA total bile acid.

Table 2. Retest results of TBA using different testing systems.

TBA testing	Our laboratory		Laboratory B	Laboratory C
Instrument	Beckman Coulter AU5800	Beckman Coulter AU5800 *	Roche	Hitachi
Reagent kit (Brand Name)	Kwork	Kwork	Reebio	Meikang
August 4th	150.5	152.8	149.9	155.9
August 5th	6.3	6.2	6.3	6.7

*** The two fully automatic biochemical analyzers in our laboratory are both Beckman Coulter AU5800.**

the liver increases bile secretion to metabolize the fat, resulting in higher TBA levels in the blood that may not correctly represent the patient's true level. It is critical for clinical staff to comply with pre-collection quality control measures, including fasting, as this forms the foundation for the accurateness and reliability of our laboratory results.

DISCUSSION

ICP is a liver disorder specific to pregnancy, typically manifesting during the mid-to-late stages of gestation. Elevated TBA levels in pregnant women not only affect maternal health but also pose significant risks to the fetus. Increased TBA can stimulate the release of prostaglandins from the uterus, triggering uterine contractions and potentially leading to preterm labor. The diagnosis of ICP primarily depends on blood tests, particularly the measurement of fasting TBA levels. Elevated TBA can cross the placenta, causing fetal hypoxia and ischemia, which increases the risk of intrauterine fetal demise. Experts recommend delivery in the 36 0/7 weeks of gestation when TBA concentration exceeds 100 μmol/L [2]. TBA concentration exceeded 100 μmol/L in a pregnant woman asymptomatic for ICP, necessitating heightened vigilance from both laboratory personnel and clinical caregivers. Laboratory personnel must not only rule out any potential factors related to laboratory testing but also systematically investigate other variables that may influence TBA levels, such as food intake and medication use. During late pregnancy, the increased burden on the liver may result in abnormal liver function indicators in pregnant women. We should not be confused by the abnormal liver function of the patient and take it for granted that the high TBA value is reasonable, thus falling into the “trap”. Doctors and nurses must also consider the patient's clinical symptoms while ensuring proper patient education on specimen collection, with emphasis on the importance of fasting for TBA testing. Collaboration between laboratory and clinical teams is essential to maintaining high laboratory testing standards and ensuring optimal patient care. The standardization of specimen collection process, including the correct timing, method, and selection of containers, falls under pre-analytical quality control. This aspect of quality control is a critical component of laboratory management, playing a significant role in improving test accuracy and ensuring patient safety.

Our case confirmed that food intake has a notable impact on TBA levels. The ingestion of food stimulates bile secretion, which may subsequently influence serum TBA concentrations. Additionally, the type of food, particularly high-fat meals, can increase bile secretion, potentially affecting TBA test results. Nonetheless, in non-pregnant individuals, even postprandial testing rarely results in such significant increase in TBA levels. This may be related to elevated progesterone levels in pregnant women, which reduce the tone of smooth muscles in the digestive tract, leading to decreased gallbladder tone and delayed emptying during pregnancy. This impairs the liver's bile excretion and causes a delay in the normalization of postprandial elevated TBA levels [12]. In this case, the patient consumed fried food on the morning of August 4th, which likely caused excessive bile secretion and contributed to the marked increase in TBA levels.

CONCLUSION

In conclusion, this case emphasizes the critical importance of adhering to fasting protocols before conducting TBA testing in pregnant women. The substantial variation in TBA levels observed in this patient, attributed to the lack of fasting and high-fat diet consumption, underscores how dietary factors can significantly affect test results. Additionally, this case also highlights the need for thorough communication between healthcare providers and patients regarding pre-test preparations and reinforces the importance of pre-analytical quality control ensure reliable diagnostic outcomes for ICP.

Declaration of Interest:
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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