Short Communication

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Effect of hemolysis on prealbumin assay

Abstract

Objectives: Hemolysis is a common problem causing interference on biochemical assays. In this study, we investigated the effect of in vitro hemolysis on the measurement of prealbumin, using normal and low prealbumin concentrations.

Methods: Serum pools containing normal and low levels of prealbumin were spiked with different dilutions of the hemolysate, which was prepared according to the classical osmotic shock procedure. The final concentrations of hemoglobin in the samples were 10.63, 6.25, 5.31, 2.66, 1.33, 0.66, 0.33 and 0 g/L. The prealbumin levels in these samples were analyzed for three times by the immunoturbidimetric method on AU680 clinical chemistry analyzer. Mean percent changes of prealbumin results were presented with interferographs.

Results: We observed that hemolysis interfered negatively with both normal and low level serum pools. This effect began to exceed the limit of 10% as a critical point in the concentration range 5.31 to 6.25 g/L hemoglobin concentration in both normal and low serum pools. The limit exceeding value was higher in the low serum pool than the normal serum pool (20% and 11%, respectively).

Conclusions: Clinical laboratories must be alert the effect on low prealbumin levels, especially in settings where hemolysis is an increasing problem.

Keywords: hemolysate; hemolysis interference; prealbumin assay; preanalytical phase; serum pool.

ÖZ


Gereç ve Yöntemler: Normal ve düşük seviyelerde prealbumin içeren serum havuzlarına, klasik oozmotik şok prosedürüne göre hazırlanan hemolizatın farklı dilüsyonları eklenildi. Numunelerdeki nihai hemoglobin konsantrasyonları 10.63, 6.25, 5.31, 2.66, 1.33, 0.66, 0.33 ve 0 g/L idi. Bu numunelerdeki prealbumin seviyeleri, immunoturbidimetrik yöntemle AU680 klinik kimya analizöründe üç kez analiz edildi. Prealbumin sonuçlarının ortalaması yüzde değişimleri interferograflarla sunuldu.

Bulgular: Normal hem de düşük seviyeli serum havuzlarına hemolizin negatif interferansını gözlemledik. Bu etki her iki serum havuzunda 5.31 ila 6.25 g/L hemoglobin konsantrasyonunda kritik bir nokta olarak %10 sınırını aşmaya başladı. Sınır aşan değer düşük serum havuzunda normal serum havuzundan daha yüksekti (srasıyla %20 ve %11).

Sonuç: Klinik laboratuar özellikle hemolizin artan bir sorun olduğu durumlarda düşük prealbumin seviyeleri üzerindeki etkisi konusunda dikkatli olmalıdır.

Anahtar Sözcüklər: hemolizat; hemoliz interferansi; Prealbumin ölçümü; preanalitik faz; serum havuzu.

Introduction

Prealbumin is one of the best indicators of the nutritional status. It has a relatively short plasma half-life of 2.5 days, it is expected that instant changes in response to protein-energy malnutrition and therapy can occur [1]. Malnutrition is a widely seen problem in hospitalized patients, especially in intensive care units [2]. The early recognition of protein malnutrition and the initiation of nutritional therapy can shorten the length of hospital stays and improve patient outcomes. For this reason, accurate determination of prealbumin concentration is needed. Prealbumin levels are...
classified as <5 mg/dL (poor prognosis), 5–10.9 mg/dL (significant risk; aggressive nutritional support indicated), 11–15 mg/dL (increased risk; monitor status biweekly) and 15–35 mg/dL (normal) [3].

Hemolysis is an undesirable condition that influences the accuracy and reliability of laboratory testing. Especially, emergency department, intensive care unit and inpatient services of hospitals have often been known as the source of hemolyzed samples [4, 5]. The most of the current automated chemistry analyzers can detect and estimate the hemolysis interference by measuring a hemolysis index (HI). It is important to evaluate the influence of hemolysis for each test and to state the limits of acceptability for each. Therefore, the clinical relevance of hemolysis estimates of the observed manufacturer document should be reviewed. Clinical and Laboratory Standards Institute (CLSI) recommends testing at least two medical decision concentrations for hemolysis interference [6]. To the best of our knowledge, there are not sufficient studies investigating the relationship between the degree of the hemolysis and the clinical decision point of prealbumin. Our aim of the study was to comprehensively evaluate the effect of in vitro hemolysis on the measurement of prealbumin, using normal and low prealbumin concentrations.

Material and methods

The samples used in this study were created in the venous blood samples accepted by routine biochemistry laboratory. All assays were carried out on residual material remaining after the completion of any diagnostic tests. The preparation of the samples was performed in the Clinical Chemistry Laboratory of Zonguldak Bülent Ecevit University Hospital in accordance with the Ethics Committee on Human Research of Bülent Ecevit University (Protocol Number: 2019-43-03/04).

Normal serum pool was prepared from non-hemolyzed samples of 10 outpatients. Two mL from each sample were transferred to a test tube to create 20 mL serum pool. Low serum pool (20 mL) was prepared as 2 mL using the non-hemolyzed samples of 10 hospitalized patients whose prealbumin levels were below critical values. The samples of interest were stored at 2–8 °C for two days until the analysis.

The whole-blood sample with EDTA was used for hemolysate. The hemolysate was prepared according to the classical osmotic shock procedure, adapted from the CLSI guidelines (CLSI EP7-A2) [6]. The plasma was decanted and the separated erythrocytes were washed three times with 0.9% cold saline to remove any trace of EDTA. After every wash, the cells were collected by centrifugation for 2 min at 150 g. Washed erythrocytes were spiked with an equal volume of distilled water, thoroughly mixed and frozen overnight at –20 °C. The day after, the sample was thawed and followed by centrifugation for 30 min to remove cell debris. The supernatant hemolysate was taken into a clean tube and then the hemoglobin value (170 g/L) was measured using the Cobas c 501 (Roche Diagnostics, Germany).

To obtain 16 fold dilution of the hemolysate, 1500 μL normal or low serum pool and 100 μL hemolysate which consisted of dilutions of the hemolysate at different hemoglobin concentrations were added to each microcentrifuge tube and then vortexed. The final concentrations of hemoglobin in the samples were 10.63, 6.25, 5.31, 3.99, 2.66, 1.33, 0.66, 0.33 and 0 g/L (100 μL normal saline was added for baseline concentration). Each of hemolyzed samples was separated into three aliquots for a single prealbumin analytical run. The serum prealbumin was analyzed by an immunoturbidimetric method on AU680 clinical chemistry analyzer (Beckman Coulter, USA) using Beckman Coulter’s serum prealbumin kit. The assay was performed according to the manufacturer’s instructions. The linearity of the assay was 3–80 mg/dL and the analytical sensitivity was 0.4 mg/dL. The within- and total-run coefficient of variation (CV) values were <2.2% and <3.2%, respectively. The internal quality control and external quality assurance were within acceptable limits throughout the study.

To compare the analyte concentrations of the baseline pool and hemolyzed pool, mean % change (bias percentage) was calculated by the formula:

\[
\frac{|C_2 - C_1|}{C_1} \times 100
\]

C1: Concentration of baseline pool, C2: Concentration of hemolyzed pool

The limit of 10% for the mean percent changes were considered as significant [7]. Also, results were compared via paired Student’s t-test. For the t-test, we considered a significant difference at a 95% confidence level, using a two-tailed analysis (p<0.05). The statistical significance testing was evaluated by using a statistical program (SPSS, version 9.0; SPSS, Chicago, Illinois, USA).

Results

We assessed the potential interference of hemolysis on prealbumin assay. When hemolysate was added to both serum pools, prealbumin levels declined from baseline values in nonhemolyzed serum in a dose-related way. We observed that mean prealbumin concentrations in normal serum pool were significantly different from the baseline value in the presence of 5.31 g/L and above of hemoglobin (p<0.05). In addition, mean prealbumin concentrations in the low serum pool were significantly different from the baseline value in the presence of 3.99 g/L and above of hemoglobin (p<0.05). The results of the study were all presented in Table 1.

The limit of 10% as critical point was exceeded in the concentration range 5.31–6.25 g/L Hb concentration in both normal and pathologic serum pool. However, the limit exceeding value was greater in the low serum pool than it was in the normal serum pool (20 and 11%, respectively). We also showed the interference effect with interferographs (Figures 1 and 2).
Discussion

Serum prealbumin level is an important parameter utilized by physicians for monitoring or evaluation of nutritional status. In this study, the effect of hemolysis on prealbumin test has been investigated by an experimental model.

Firstly, we found that there has been a negative interference on prealbumin test with hemolysis effect. It has been reported many times that hemolysis is an important interference factor in biochemical and immunochemical analysis. There are two central mechanisms of interference by hemolysis; the spectral interference due to release hemoglobin and the chemical interference due to release intrarerythrocytic components. The presence of cell debris (e.g., cell membranes) and other potential interferents from cells can dilute analytes and result in decreased values [8]. If the level of interference from serum hemoglobin is high, all these interference effects might coexist, it can result in overestimation or underestimation [9, 10]. Although it is difficult to identify which mechanism of hemolysis affects prealbumin results in our study, the dilution effect seems possible.

Some researchers have reported that even severe hemolysis (Hb: 10 g/L) does not have any effect on prealbumin assays in Roche analyzer [11]. However, they assessed hemolysis interference only on one single level of concentration. According to another study on Roche analyzer, prealbumin was affected at 4 gr/L hemoglobin [12]. Unfortunately, there is no information on which analyte level was used in the study. Whereas, it is necessary to evaluate the influence of hemolysis for each test and their different concentrations. The degree of interference, especially measured at a low analyte concentration, should be noticed in our study. Surely, manufacturers’ suggestions about the use of the specified cut off limits for the test results of hemolyzed specimens must also be considered. But, the hemolysis effect on low prealbumin

Table 1: Results of prealbumin levels according to the different hemoglobin concentration and the hemolysis index (HI) of Beckman Coulter AU680 analyzer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hemoglobin (g/L)</th>
<th>Beckman Coulter AU680 HI</th>
<th>Prealbumin (mg/dL)</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
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<tr>
<td>Low level pool</td>
<td>0</td>
<td>None</td>
<td>6.0</td>
<td>0.06</td>
<td>&lt;1</td>
<td></td>
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<tr>
<td></td>
<td>0.33</td>
<td>None</td>
<td>6.1</td>
<td>0.06</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.66</td>
<td>+</td>
<td>6.0</td>
<td>0.1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.33</td>
<td>++</td>
<td>6.0</td>
<td>0.1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.66</td>
<td>+++</td>
<td>5.8</td>
<td>0.06</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.99</td>
<td>++++</td>
<td>5.7</td>
<td>0.06</td>
<td>1</td>
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</tr>
<tr>
<td></td>
<td>5.31</td>
<td>++++</td>
<td>5.6</td>
<td>0.06</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>++++</td>
<td>4.8</td>
<td>0.1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.63</td>
<td>++++</td>
<td>3.6</td>
<td>0.2</td>
<td>5.5</td>
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<tr>
<td>Normal level pool</td>
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<tr>
<td></td>
<td>0.33</td>
<td>None</td>
<td>20.2</td>
<td>0.4</td>
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<tr>
<td></td>
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<td>+</td>
<td>20.1</td>
<td>0.4</td>
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<td>15.7</td>
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</table>

Figure 1: Interferogram showing the effect of hemolysis in normal prealbumin levels.
levels in our study does not comply with the manufacturer’s recommendation, which is less than 3% interference in hemoglobin measured below 5 g/L. Therefore, we recommend that each laboratory should establish the individual HI cutoff for its specific prealbumin assay.

The main limitations of our study is the use of a single analytical platform and including a small number of samples in pools.

**Conclusion**

Clinical laboratories must be alert about the effect on low prealbumin levels, especially in settings where hemolysis is an increasing problem.

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**Author contributions:** BG researched literature, conceived the study and wrote manuscript. BG and AT were involved in gaining ethical approval, collection of sample and data analysis. MC approved the final version of the manuscript.

**Competing interests:** The authors declare that they have no competing interests.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**References**


