ARAŞTIRMA / RESEARCH

Examining the Effect of Venipuncture Technique with Vacutainer and Injector on the Rate of Hemolysis

Vacutainer ve Enjektör ile Kan Alma Tekniğinin Hemoliz Oranına Etkisinin İncelenmesi

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Abstract

Objective: This study was conducted to determine hemolysis rates for venous blood samples drawn by injector and vacutainer holder and to assess the effect of the venipuncture technique on the process of hemolysis.

Material and Method: This cross-sectional and analytical study was conducted with 128 patients who were admitted to the Cardiology and Angio clinics of a university hospital and met the inclusion criteria. One group of patients who were to have routine biochemical tests underwent venipuncture with an injector, and another group with a vacutainer.

Results: The serum hemolysis level is not significantly different by gender. No significant correlation was found between blood serum hemolysis levels and age or Body Mass Index with blood drawn with an injector or with a vacutainer. While the serum hemolysis level was on average 0.11 g/dl in blood drawn with an injector, the level in blood drawn with a vacutainer was 0.06 g/dl; the difference was not statistically significant.

Conclusion: This study revealed that there was no statistically significant difference between hemolysis rates in blood drawn with an injector or with a vacutainer. However, the hemolysis rates in blood drawn with an injector were almost twice the rates noted in blood drawn with a vacutainer, and this difference may be clinically significant.

Keywords: Hemolysis, nursing practice, phlebotomy, technique, venipuncture.

Öz

Amaç: Bu araştırma enjektör ve vacutainer kullanılarak alınan venöz kanlarda hemoliz oranını belirlemek ve kan alma tekniğinin hemoliz gelişimi üzerindeki etkisini değerlendirmek amacıyla yapılmıştır.

Gereç ve Yöntem: Kesitsel ve analitik tipte olan bu araştırma, bir üniversite hastanesinin Kardiyoloji ve Anjiyo kliniklerine yatışı yapılan ve araştırmaya dahil edilme kriterlerine uyan 128 hasta ile yürütülmüştür. Rutin biyokimyasal tetkikleri istenilen bir grup hastadan enjektörle, diğer gruptaki hastalardan vacutainer ile kan alınmıştır.

Bulgular: Serum hemoliz düzeyi cinsiyetler arasında anlamlı olarak farklı değildir. Enjektör ve vacutainer ile alınan kan örneklerindeki serum hemoliz düzeyleri ile yaş ve Beden Kütle İndeksi arasında anlamlı korelasyon saptanmamıştır. Serum hemoliz düzeyi enjektör ile alınan kanlarda ortalama 0.11 g/dl iken vacutainer ile alınan kanlarda ortalama 0.06 g/dl olup bu fark istatistiksel olarak anlamlı bulunmamıştır.

Sonuç: Bu araştırmada enjektör ve vacutainer ile alınan kan örneklerindeki hemoliz oranları istatistiksel olarak anlamlı farklılık göstermemiştir. Ancak enjektörle alınan kanlarda ortalama hemoliz oranı vacutainer ile alınan kanlardan neredeyse iki kat fazladır, bu fark klinik açıdan anlamlı olabilmektedir.

Anahtar Kelimeler: Kan alma, teknik, hemoliz, hemşirelik uygulaması.

1. Introduction

Venipuncture is one of the most common hospital procedures that almost all hospitalized patients encounter at one time or another. Furthermore, some patients undergo the procedure at least once a day; 25% of patients admitted into the hospital are administered the procedure three or even more times a day (1). Laboratory-based blood sample analysis results play a determining role in 60%-70% of hospital admittances, discharges, and all drug-related clinical decisions (2).

Venipuncture is an intervention that is widely practiced in the pre-analytical phase of the blood sampling process (2). A large percentage of errors made during the entire testing process (70%-80%) are known to occur during the pre-analytical phase (3, 4, 5). This phase consists of the processes of drawing blood, labeling the specimen, and transporting it (4). It has been emphasized that most pre-analytical errors result from hemolyzed samples, which actually originate from the process of drawing blood (4, 6). Studies have pointed to the discovery that hemolysis is the most frequent reason blood samples can be rejected (7, 8, 9).

It is reported that pre-analytical (in vitro) hemolysis may occur due to the use of small-gauge needles, inappropriate blood sampling equipment, thin and sensitive veins, drawing blood without waiting for antiseptics such as alcohol to dry, overuse of the tourniquet, the motions of opening and closing the fist, not using a vacuum system, causing the blood drawn into the injector to hit the wall of the tube with pressure or a situation where the blood in the tube is forcefully jostled or mixed (6, 10, 11). Many test results are affected by hemolysis and for this reason, a repeat of blood drawings needs to be made, leading to a delay in diagnosis, increased cost, diminished patient safety, and unnecessary discomfort and pain (4, 10).

1.1. Background

Hemolysis is defined as erythrocyte rupture resulting from the release of intracellular components into the surrounding plasma or serum. Erroneous rises in intracellular plasma/serum components such as potassium (K), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) can affect test results (12). One study has explored the fact that while levels of concentration of K, AST, LDH, and PO4- are higher in the erythrocyte cell than in plasma, hemolysis raises these levels even more. Similarly, while intracellular erythrocyte concentrations of parameters such as glucose, sodium, chloride, calcium, and albumin are lower than in plasma, these concentrations can be found to be even lower due to dilution (6).

In a study where hemolysis occurring in blood samples taken with an intravenous (IV) catheter or a butterfly needle was evaluated, it was found that while the hemolysis rate in blood taken with an IV catheter was 14.6%, the rate was 2.7% in blood taken with a butterfly needle (13). In a meta-analysis, it was shown that drawing blood with an intravenous catheter increased the hemolysis rate, and it was reported that guidelines recommend that blood samples should be taken from a peripheral intravenous cannula only when the cannula is inserted only once at first and not routinely at other times (14).

Another study examined the effect of using butterfly needles of different gauges (21, 23 and 25G) on coagulation tests and platelet counts. The results (except for D-dimer) showed that using 23G and 25G needles tended to produce lower values but a sample taken with a 21G needle did not produce significantly different values (15). The effect on routine clinical biochemistry tests of using different needle gauge sizes was explored in another study by Lippi et al. it was found that compared with 21G needles, 23G size needles when used correctly, did not produce any statistically or clinically significant error of measurement. Increased variability was seen in potassium results from a 25G compared to a 23G needle. Outside various specific situations such as patients with problematic venous access and newborns, it is universally not recommended for blood to be drawn for clinical biochemistry tests with a 25G needle or with needles with smaller lumen diameters (16).

1.2. Objective of the Study

Based on the knowledge that vacuuming (that is, rapidly transferring the blood into a tube) can increase hemolysis, this study aimed to investigate the differences in terms of hemolysis when blood is drawn with an injector or a vacutainer.

2. Materials and Method

2.1. Study Design and Setting, Participants

This study used a cross-sectional and analytical design. The study universe consisted of patients, ages 18-64, who had been admitted to the Cardiology and Angio units of a university hospital over the period October 2018 – to April 2019. Since no studies investigating similar variables could be accessed, the smallest sample size was computed as 64 individuals for each group in the power analysis for Cohen's t-test, using the recommended estimated medium effect size (d=.50) at a 95% confidence interval and 80% power (17). The total target was 128 individuals (17). The study was completed with 128 participants, 66 having venipuncture performed with an injector and 62 with a vacutainer. Eighteen of the participants were excluded because the venipuncture could not be performed successfully on the first try.

The study data were collected from patients hospitalized at the Cardiology or Angio clinics of a training and research hospital who were of the ages 18-64, whose doctor had ordered biochemical blood tests, who consented to participate in the research and had no hematoma, redness or swelling in the antecubital fossa where the blood was to be drawn. The participants and their blood samples were excluded from the study if the blood could not be drawn successfully on the first try.

2.2. Data Collection

The hemolysis level of the blood samples drawn for routine biochemical blood tests from patients was checked; no further venipuncture procedure was performed on patients for the study. One group of patients underwent venipuncture with an injector (10 ml) (Figure 1, Figure 2), and the other group with a vacutainer holder (Figure 3). The decision as to which technique was to be employed and on which day was made with block randomization so that outside of the venipuncture method itself (Vacutainer or Injector), age, gender, and other characteristics would show similar distribution (https://www.randomizer.org/). To avoid bias, the researchers did not carry out the venipuncture procedure, which was performed by clinical nurses. Information was given to the clinical nurses about the purpose of the blood drawings, which technique was to be used and which tube the blood should be drawn into, as well as the points to take into consideration during the venipuncture. For routine biochemical tests, the blood was drawn into biochemical red-top tubes with a gel barrier.

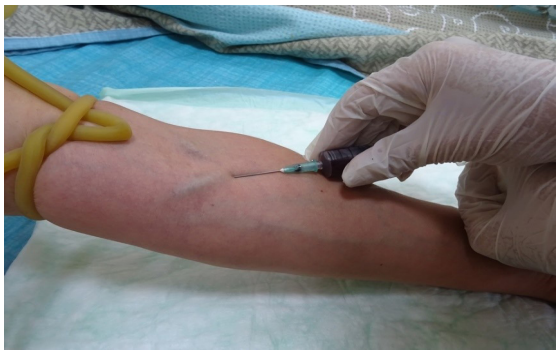

Figure 1. Venipuncture by 10 ml injector

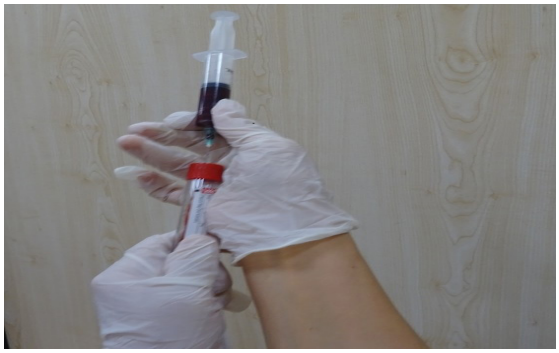

Figure 2. Transferring into the tube the blood taken by the injector

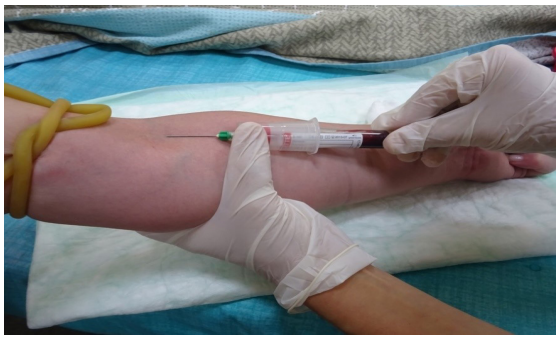

Figure 3. Venipuncture by vacutainer holder

Then the research team immediately (within a maximum of 1 hour), transported the blood specimens to the biochemical laboratory of the same hospital by taking care that the lids of the tube were facing upward, and no jostling took place. The blood specimens received at the laboratory were centrifuged at a 4000 cycle for 10 minutes in preparation for the testing. An amount of 0.3 ml serum was taken from the test tube to test for hemoglobin, after which the blood tube was given to the laboratory technicians to work on the routine tests ordered for the patient.

The following were performed to eliminate the factors that could affect hemolysis during the blood drawing procedure and the clinical nurses were informed accordingly.

- It was expected that before initiating the venipuncture, an appropriate pause would be given so that antiseptics such as alcohol used in cleaning the venipuncture site could dry.

- The application of the tourniquet did not take more than 1 minute.

- Needles of 21-gauge (green-tipped) were used.

- Care was taken so that the blood would not be abruptly shaken after being transferred into the tube (18, 19).

2.3. Assessment Criteria

All the blood samples were spun in a centrifuge for serum separation, then tested for hemoglobin using the Drabkin method as a hemolysis indicator.

The Drabkin reagent was prepared with the consumable chemicals found in the biochemistry laboratory: 100 milliliters (ml) of sodium bicarbonate, 20 ml of potassium ferricyanide, and 5 ml of potassium cyanide were dissolved in 1000 ml distilled water. After the mixture was homogenized, it was transferred into dark bottles. Three ml of Drabkin reagent was placed into the cuvette. The 200 microliters of serum that was to be tested for hemoglobin was added to the tube, which was then incubated for 10 minutes at room temperature. The same procedure was performed for each sample. Using the Drabkin method, an absorbance determination was made at 540 nanometers with an Architec 18000 model (Abbott, USA) automatic analyzer. A hemoglobin calibration of 1 g/dl was used to achieve a standard curve to determine hemoglobin concentration. The results were expressed as gram/dl. To prevent bias, each tube was given a number and the analysis was performed without knowing which technique had been used in drawing the blood.

2.4. Statistical Analysis Methods

The IBM SPSS 24 Statistical Package Program (Chicago, IL, USA) was used in the data analysis. Descriptive statistics were presented in the form of numbers, percentages, means, and standard deviation. The Mann-Whitney U test was used to determine the differences between serum hemolysis levels according to gender and venipuncture technique: Spearman's rho correlation analysis was employed to assess the relationship between serum hemolysis levels in terms of age and BMI. The level of statistical significance was accepted as $p<0.05$.

3. Results

The participants were between the ages of 32-96; their mean age was 64.70±13.32 years. Of the participants, 64.1% were male (n=82); the mean BMI was 27.35±4.75. Blood was drawn from 48.4% of the participants by vacutainer, from 51.6% by injector.

The characteristics of the participants by venipuncture technique can be seen in Table 1. Additionally, 65.2% of the blood samples drawn by injector and 62.9% of the blood drawn by vacutainer were taken from males (not shown in the data table).

Table 1. Characteristics of Participants by Venipuncture Technique

Characteristics	Venipuncture Technique	N	Mean (SD*)
Age	Injector	66	66.26 (12.58)
	Vacutainer	62	63.05 (13.98)
Height (cm)	Injector	66	164.36 (9.35)
	Vacutainer	62	166.32(10.83)
Weight (kg)	Injector	66	72.49 (10.86)
	Vacutainer	62	76.36 (13.22)
BMI	Injector	66	27.03 (4.88)
	Vacutainer	62	27.68 (4.63)

*: Standard Deviation

A very weak and negative correlation was found between the serum hemolysis and the participant's age (Table 2).

Table 2. Serum Hemolysis Level Correlation Coefficients, by Age and BMI

	Age	BMI
Correlation coefficient	- 0.195	0.022
p value	0.027*	0.809
N	128	128

*: $p<0.05$

The correlation between the serum hemolysis level and age was negative, and the correlation with BMI was positive in blood drawn with an injector. In blood drawn with a vacutainer, however, a negative correlation was found between the serum hemolysis level and both age and BMI; these correlations however were not found to be significant (p>0.05) (Table 3).

Table 3. Serum Hemolysis Level Correlation Coefficients, by Age and BMI according to the Venipuncture Technique

Venipuncture Technique		Age	BMI
Injector	Correlation coefficient	- 0.234	0.071
	p value	0.058	0.571
	N	66	66
Vacutainer	Correlation coefficient	- 0.175	- .042
	p value	0.173	0 .747
	N	62	62

Serum hemolysis values by gender are shown in Table 4. The results of the analysis did not indicate a significant difference between serum hemolysis levels by gender (Table 4).

Table 4. Serum Hemolysis Levels by Gender

Gender	N	Serum Hemolysis Level (g/dl)			
		Mean (SD)	Median	Range	p*
Male	82	0.090 (0.13)	0.054	0.019 - 0.91	0.937
Female	46	0.097 (0.10)	0.053	0.010 - .050	

* Mann Whitney U test

While the serum hemolysis level was on average 0.11 g/dl in blood drawn with an injector, the level in blood drawn with a vacutainer was on average 0.06 g/dl. No significant difference was seen between serum hemolysis levels according to the venipuncture technique used (Table 5).

Table 5. Serum Hemolysis Levels According to Venipuncture Technique

Venipuncture Technique	N	Serum Hemolysis Level (g/dl)			
		Mean (SD)	Median	Range	p*
Injector	66	0.11 (0.16)	0.053	0.02 - 0.91	0.270
Vacutainer	62	0 .06 (0.05)	0.056	0.01 - 0.39	

* Mann Whitney U test

4. Discussion

Hemolysis percentages in blood specimens taken by injector and vacutainer were evaluated in this study. It was found that hemolysis percentage means were 0.11 g/dl in blood drawn with an injector and 0.06 g/dl in blood drawn with a vacutainer. Although the statistical difference between hemolysis percentages was not significant according to either venipuncture technique, it is of clinical significance to note that the mean hemolysis percentage in the blood drawn with an injector was almost twice that of the blood drawn with a vacutainer. Hemolysis is defined in the literature as the state in which the cell-free hemoglobin concentration after centrifuging exceeds 0.30 g/L (0.03 g/dl) (20). In this study, compared with the hemolysis threshold value accepted as 0.03 g/dl, the hemolysis percentage found in the blood specimens taken with an injector displayed approximately 3.5 times this value while those taken with a vacutainer revealed twice the value. Expressed differently, hemolysis was seen in both techniques. The hemolysis noted in the blood taken with both techniques may have been caused by in vivo factors such as the patient's current medical condition or the medicines the patient was taking at the time. Another study has accepted a free hemoglobin threshold value of 0.5 g/L, reporting that a hemolysis value of over 1 g/L is an indication of severe hemolysis (21). When this is taken into consideration, it can be said that the hemolysis rate found in blood specimens drawn with an injector is severe hemolysis. In many clinics, the procedure of transferring blood drawn with an injector into a tube is rushed and for this reason, the process is usually carried out without removing the needle from the injector. Especially when black- and green-tipped needles with small diameters are used, blood cells are forced through the needle with the pressure applied and hemolysis becomes inevitable. Biochemists and laboratory technicians working in clinical laboratories cancel blood samples when hemolysis is observed after centrifuging and ask for a new specimen due to this hemolysis. The process of taking a new blood sample is an unwanted situation due to matters of cost, time consumption, and causing discomfort to the patient.

It is reported in one study that hemolysis rates in blood drawn with an intravenous catheter from all areas, including antecubital fossa, hands, forearms, and wrists, are significantly higher than in venipuncture performed with a butterfly needle (22). It was stated in a similar study where the hemolysis rate in blood drawn with a butterfly needle was compared to blood drawn with an intravenous catheter that the hemolysis rate was lower by more than half with the butterfly needle (23). In another study, it was shown that if IV catheters were used in venipuncture, the hemolysis rate was directly related to the vacuum inside the tube and that the highest hemolysis rates were seen in full-draw evacuated tubes (24). This finding demonstrates that the blood tubes used are at least as important as the venipuncture technique as far as in vitro hemolysis is concerned.

It was found in one study that hemolysis rates of blood drawn by nurses were lower than in blood drawn by doctors (21). A study conducted in Croatia to determine the opinions of nurses regarding hemolysis indicated that nurses knew the term "hemolysis" but had insufficient knowledge about the factors causing it. It was emphasized in the article that nurses were eager to increase their knowledge on this subject (10). Being aware of the parameters affected by venipuncture and the difference between in vivo and in vitro hemolysis will increase patient safety (4). It has been reported that a traumatized venipuncture site, blood drawn through an intravenous catheter or from the capillaries, the needle gauge, the motion of the needle, antiseptic procedures, and the tube into which the blood is drawn are all factors affecting hemolysis (20). It is therefore necessary for patient safety that the venipuncture is carried out by experienced professionals trained in the intricacies of this procedure.

4.1. Study Limitations

It is a limitation that in this research, the hemolysis rate cannot be measured by taking blood from the same patient with both an injector and a vacutainer holder. Another limitation is that the test values of K, LDH, and AST, which are the parameters most frequently affected by hemolysis, cannot be compared from blood samples taken from both types of the same patient.

5. Conclusion

In this study, patient prepping, blood tubes, and other basic sources of preanalytical and analytical variation were standardized. The study revealed no statistically significant difference between hemolysis rates in blood drawn with an injector or with a vacutainer, but on the other hand, the existing difference was clinically significant. In terms of both patient safety and the improvement of hospital quality procedures, it is our belief that the technique of drawing blood with an injector should be replaced by the use of a vacutainer. In order to apply these results to practice, there is a need for more research in the nursing field and for studies based on larger samples.

Contribution to the Field

Blood analysis results are of vital importance in the treatment of most patients and in terms of determining the direction of the treatment. Although there is not yet reliable evidence or definitive indicators regarding the effect on routine biochemical test results of injectors and vacutainers in the procedure of drawing venous blood, it is recognized that a state of extreme vacuum should be avoided and that the blood should be transferred into the tube carefully. The results of the present study provide the literature with evidence-based data and confirm the hypotheses.

The procedure of venipuncture is carried out in Turkey by clinical/intensive care/emergency room nurses and other nurses and drawing blood with an injector is a technique that is still widely used. Raising the awareness of nurses about the factors affecting hemolysis depending on the technique of venipuncture will constitute a significant initiative that will lead to the resolving of this issue.

Research Ethics

Prior to the conduction of the study, ethical approval was obtained from the Clinical Studies Ethics Committee of a university hospital (09.08.2018/ethical approval no:101), institutional permission was granted by the hospital and the clinical supervising physician, and the written consent of the patients agreeing to participate in the study was received.

Conflict of Interest

This article did not receive any financial fund. There is no conflict of interest regarding any person and/or institution.

Authorship Contribution

Concept: BC; **Design:** BC, SA, AA, DUY, EA; **Supervision:** BC, SA, AA; **Funding:** SA, BC, AA, DUY; **Materials:** None; **Data Collection/ Processing:** BC, AA, DUY; **Analysis/ Interpretation:** BC, SA; **Literature Review:** BC, SA; **Manuscript Writing:** BC, SA, AA; **Critical Review:** SA, BC, AA, DUY, EA.

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