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Tourniquet Application Time During Phlebotomy and The Influence on Clinical Chemistry Testing; Is It Negligible?

[Turnike Uygulama Süresi ve Klinik Kimya Testlerine Etkisi: İhmal Edilebilir Mi?]

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ABSTRACT

Objectives: This study was aimed to investigate the influence of tourniquet application performed by two different groups involving expert and non-expert phlebotomists on the biochemical laboratory testing.

Materials and Methods: Ten experienced and ten inexperienced phlebotomist responsible of venipuncture were included in the study. The effects of the tourniquet application time recorded for each group were evaluated for laboratory testing.

Results: Tourniquet application time was found as 18.9 ± 9.8 sec for experienced and 37.4 ± 11.2 sec for inexperienced staff ($p<0.001$). Biochemistry and immunological tests including glucose, urea, creatinine, uric acid, cholesterol, triglyceride, total protein, albumin, bilirubins, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, amylase, creatine kinase, lactate dehydrogenase, alkaline phosphatase, Na, K, thyroid stimulating hormone, free thyroxin, estradiol, HCG performed with the blood obtained after 30 sec and 60 sec of tourniquet application time. None of these parameters showed any statistically significant differences and had lower analytical precision levels when compared to the values of Proficiency Testing Criteria for Acceptable Performance.

Conclusion: According to the results, the acceptable period for tourniquet application might be recommended as 30-60 sec. Biochemical tests remained within the analytical precision limits when analyzed on blood drawn after venous stasis in determined periods. In conclusion, present study confirmed that tourniquet placing might affect biochemical and immunological tests minimally that could be neglected.

Key Words: venipuncture, tourniquet time, laboratory test, analytical precision

ÖZET

Amaç: Bu çalışmada öncelikle uzman ve uzman olmayan flebotomistler için turnike uygulama süresinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu amaç için on uzman ve on deneyimsiz flebotomist çalışmaya alındı. Elde edilen turnike uygulama sürelerine göre de laboratuvar testlerine etkileri araştırıldı.

Sonuçlar: Turnike uygulama zamanı uzman personel flebotomist için 18,9 ± 9.8 sn, deneyimsiz personel grubu için ise 37,4 ± 11.2 sn olarak tespit edildi ($p<0.001$). 30 ve 60 sn turnike uygulama süresiyle alınan kanlarda biyokimyasal ve immünolojik testlerden glukoz, üre, kreatinin, urik asit, kolesterol, trigliserit, total protein, albumin, bilirubinler, alanin aminotransferaz, aspartat aminotransferaz, γ-glutamil transpeptidaz, amilaz, kreatin kinaz, laktat dehydrogenaz, alkalen fosfataz, Na, K, tiroid uyarıcı hormon, serbest tiroksin, estradiol, HCG ölçümleri yapıldı. Bu testlerin hiçbirinde istatistiki değişim saptanmamıştır ve Kabul Edilebilirlik Performanslar İçin Yeterlilik Test Kriterlerine göre analitik tekrarlanabilirlik değerlerinin düşük olduğu tespit edilmiştir.

Sonuçlar: Bu sonuçlara göre kabul edilebilir turnike uygulama süresi 30-60 sn arasındadır. Bu uygulama süresinde biyokimyasal test sonuçları analitik tekrarlanabilirlik sınırları içerisindedir. Sonuç olarak, bu çalışmada, turnike uygulamalarının biyokimyasal sonuçları anlamlı düzeyde etkilemediği ve bu etkilenmenin ihmal edilebilir seviyede olduğu değerlendirilmiştir.

Anahtar Kelimeler: Kan alımı, turnike zamanı, laboratuvar testleri, analitik tekrarlanabilirlik

Introduction

In the field of clinical biochemistry, laboratory errors might originate from preanalytical, analytical and post analytical sources. Preanalytical errors during collection of samples, specimen handling, storage and delivery of the specimens, and analytical errors have been the main targets to be minimized into the acceptable limit of numbers (1,2,3).

The type and time of tourniquet application before the blood is drawn has been one common source of preanalytical errors. As reported by many authors, tourniquet placement during venipuncture might provide alterations in the results of several traditional biochemical analytes due to the prolonged venous stasis (1,2,3). However, time periods for tourniquet placement were given as 1, 3 or 6 minutes. The aim of this study was to assess the effect of different tourniquet application times on several routine biochemical testing according to the experience status of the staff performing phlebotomy (1,2).

Methods

Blood samples were drawn after written informed consent was obtained from apparently healthy volunteers (60 women, 60 men; mean age 45.4 ± 12.45 years) by phlebotomists using a butterfly needle connected to an evacuated tube holder. Serum was obtained after clotting for 30 min at room temperature, followed by centrifugation at 2000 x *g* for 5 min. All specimens were processed within 2 h of collection.

Venipunctures were done on different veins of antecubital site to exclude any interference originating from the previous tourniquet. The first phlebotomy was carried out without any tourniquet application, whereas the second and third venipunctures were performed on 30 sec-stasis and 60 sec-stasis by 6 experienced phlebotomist and other 6 non-expert blood-drawing staff. Here, experienced staff was nominated as those who were assigned for venipuncture for three years, and non-experts were medical and nursing students on phlebotomy training. The blood samples were drawn with 21-gauge straight needle. All blood collection procedure was completed within 10 minutes from the first draw. Blood samples were centrifuged at 3000 x g for 10 minutes for serum separation, stored in aliquots and kept at -70 ºC until assay.

Routine biochemical tests including the measurement of glucose, urea, creatinine, uric acid, cholesterol, triglyceride, total protein, albumin, conjugated / unconjugated bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), amylase, creatine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), sodium (Na), potassium (K) were performed on AU 2400 autoanalyzer (Olympus, Japan) by using the commercially available kits. The levels of thyroid stimulating hormone (TSH), thyroxine, estradiol and HCG were measured on Roche E-170 modular immunoanalyzer (Roche Diagnostica, Germany) by using electrochemiluminescence technique. The instrument was calibrated against appropriate reference standard material and controlled daily by the use of control sera. All measurements were performed in triplicate.

All results were compared with average CV and Health Care Financing Administration/Clinical Improvement Amendments (HCFA/CLIA) Proficiency Testing Criteria for Acceptable Performance. Avarage coefficients of variation [(intraassay CV % + interassay CV %) /2)] were calculated by the measurements of control sera with two different concentrations for seven times in a day and in different days (4).

Statistical analyses were performed by using SPSS 15.0 for Windows. Significance of differences between samples was evaluated by Wilcoxon and Mann Whitney U test and $p<0.05$ was accepted as the level of statistical significant changes.

Results

When the time of tourniquet use by non-experts and experts were compared, the duration of venous-statis during the phlebotomy by experienced staff was found statistically shorter ($p<0.001$). Experts' time of use was 18.9 ± 9.8 sec, whereas non-experts' time was 37.4 ± 11.2 sec (Figure 1). Tourniquet time lasted more than one minute in 3 samples (5 %), however, this time was recorded below 30 sec when performed 52 times by experienced phlebotomist (87 %) and 23 by non-experts (38 %). These results showed that time of tourniquet application by phlebotomists were less than 30 sec in routine use.

Table 1 and Figure 2 illustrate the changes in routine biochemical test results affected by tourniquet application for 30 sec, 60 sec and average CV %. In this regard, no statistical difference was found between the results obtained in the specimens drawn following a tourniquet application for 30 sec and 60 sec. Additionally, all tourniquet applications gave no different results in parameters when compared those obtained at tourniquet applied for the time of intraassay CV %. The results found were lower than the values of intraindividual CV % (Table 1 and Figure 1). Thus, these results were lower than that found as analytical error and those found in levels in HCFA/CLIA Proficiency Testing Criteria for Acceptable Performance Criteria.

Discussion

The development in laboratory automation technology and quality control performance of the testing process has improved the errors in analytical phase and caused the laboratory staff to be more interested and alerted on preanalytical errors within laboratory testing process (1, 2). So, overall prevalence of analytical error has been reduced as the improvement in laboratory automation has been accelerated.

Table 1. Comparison of mean values of alterations on test results run after 30 sec and 60 sec tourniquet application with the values of intraindividual CV %, interassay CV % and HCFA/CLIA.

	Alteration (%)		interassay CV %	Intraindividual CV %	HCFA/ CLIA
	30 sec	60 sec			
Glucose	-2.3	-2.2	3.9	10	8.3
Urea	1.1	0.1	2.1	9	18
Creatinine	-1.8	-1.2	2.5	15	6.8
Uric acid	-2.8	-2.9	3.1	17	9
T. Cholesterol	0.1	-2.3	2.5	10	8.2
Triglyceride	-2.1	0.1	2.6	25	28.8
Bilirubin (conj.)	-4.8	-5.2	4.2	20	24.6
Bilirubin (unconj.)	2.2	4.1	4.6	20	
AST	3.1	3.3	3.4	20	15.1
ALT	2.1	-1.0	3.3	20	23.7
ALP	-0.9	-1.2	3.4	30	4.4
Total Protein	-0.7	-2.1	2.6	10	3.5
Albumin	-0.8	-0.9	2.5	10	2.8
LDH	-1.2	-4.3	3.7	20	7.9
CK	1.0	-1.2	3.6	30	22.8
Sodium	0.1	-1.1	1.9	5	1.6
Potassium	3.1	1.9	2.3	5	5.4
Calcium	-0.8	-2.1	2.1	10	3.3
TSH	2.1	3.1	3.4	20	24
Thyroxine	1.3	2.4	2.8	20	25
Estradiol	-1.4	-1.9	2.4	20	
HCG	-2.5	3	3.6	20	

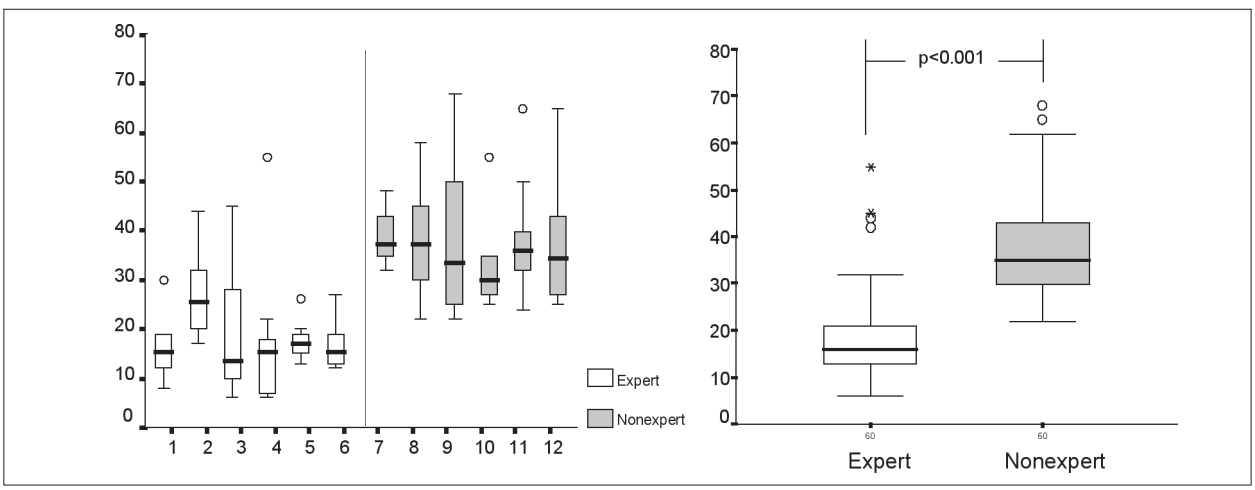

Figure 1. Tourniquet time for experts and nonexperts staff

As the duration of tourniquet placement extends, the effect of increased intravenous pressure and hypoxia on vascular endothelium triggers the infiltration of small molecules and fluid from lumen to the peripheral tissues. Proteins (involving lipoproteins), erythrocytes and other blood cells cannot pass through the membrane at the same rate. Therefore, their concentrations in plasma will increase. However, the relative levels of proteins like immunoglobulins will also increase in sera. In addition, proteins and protein-bound substances in plasma are also largely influenced by venous stasis leading to an increase in protein-bound calcium levels. Thus, for calcium analysis, blood should be drawn without tourniquet placement if any increase was noted previously. In a similar manner, protein-bound hormone measurements are also under effect of stasis time. Hypoxia due to the elongated stasis may also cause intracellular elements to infiltrate into the plasma (1,5).

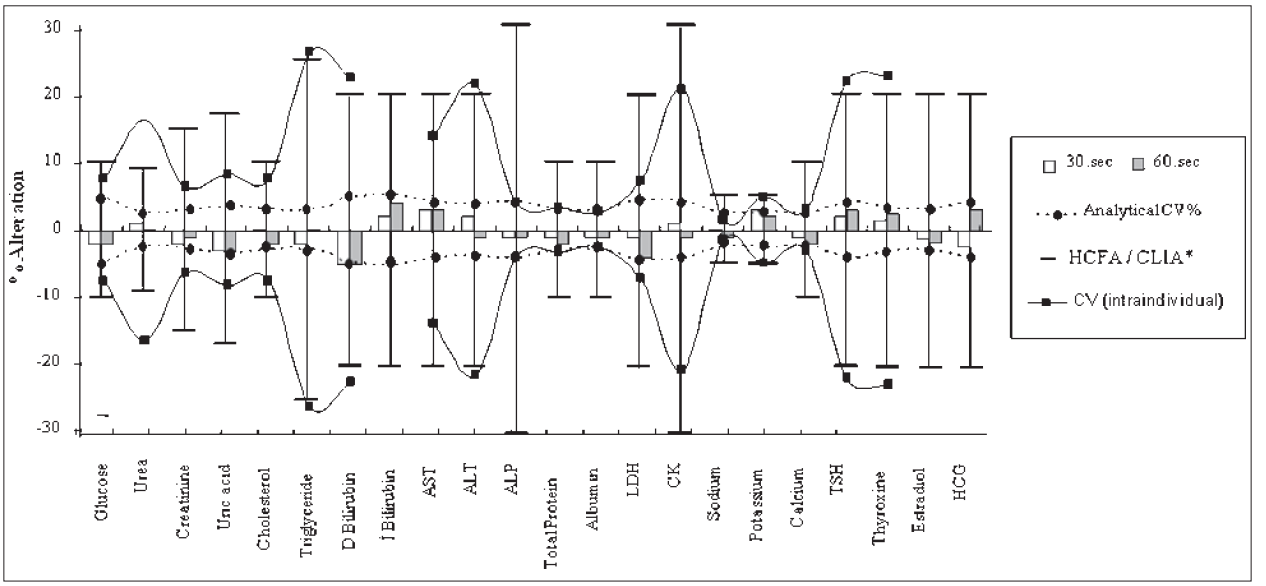

Figure 2. This figure illustrates the changes in values of biochemical parameters and total coefficients of variation (% CV) and individual variation in all biochemical testing parameters depending on various application time (30 and 60 sec) indicated below.

A suitable blood-drawing technique requires a fulfillment of rigorous and standardized criteria. The time for tourniquet application differs from patient to patient. For instance, venous stasis time may be longer for obese patients whose veins are not easily visible for blood drawing. In this study, the applications time was less than one minute even for non-expert staff. Even in cases with 60 sec-tourniquet application, changes were found lower than intraassay variation values for some biochemical tests reported in previous studies. The reason might be the longer duration of tourniquet placement (for 3 min or 6 min).

It was reported that levels of some biochemical tests including total protein, iron, cholesterol, AST and bilirubin were found to be increased due to the venous stasis, especially potassium levels were changed in the range of 4.9-9.3 % (5).

In another study, Lippi et al. assessed the changes at levels of 12 testing parameters after a tourniquet placement for 1-3 min and found significant changes in ALT, albumin, potassium, iron, glucose, creatinine kinase where a sphygmomanometer was used for venous stasis (6). Our study aimed to find out the effect of tourniquet time on the change of biochemical tests and to evaluate this effect on the analytical precision of test parameters under conditions of routine venipuncture application showed no variation in the test results . The changes in biochemical tests were found lower than the values for intraindividual % CV as noted in Table 1 and Figure 1, and the effects on CLIA values were also minimal. Since intraindividual values were lower and had no significant differences when compared with intraassay CV % values, it could be pointed out that tourniquet application performed up to 60 sec had no significant on results. The main aspect of present study differing from the other related studies was the determination of real tourniquet application time and focusing on the changes for the 30-60 sec application time.

However, we used tourniquet placement to provide a real-life experience which was almost different than previous studies, and that was the reason of the lower incidence of venous stasis effect. Consequently, time for tourniquet application was commonly found below than 30 sec. In addition to that, this performance time may also be stated as less than one minute even for inexperienced phlebotomist and patients whose blood-drawing is almost tedious, and this duration may not influence the laboratory test results.

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