

Effect of prolonged venostasis on lipid profile parameters among apparently healthy University students: A case-control study in Ghana

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ABSTRACT

Background: The effect of prolonged tourniquet application on lipid profile parameters (total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c)) among healthy students in the University of Cape Coast was assessed in this study.

Methods: A total of 30 participants were sampled and venous blood was taken from both arms at different duration of tourniquet application. Blood was allowed to clot, centrifuged and serum separated and stored until assay. Lipid profile parameters were measured using the enzymatic techniques with the aid of a spectrophotometer. Lipid parameters were expressed in mmol/L. The relationship between the concentrations was analyzed using GraphPad Prism version 6.0.

Results: The mean serum levels of total cholesterol and HDL-c were significantly ($p < 0.05$) elevated after long duration (13.44 ± 2.40 s) of tourniquet application compared to short duration (11.20 ± 0.38 s) of tourniquet application. Despite the fact that the serum levels of triglyceride and LDL-c increased after long duration of tourniquet application compared to short duration, the difference was not significant ($p > 0.05$). There was significant % mean differences of 7.0% ($p < 0.0001$) and 11.2% ($p = 0.0082$) in the concentrations of total cholesterol and HDL-c respectively following prolonged tourniquet application of 120-180 s. A significant increase of up to 7% (5.01 ± 0.04 , 5.352 ± 0.05 , $p < 0.0001$) and 11% (2.41 ± 0.07 , 2.674 ± 0.07 , $p = 0.0082$) in the concentrations of total cholesterol and HDL-c respectively was also recorded.

Conclusion: Prolonged venostasis has a direct effect on the concentration of serum total cholesterol and HDL-c but not triglyceride and LDL-c. Non-application of tourniquet in patients with prominent veins, the application of standardized external pressure as well the early release of tourniquet after needle insertion in the vein should be followed during sample taking for lipid profile.

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Keywords: Venostasis, lipid profile, tourniquet, prolonged, application

INTRODUCTION

In most clinical and laboratory set-up, phlebotomists apply tourniquet during venipuncture. This is mostly seen when taking blood samples of infants, the obese, immunosuppressed or the geriatrics whose veins are difficult to locate. Although blood collection is supposed to be as fast as possible, sev-

eral concurrent causes might contribute to lengthening the time up to 180 seconds after tourniquet fastening, such as location of appropriate venous access, selection of the most suited blood collection system, needle insertion into the vein and collection of many samples.

A greater concern has been raised on the type and duration of the tourniquet application. This has been considered as a source of pre-analytical error (Lippi *et al.*, 2005). Studies have indicated that, prolonged venostasis might cause alterations in the results of several biochemistry analytes (McMullan *et al.*, 1990; Young *et al.*, 2006). Lippi *et al.*, (2005) commented on the significant alterations in alanine transaminase (ALT), total calcium, sodium, urea, total glycerol, albumin, bilirubin, potassium, glucose, iron and creatine kinase after applying a sphygmomanometer at the same pressure. This alteration can then be traced to the prolonged venostasis and neither pressure on the vascular endothelium nor hypoxia as suggested by Young *et al.*, (2006).

It has also been reported that most of these alterations occur to protein bound substances in plasma including calcium (Sedar *et al.*, 2008).

Medical and laboratory personnel worldwide apply tourniquet during venipuncture of patients especially the obese, pediatrics, aged and the immunosuppressed. However, a clearly defined effect of the tourniquet application is not revealed. Lippi *et al.*, (2005) opined that, the effect caused by venostasis on protein bound substances is appreciably significant upon 60 – 180 seconds of tourniquet application. On the other hand, Serdar *et al.*, (2008) intimated that this effect is not statistically significant upon less than 60 seconds of tourniquet application.

Much attention has been given to the effect of prolonged tourniquet application time on intracellular ions (K, Ca, Na), albumin, ALT, glucose and urea (Stankovic and Smith, 2004). However, little is known and documented on the effect of tourniquet application time on lipoproteins and lipids. This study aimed at determining the effect of prolonged tourniquet application on lipid profile parameters.

MATERIALS AND METHODS

Study Design/ Recruitment of participants

Simple random sampling was used to recruit 30 apparently healthy students (10 females and 20

males) from the Department of Laboratory Technology, University of Cape Coast who gave written informed consent before sampling. Blood was drawn from 2 different veins of the upper arms from alternating sides of each participant in the morning following overnight fasting. The first and second venipunctures were performed following tourniquet application for less than 30 sec and 120- 180 sec respectively.

Ethical issues

Protocols used for the study were approved by the University of Cape Coast Institutional Review Board. Also, permission was sought from the Department of Laboratory Technology, University of Cape Coast prior to the study. The data collected was treated with enough confidentiality during analysis and even after analysis. Thus, the details of the participants were held confidential.

Blood sample collection and analysis

Blood was collected by only one phlebotomist by venipuncture with 20-gauge needles (Anhui Thankang Medical Product, Anhui, China) and dispensed into serum separating vacuum tubes containing gel and clot activator (Anhui Thankang Medical Product, Anhui, China). After centrifugation (NF 200, Ankara, China) at 3000 rpm for 10 min at room temperature, serum was separated in aliquots and kept frozen until assay.

The concentrations of total cholesterol, triglycerides and HDL-cholesterol were estimated manually by enzymatic procedure by measuring the absorbance using a spectrophotometer (UV mini-1240, Shimadzu) at wavelength of 510 nm. The concentration of LDL-cholesterol was then calculated using the Friedewald equation, under the condition that, triglyceride is not greater than 4.5 mmol/L (Dittmann, 2001). All measurements were duplicated within a single analytical session. The results were finally reported as the mean of paired measurement.

Statistical Analysis

GraphPad Prism vision 6.0 was used to analyze the data. A graph plots and limits of agreement were

used to compare the results of the independent measurement and plot differences were finally reported as percentages. The graphical presentations suit to reveal the potential increase in variability of the differences. The differences between continuous variables was assessed by a paired student t-test. P-value less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows Lipid profile categorized according to the duration of tourniquet application. The mean serum levels of total cholesterol and HDL-c were significantly elevated after long duration (13.44±2.40 s) of tourniquet application compared to short duration (11.20±0.38 s) of tourniquet application (p<0.05).

The percent (%) mean difference for total cholesterol was determined to be 7.0%. In the values of HDL-c, the % mean difference was found to be 11.2%. These two parameters measured in lipid profile showed significant statistical % mean difference (Table 1).

Triglyceride and LDL-c, though they showed percent (%) mean differences of 7.4% and 1.5% respectively, the difference in serum concentration following the respective tourniquet application time were not statistically significant (p<0.05) (Table 1).

As shown in Figure 1, the mean level of total cholesterol was significantly elevated when tourniquet was applied for a longer duration compared to the short duration (p<0.0001).

As shown in Figure 2, the mean level of serum triglyceride was elevated when tourniquet was applied for a longer duration compared to the short duration. However, there was no statistically significant difference (p=0.3091) in serum triglyceride levels following the different tourniquet application times.

As shown in Figure 3, the mean level of HDL-c was significantly elevated when tourniquet was applied for a longer duration (13.44±2.40 s) compared to the short duration (p=0.0082).

Table 1: Lipid profile categorized according to the duration for tourniquet application

Lipid profile	Average duration		P-value	Mean difference (95% CI)	% Mean difference
	11.20 ± 0.38 s (N=30)	13.44 ± 2.40 s (N=30)			
Total cholesterol (mmol/l)	5.01 ± 0.04	5.35 ± 0.05	< 0.0001	0.35 ± 0.06 (-0.46 to 0.22)	7.0%
Triglyceride (mmol/l)	1.36 ± 0.07	1.46 ± 0.07	0.3091	0.10 ± 0.10 (-0.30 to 0.09)	7.4%
HDL-C (mmol/l)	2.41 ± 0.07	2.67 ± 0.07	0.0082	0.27 ± 0.09 (-0.46 to -0.07)	11.2%
LDL-C (mmol/l)	1.98 ± 0.06	2.01 ± 0.06	0.6876	0.03 ± 0.08 (-0.19 to 0.12)	1.5%

Data presented as mean ± standard deviation and percentages

As shown in Figure 4, the mean level of LDL-cholesterol was elevated when tourniquet was applied for a longer duration (13.44 ± 2.40 s) compared to the short duration (11.20 ± 0.38 s). However, there was no statistically significant difference ($p=0.6876$).

DISCUSSION

This research primarily analyzed the effects of prolonged tourniquet application on lipid profile. Our findings showed that prolonged tourniquet applica-

tion has a direct significant effect on total cholesterol, and HDL-Cholesterol but not triglyceride and LDL cholesterol. In a similar study by Sedar *et al*, (2008) on the effect of tourniquet application, the duration of tourniquet application was compared between expert and non-expert phlebotomists.

They recorded 18.9 ± 9.8 sec and 37.4 ± 11.2 sec respectively and therefore reported no significant difference in total cholesterol, triglyceride and other biochemical parameters including alanine trans-

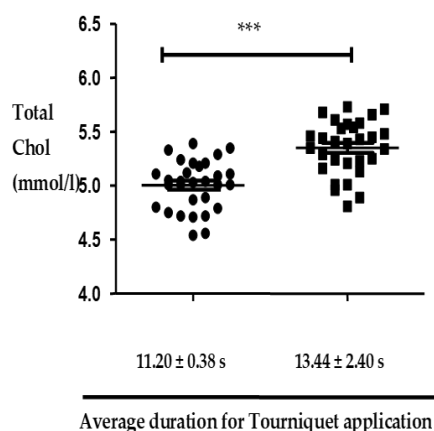


Figure 1: Levels of total cholesterol categorized according to the duration of tourniquet application

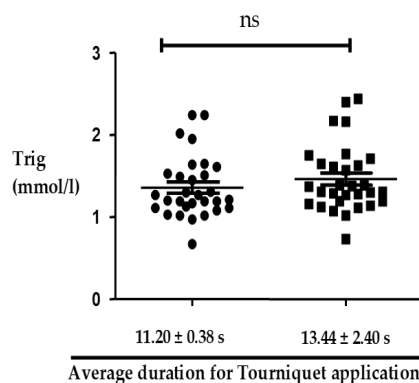


Figure 2: Levels of triglyceride categorized according to the duration of tourniquet application

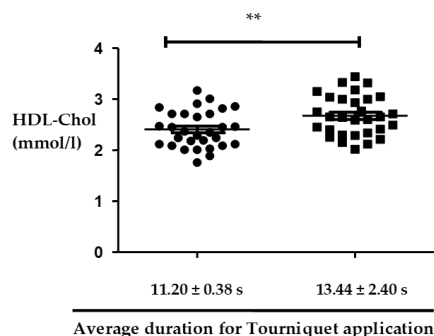


Figure 3: Levels of HDL-cholesterol categorized according to the duration of tourniquet application

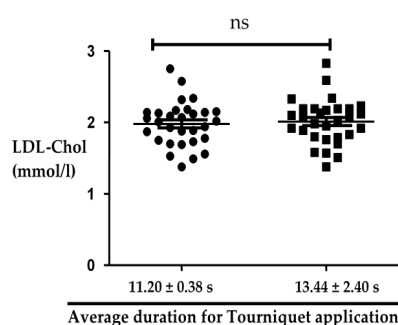


Figure 4: Levels of HDL-cholesterol categorized according to the duration of tourniquet application

aminase, creatine kinase, iron, aspartate transaminase, amylase, gamma glutamyl transferase, uric acid, creatinine, lactate dehydrogenase and urea following the duration of tourniquet application used in their research (less than 60 sec). They were more particular of the determination of real tourniquet application time and focused on the changes for the 30-60 sec application time. Aside the duration of the tourniquet application, differences in the methods such as the use of AU 2400 autoanalyzer (Olympus, Japan) which employs automation and hence different margin of error for the measurement of parameters used could contribute to such slight variation between the two studies.

However, the finding of this work is consistent with earlier studies (Cooper *et al.*, 1992; Lippi *et al.*, 2005). In this work, a total of 30 healthy volunteers were involved where a statistical difference of up to 7% (5.01 ± 0.04 , 5.352 ± 0.05) and 11.2% (2.41 ± 0.07 , 2.674 ± 0.07) were found in the values of total cholesterol and HDL-c. Also, this significant difference is evident in the % mean difference of 7.0% and 11.2% in the concentrations of total cholesterol and HDL-c respectively.

This partly agrees with the findings of Lippi *et al.*, (2005) in which 23 healthy volunteers were involved in the research and sphygmomanometer cuff application for 60 – 180 seconds recorded a significant difference up to 10% in the total cholesterol concentration and the other biochemical parameters. In the work of Cooper *et al.*, (1992), significant difference in the concentrations of total cholesterol, calcium and alkaline phosphatase, ranged from 5-10% following up to 180 seconds of tourniquet application. In a similar work by Statland *et al.*, (1974) it was stated that the level of total cholesterol and other biochemical parameters were found to be increased due to the venostasis in the range of 4.9-9.3%. Although, there is an agreement between these studies, there are minor differences in the ranges of percentage increment.

Notable among the contributing factors is the use of sphygmomanometer at standardized pressure of 60mmHg in the work of Lippi *et al.*, (2005) while in

this current work, no sphygmomanometer was used and that, there is tendency of variable pressure application. Also, the inter-individual variability in procedures used to reproduce venous stasis during venipuncture is also a major limiting factor that might have influenced the slight difference in the results.

Again, the methods used in the estimation of the parameters could be a contributing factor. In this study, all the absorbances were manually measured with a spectrophotometer (UV mini-1240, Shimadzu) in contrast to the auto analyzer [Roche/Hitachi Modular System P (Roche Diagnostics GmbH, Mannheim, Germany)] employed by Lippi *et al.*, (2005). Also, Lippi and colleagues used 20-gauge (G) straight needles (Terumo Europe NV, Leuven, Belgium) to draw blood directly into vacuum tubes containing Gel q45 USP U Lithium Heparin (Terumo Europe, Haasrode, Belgium) hence the sample used was plasma. In this current study, 20-gauge needles (Anhui Thankang Medical Product, Anhui, China) was used to draw blood into serum separating vacuum tube containing gel and clot activator (Anhui Thankang Medical Product, Anhui, China) hence serum was used in this current work. Difference in percentages could therefore be attributed to different samples used.

Conversely, the concentrations of triglyceride and LDL-c determined in this study found no significant difference regardless of the duration of tourniquet application (120-180 s). Their % mean differences were however found to be 7.4% and 1.5% respectively. This partly agrees with the work of Sedar *et al.*, (2008) though tourniquet application was shorter and prolonged in their work and ours respectively. Also, the insignificant variation in the concentrations of triglyceride and LDL-cholesterol is consistent with earlier findings where blood sampling technique similar to what was employed in our study produced unaltered values for routine blood gases, hematological testing but not lipid parameters (Cengiz *et al.*, 2009).

Taken together results of the present investigation confirm that variations in the serum concentration

of several biochemical analytes including total cholesterol and HDL-Cholesterol due to inappropriate standardization of the pre-analytical phase, such as prolonged tourniquet placement, may be clinically meaningful. As these observations are dependent on the length of stasis applied during venipuncture and the biochemical or metabolic characteristics of the parameter, these variations could likely be anticipated. Thus, the most appropriate preventive measure for minimizing the influence of tourniquet placement can be adopted, including non-application in patients with large or prominent veins, palpating for prominent veins for venipuncture before tourniquet application, standardization of the external pressure and early release after needle insertion in the vein.

Following current practices, the tourniquet should be applied at approximately 7.5 cm above the anticipated puncture site, should be tight enough to stop venous blood flow, but should not hamper arterial blood flow, i.e., approximately 20-30 mmHg below the systolic blood pressure. It should be removed as soon as blood flow is established in the collection system, and under no circumstances should it be left in place for more than 60 seconds. When more time is required, the tourniquet must be released, so that blood flow can resume and normal skin colour returns to the extremities (Dittmann, 2001). This arises from consolidated evidence that venous stasis, as induced by prolonged tourniquet application might exert considerable influence on the concentration of total cholesterol, HDL-cholesterol and other analytes in serum.

CONCLUSION

Prolonged venostasis has a direct effect on the concentration of serum total cholesterol and HDL-c but not triglyceride and LDL-c. This is estimated up to 7% and 11% in total cholesterol and HDL-c respectively.

COMPETING INTEREST

Authors declare that they have no competing interests

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AUTHOR CONTRIBUTIONS

RKDE, SAA, AAA: Design of study, interpretation of results and original manuscript. EOA: Data analysis. RKDE: Revision of manuscript for important intellectual content. All of the above authors read and approved the final manuscript.

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