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Original Research Paper

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TOURNIQUET APPLICATION TIME DURING PHLEBOTOMY AFFECTS BIOCHEMICAL TESTING

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ABSTRACT

Introduction: Venous blood sampling is usually performed using a tourniquet to help locate and define peripheral veins to achieve successful and safe venepuncture. In developing countries like India, there is lack of understanding about good laboratory practices and inadequate training to phlebotomists, compelling them to make errors during phlebotomy. The improper venous accesses or prolonged venous stasis created by tourniquet application will result in collection of unsuitable blood sample. **Methods:** The present study was conducted at one of the tertiary care hospital at State Capital to find out the effect of tourniquet application time on 17 common biochemical analytes in 20 fasting healthy adult volunteers. Sequential venepunctures were performed by a single expert phlebotomist either without venous stasis or following the application of standardized external pressure of 60 mm Hg using a sphygmomanometer for 1, 3 and 6 minutes. Biochemical parameters were analyzed on Fully Automated Analyzer Olympus AU 680 by using standard protocol of commercially available kits. **Results:** ALT concentration was significantly high in samples collected after application of external pressure for 3min ($P<0.05$) and 6min ($P<0.01$); Alkaline phosphatase, LDH, Creatine kinase, Total Cholesterol, Triglyceride, Total Protein, Albumin and Calcium concentrations were significantly high in samples collected after application of external pressure for 6min ($P<0.05$) as compared to samples collected without venous stasis. There were no significant alterations in rest biochemical analytes concentrations (Glucose, Urea, Creatinine, AST, Uric Acid, Phosphorus, Sodium, and Potassium) even after 6 min venous stasis. **Conclusion:** Venous stasis from tourniquet placement during venepuncture should be minimized, as it accounts for spurious and significant variations for several biochemical analytes.

KEYWORDS : Tourniquet, Venous Stasis, Biochemical Analytes, Phlebotomy

INTRODUCTION

Previously results of analysis were dependent on analytical variation, but nowadays control and standardization of pre-analytical variability is a critical factor for achieving accuracy and precision in laboratory testing because about 32% to 75% laboratory errors are made in pre-analytical phase and majority of them are preventable (1,2). Venous blood sampling is usually performed using a tourniquet to help locate and define peripheral veins to achieve successful and safe venipuncture. During the last two decades significant improvements in blood sampling technique and equipments e.g. positive patient identification, vacuum tubes for blood sampling, improved needles for phlebotomy and operator safety are made, it is very important to give proper attention and careful supervision to prevent errors during phlebotomy procedure (3).

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. Tourniquet is widely used for venipuncture by medical and laboratory staff but very few are aware of effect of tourniquet application on laboratory parameters. Ideally, the tourniquet should be applied if necessary and quickly removed when the needle is safely in the vein. Tourniquet application should be minimized because it causes spurious and significant variation of several plasma analytes (4). For example prolonged venous stasis can cause 7% increase in prevalence of hypercholesterolemia and thus unnecessary medication can be introduced (5). Prolonged tourniquet application can lead to local hypoxia and thus acidosis, which can affect potassium measurement (6). With venous stasis, protein and protein bound constituents can increase upto 15% (7).

However, in clinical practice the tourniquet is rarely released before blood drawing is complete to avoid vein collapse. Although blood collection is supposed to be as fast as possible, several concurrent causes might contribute to lengthen the time up to 3 min 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. To the best of our knowledge, there are no definitive working guidelines for tourniquet application and only a few reliable studies investigated the influence of venous stasis on imprecision in clinical chemistry testing (8). In addition, in most cases, results of these earlier investigations were influenced by substantial inter-individual variations in the procedure used to reproduce venous stasis during venepuncture, as the pressure applied to the tourniquet could barely be standardized. A greater concern has been raised on the type and duration of the tourniquet application.

Therefore, the main purpose of this study was to assess the influence of 1-,3- min and 6-min stasis, on the measurement of 17 common analytes, including the most representative proteins, protein-bound substances, enzymes and electrolytes.

MATERIALS AND METHODS

The study was conducted on 20 normal healthy subjects of Jaipur, Rajasthan (India) population. Care was taken to ensure that all the normal healthy subjects included in this study are Symptomless for any disease, Free from any abnormality on routine examination, Non-smoker, Non-alcoholic, Not taking Medications. Physical Examination included age, sex, personal history regarding occupation, socio-economic status; habits like food, smoking, alcohol intake, tobacco chewing and drugs of all subjects were carefully recorded. Samples were collected from healthy adult volunteers ranging in age about 25-50 years and informed consent was obtained from each participant. The volunteers were instructed to fast overnight (until blood collection was completed) and to avoid vigorous exercise upon walking. The blood samples of each subject were collected by venepuncture from the anticubital vein after an overnight fast of 10-12 hours.

To verify the potential influence of tourniquet placement on laboratory testing, we measured the variation of 17 common

biochemical analytes in 20 fasting healthy adult volunteers after short term venous stasis. Sequential venepunctures were performed on four different veins of the upper arms, alternating one arm with the other, to minimize either interference from earlier stasis or contamination from endovascular material released from earlier venepunctures. The first venepuncture was carried out without venous stasis (no stasis), whereas the second, third and fourth venepunctures were performed following the application of standardized external pressure of 60 mm Hg using a sphygmomanometer for I min (1 min stasis), 3 min (3 min stasis) and 6 min (6 min stasis). Blood was always collected by a single expert phlebotomist. All phases of the sample collection were accurately standardized, including an identical rest time for the subjects (>10 min) and the use of needles & tubes for the same lot. No specimen needed to be discarded due to unsatisfactory attempts, difficulty in locating easy venous access, missing veins or manifest hemolysis or lipemia. Blood was then allowed to clot for 60 minutes at room temperature; centrifuged at 1500xg for 10 minutes; serum was separated and immediately analyzed for 17 routine biochemical parameters which includes Glucose, Urea, Creatinine, Aspartate transaminase, Alanine transaminase, Alkaline Phosphatase, Lactate dehydrogenase, Creatine Kinase, Total Cholesterol, Triglyceride, Total Protein, Albumin, Uric Acid, Calcium, Phosphorus, Sodium, Potassium on Fully Automated Analyzer Beckman AU 680 by using standard protocol of commercially available kits.

Statistical Analysis

All analyses were performed using Statistical Package for Social Sciences statistical package (SPSS,version 15.0 for Windows XP) (9). The clinical significance was calculated as Mean difference (95% CI) and Mean % difference. The significance of differences between samples was assessed by a paired Student t-test; the level of statistical significance was set at $P<0.05$.

RESULTS

In this study, the influence of short-term venous stasis (tourniquet application) on routine biochemical testing was investigated by measuring the concentration of 17 routine biochemical analytes, including proteins, protein-bound substances, enzymes and electrolytes, in serum specimens collected either without venous stasis or following the application of standardized external pressure of 60 mm Hg using a sphygmomanometer for 1, 3 and 6 minutes (Table 1,2&3).

We have observed clinically significant increase in the concentration of Alanine transaminase (ALT) after 3-min ($P<0.05$) and 6-min ($P<0.01$) venous stasis. The percent (%) mean difference for Alanine transaminase (ALT) after 3-min to be 15.1% and and 6-min to be 21.91% which showed significant statistical % mean difference (Table2&3). Percent (%) mean difference for Alkaline phosphatase (3.99%), LDH (1.65%), Creatine kinase (2.97%), Total Cholesterol (1.67%), Triglyceride (1.42%), Total Protein (2.70%), Albumin (3.63%) and Calcium (1.07%) were found non-significant ($P>0.05$) after 3-min venous stasis as compared to without venous stasis, but percent (%) mean difference for Alkaline phosphatase (6.0%), LDH (3.03%), Creatine kinase (4.64%), Total Cholesterol (2.19%), Triglyceride (2.54%), Total Protein (5.63%), Albumin (5.0%) and Calcium (1.82%) were found significant ($P<0.05$) after 6min venous stasis as compared to without venous stasis (Table 2 &3). Glucose, Urea, Creatinine, Aspartate transaminase (AST), Uric acid, Sodium, Potassium and Phosphorus concentrations were found non-significant even after 6-min venous stasis (Table 3).

Table 1: Comparison Of Biochemical Parameters Without Venous Stasis And After Application Of External Pressure For 1 Minute

Analytes	No Stasis	1-min Stasis	P-value	Mean difference (95% CI)	Mean % difference
	Mean±SD	Mean±SD			
Glucose (mg/dl)	86.54±3.7	86.23±3.5	0.7869	-0.31 (-2.61 to 1.99)	-0.35
Urea (mg/dl)	31.24±2.3	31.17±2.4	0.9255	-0.07 (-1.57 to 1.43)	-0.22
Creatinine (mg/dl)	0.75±0.15	0.75±0.16	1.0000	0.0 (-0.09 to 0.09)	0.0
Aspartate transaminase AST (U/L)	22.82±3.7	23.01±3.8	0.8736	0.19 (-2.21 to 2.59)	0.83
Alanine transaminase ALT (U/L)	20.21±4.3	20.93±4.4	0.6038	0.72 (-2.06 to 3.50)	1.05
Alkaline Phosphatase (U/L)	68.15±5.8	68.98±5.7	0.6507	0.83 (-2.85 to 4.51)	1.21
Lactate dehydrogenase LDH (U/L)	187.7±7.5	188.3±7.6	0.8209	0.60 (-4.23 to 5.43)	0.31
Creatine Kinase (U/L)	114.2±6.2	115.8±6.3	0.4233	1.60 (-2.40 to 5.60)	1.40
Total Cholesterol (mg/dl)	173.4±4.8	174.2±4.7	0.5974	0.80 (-2.24 to 3.84)	0.46
Triglyceride (mg/dl)	98.3±3.5	98.9±3.6	0.5962	0.60 (-1.67 to 2.87)	0.61
Total Protein (g/dl)	7.4±0.48	7.4±0.47	1.0000	0.0 (-0.30 to 0.30)	0.0
Albumin (g/dl)	4.40±0.32	4.49±0.33	0.3867	0.09 (-0.11 to 0.29)	2.0
Uric Acid (mg/dl)	4.62±0.48	4.63±0.47	0.9473	0.01 (-0.29 to 0.31)	0.21
Calcium (mg/dl)	9.32±0.24	9.36±0.25	0.6087	0.04 (-0.11 to 0.19)	0.42
Phosphorus (mg/dl)	3.45±0.28	3.44±0.27	0.9091	-0.01 (-0.18 to 0.16)	-0.28
Sodium (mmol/L)	141.1±1.6	141.4±1.5	0.5444	0.30 (-0.69 to 1.29)	0.21
Potassium (mmol/L)	4.21±0.24	4.20±0.25	0.8980	-0.01 (-0.16 to 0.14)	-0.23

Table 2: Comparison Of Biochemical Parameters Without Venous Stasis And After Application Of External Pressure For 3 Minutes

Analytes	No Stasis	3-min Stasis	P-value	Mean difference (95% CI)	Mean % difference
	Mean±SD	Mean±SD			
Glucose (mg/dl)	86.54±3.7	85.47±3.6	0.3598	-1.07 (-3.40 to 1.26)	-1.23
Urea (mg/dl)	31.24±2.3	31.06±2.2	0.8017	-0.18 (-1.62 to 1.26)	-0.57
Creatinine (mg/dl)	0.75±0.15	0.74±0.14	0.8286	-0.01 (-0.10 to 0.08)	-1.33
Aspartate transaminase AST (U/L)	22.82±3.7	23.74±3.6	0.4304	0.92 (-1.41 to 3.25)	4.03
Alanine transaminase ALT (U/L)	20.21±4.3	23.28±4.5	0.0335	3.07 (0.25 to 5.88)	15.1
Alkaline Phosphatase (U/L)	68.15±5.8	70.87±5.6	0.1396	2.72 (-0.92 to 6.36)	3.99
Lactate dehydrogenase LDH (U/L)	187.7±7.5	190.8±7.4	0.1961	3.10 (-1.66 to 7.86)	1.65
Creatine Kinase (U/L)	114.2±6.2	117.6±6.5	0.0987	3.40 (-0.66 to 7.46)	2.97
Total Cholesterol (mg/dl)	173.4±4.8	176.3±4.8	0.0636	2.90 (-0.17 to 5.97)	1.67
Triglyceride (mg/dl)	98.3±3.5	99.7±3.4	0.2072	1.40 (-0.80 to 3.60)	1.42
Total Protein (g/dl)	7.4±0.48	7.6±0.46	0.1865	0.20 (-0.10 to 0.50)	2.70

Albumin (g/dl)	4.40±0.32	4.56±0.32	0.1221	0.16 (-0.04 to 0.36)	3.63
Uric Acid (mg/dl)	4.62±0.48	4.67±0.48	0.7437	0.05 (-0.25 to 0.35)	1.08
Calcium (mg/dl)	9.32±0.24	9.42±0.23	0.1865	0.10 (-0.05 to 0.25)	1.07
Phosphorus (mg/dl)	3.45±0.28	3.40±0.26	0.5619	-0.05 (-0.22 to 0.12)	-1.4
Sodium (mmol/L)	141.1±1.6	141.9±1.6	0.1221	0.80 (-0.22 to 1.82)	0.56
Potassium (mmol/L)	4.21±0.24	4.17±0.26	0.6161	-0.04 (-0.20 to 0.12)	-0.95

Table 3: Comparison Of Biochemical Parameters Without Venous Stasis And After Application Of External Pressure For 6 Minutes

Analytes	No Stasis	6-min Stasis	P-value	Mean difference (95% CI)	Mean % difference
	Mean±SD	Mean±SD			
Glucose (mg/dl)	86.54±3.7	84.34±3.5	0.0609	-2.20 (-4.50 to 0.10)	-2.54
Urea (mg/dl)	31.24±2.3	30.92±2.4	0.6693	-0.32 (-1.82 to 1.18)	-1.02
Creatinine (mg/dl)	0.75±0.15	0.73±0.15	0.6757	-0.02 (-0.11 to 0.07)	-2.66
Aspartate transaminase AST (U/L)	22.82±3.7	24.10±3.7	0.2809	1.28 (-1.08 to 3.64)	5.60
Alanine transaminase ALT (U/L)	20.21±4.3	24.64±4.4	0.0026	4.43 (1.64 to 7.21)	21.91
Alkaline Phosphatase (U/L)	68.15±5.8	72.24±5.8	0.0317	4.09 (0.37 to 7.80)	6.0
Lactate dehydrogenase LDH (U/L)	187.7±7.5	193.4±7.5	0.0212	5.70 (0.89 to 10.5)	3.03
Creatine Kinase (U/L)	114.2±6.2	119.5±6.4	0.0114	5.30 (1.26 to 9.33)	4.64
Total Cholesterol (mg/dl)	173.4±4.8	177.2±4.9	0.0178	3.80 (0.69 to 6.90)	2.19
Triglyceride (mg/dl)	98.3±3.5	100.8±3.5	0.0297	2.50 (0.25 to 4.74)	2.54
Total Protein (g/dl)	7.4±0.48	7.8±0.49	0.0129	0.40 (0.08 to 0.71)	5.63
Albumin (g/dl)	4.40±0.32	4.62±0.34	0.0418	0.22 (0.008 to 0.43)	5.0
Uric Acid (mg/dl)	4.62±0.48	4.71±0.49	0.5608	0.09 (-0.22 to 0.40)	1.94
Calcium (mg/dl)	9.32±0.24	9.49±0.24	0.0310	0.17 (0.016 to 0.32)	1.82
Phosphorus (mg/dl)	3.45±0.28	3.35±0.27	0.2574	-0.10 (-0.27 to 0.07)	-2.89
Sodium (mmol/L)	141.1±1.6	142.1±1.6	0.0554	1.0 (-0.02 to 2.02)	0.70
Potassium (mmol/L)	4.21±0.24	4.15±0.24	0.4341	-0.06 (-0.21 to 0.09)	-1.42

DISCUSSION

The tourniquet application is an important controllable variable of pre analytical factor that can affect laboratory results. It is usual practice that tourniquet remains in place during blood collection so that continued venous dilation allows rapid sample collection which may result in its prolonged application. This application time is rarely regarded as a potential source of laboratory variability that may be lengthen due to difficult location of vein, selection of proper blood collection system, insertion of needle into the vein and collection of many tubes (10) .

A suitable technique for drawing blood requires that rigorous procedural criteria are fulfilled to ensure accuracy and precision of laboratory testing. Before venepuncture, a tourniquet is always used to assist the phlebotomist in locating a suitable vein. According to current practice, the tourniquet, applied at approximately one hand-width (7.5 cm) above the anticipated puncture site, should be tight enough to stop venous blood flow, but not to 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 1 min. When more time is required, the tourniquet must be released so that blood flow can resume and normal skin colour returns to the extremities. Hemoconcentration is recognized as a major factor contributing to increased concentration of large molecules, such as proteins and protein-bound substances, cells and coagulation factors (11).

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 (12).

Some earlier reports indicated that short-term tourniquet application had no significant influence on routine laboratory testing (13) and that up to 6-min tourniquet application did not substantially modify sodium, potassium, carbon dioxide, creatinine, uric acid and the ratio of electrophoretic fractions but might increase by an average of 4-9% the red cell count, hemoglobin, packed cell volume, total protein, albumin, GGT, alkaline phosphatase, lactate dehydrogenase, CK, bilirubin, cholesterol, total glycerol and calcium (14). According to McMullan et al, total calcium and albumin in serum increased modestly on application of a standard tourniquet (15).

In a similar study, Lippi et al (4) recorded substantial and clinically significant variations in albumin, calcium and potassium, even after 1-min venous stasis. Whereas the concentration of most large analytes (albumin and enzymes) or protein bound substances (calcium and iron) tended to increase consistently, that of smaller molecules, such as electrolytes, behaved rather differently and showed a consistent decrease, achieving both statistical and clinical significance fir potassium (after 1-min and 3-min stasis) and chloride (after 3-min stasis).

Serdar et al (16) investigated the influence of tourniquet application performed by two different groups involving expert and non-expert phlebotomists on the biochemical laboratory testing. 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, -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.

Sonoli performed study on five routinely advised analytes and showed a mean increase of 0.18g/dl (p=0.006) in protein measurement after one minute of tourniquet use (17). Richard et al findings showed that prolonged tourniquet application has a direct significant effect on total cholesterol, and HDL-Cholesterol but not triglyceride and LDL cholesterol (18). Differences between the results may be due to difference in technique employed to apply tourniquet, biochemical characteristics of the measuring analytes. The studies were also conducted on different population with different instruments.

CONCLUSION

Taken together, results of the present investigation confirm that variations in the plasma concentration of several biochemical analytes due to inappropriate standardization of the preanalytical 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.

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