Comparison of ethanol concentrations in blood samples collected from left subclavian vein, right subclavian vein and femoral vein

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Abstract

Femoral blood is normally recommended for toxicological analysis of ethanol and other drugs. In some circumstance, blood samples cannot be collected from this site, alternative peripheral sites are considered to be used such as subclavian vein which comprises left and right sides.This study aims to compare blood ethanol concentration collected from left subclavian vein, right subclavian vein and femoral vein. Blood samples were collected from 3 sites of 38 Thai corpses that were autopsied at the Institute of Forensic Medicine, Police General Hospital. Blood ethanol concentrations were analyzed usingheadspace gas chromatography technique.The data were analyzed by Pearson's correlation and paired t-test. The results showed that ethanol concentration in blood samples collected from left subclavian vein and right subclavian vein was not significantly different ($p = 0.269$) and the concentrations of ethanol in blood of both sides were not significantly different from those collected from the femoral vein ($p = 0.915$ and $p = 0.399$, respectively). Ethanol concentration in blood collected from left and right subclavian veins was linearly correlated ($r = 0.995$, $p<0.001$) and both were linearly correlated to ethanol concentration in femoral blood ($r = 0.988$, $p<0.001$ and $r = 0.988$, $p<0.001$, respectively). Therefore, both sides of subclavian vein can be used as an alternative site when femoral blood is not available for ethanol analysis.

Keywords : blood ethanol concentration, left subclavian vein, right subclavian vein, femoral vein

INTRODUCTION

Traffic accidents become one of the main causes of severe injuries and deaths. Most of the cases are associated with the use of alcoholic beverages before driving. Postmortem blood alcohol concentrations (BACs) are usually used as evidence in criminal and civil litigation to investigate the association of alcohol and the cause of death or the injury due to reduced decision performance. Postmortem blood specimens from two sites, heart and peripheral blood specimens are recommended to be collected at autopsy. Femoral venous blood is most recommended as peripheral blood specimens for toxicological analysis of ethanol and other drugs [1]. However, in some circumstance, femoral blood samples are insufficient in quantity or unobtainable, thus alternative sites of blood collection for postmortem ethanol analysis have been an issue of interest.

Many researcher groups performed the studies comparing BAC in femoral venous blood and in various sites/samples such as cardiac blood [2], subclavian blood [3], vitreous humor [4, 5, 6], synovial fluid [7], superior sagittal sinus [8], etc. Those proposed sites/samples of blood collection for postmortem ethanol analysis possess different advantages and limitations. Subclavian vein is the site of interest of our team because blood sampling at this site is easy particularly during an examination at the scene that autopsy is refused. Ethanol concentrations in subclavian blood were found to be closely correlated to BAC in femoral blood and were not influenced by the degree of putrefaction, cardiac resuscitation and gastric ethanol interference [3]. However, subclavian veins comprise left and right sides and the difference of concentration of ethanol in both sides has not been clarified. Since anatomical position of left subclavian vein is more close to the heart than the right one, impact of ethanol redistribution from the heart is questioned. Therefore, in this study, we aim to compare the concentrationsof ethanol in blood samples collected from left subclavian vein, right subclavian vein to the standard site of blood collection, the femoral vein so as to be an alternate site when femoral blood cannot be collected for ethanol analysis.

MATERIALS AND METHODS

Blood samples

The 38Thai subjects were deceased persons whose bodies were sent for autopsy at the Institute of Forensic Medicine, Police General Hospital, Bangkok, Thailand, during January to December 2014. Inclusion criteria are as follow: corpses of both sexes with any ages, lack of putrefaction as well as open wounds.The subjects with greenish discoloration of the skin, the evidences of open wound on the body surface, ruptured internal organs, insufficient amount of blood samples, are excluded. Blood samples were collected from left subclavian vein (LSV), right subclavian vein (RSV) and femoral vein (FV). All blood samples were kept in tubes containing 1% sodium fluoride. The time between death and autopsy was less than 24 h. The blood samples were stored at 4 °C until analysis.

Determination of ethanol concentration in blood samples

BAC was determined by headspace gas chromatography (GC) technique. GC equipped with flame ionization detector (Schimadzu model GC-2010, Japan) and double capillary columns (column 1: Restek® Rtx-BAC2 30 m × 0.53 mm ID × 2 µm df; column 2: Phenomenex® ZB-BAC-1 30 m × 0.53 mm ID × 3 µmdf) were used. The internal standard was tertiary butanol (EMSURE®, Merck KGaA Co. Ltd., Germany) at 0.78 mg/100 mL. Two hundred microliters of internal standard and 200 µL of each sample or the standard ethanol solution were added to 20 mL-headspace vial. Each sample or standard solution was performed in duplicated. The headspace condition settings were as follows: vial incubation temperature and injection temperature at 70 °C. Injection was done in split mode with a division ratio of 1/5. The carrier gas was helium at a constant flow of 69.0 mL/min. The oven temperature was 40 °C, isothermal time 4.7 min. The detector temperature was 255 °C. The retention times of ethanol and t-butanol from capillary column 1were 2.421 and 3.212, respectively. The retention times of ethanol and t-butanol from capillary column 2 were1.314 and 1.864, respectively. The limit of detection (LOD) for ethanol was 1 mg/dL and the limit of quantification (LOQ) was10 mg/dL. The standard curve of ethanol was performed with 5 concentrations of the standard ethanol (CerilliantR, Sigma-Aldrich, Inc, USA): 10, 25, 50, 150, 400 mg/dL The linearity range between 10-400 mg/dL demonstrated the coefficient of determination (R2) of 0.9992 and 0.9994 from column 1 and 2, respectively.

Statistical analysis

Correlation between BAC in LSV or RSV and FV were analyzed using Pearson's correlation test and the degree of correlation was shown by the correlation coefficient (r). Paired t-test was used to compare BAC in LSV or RSV and FV. Statistical analysis was performed using SPSS for Windows, version 22.0. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The subjects used in this study were mostly male (n= 36; 94.74%) while the remaining were female (n=2; 5.26%). Mean ± SD of the subject ages were ..... The causes of death were mostly due to respiratory system and blood circulation failure. BAC of the subjects are range between 21-435, 17-426 and 12-490 mg/dL in the blood collected from LSV, RSV and FV, respectively.

According to the Pearson's correlation test, the results demonstrated that BAC in both LSV and RSV are closely correlated to the BAC in FV (LSV vs FV, $r=0.988$, $p<0.001$, Figure 1; RSV vs FV, $r=0.988$, $p<0.001$, Figure 2). BAC in LSV was also closely correlated to the BAC in RSV($r=0.995$, $p<0.001$, Figure 3).

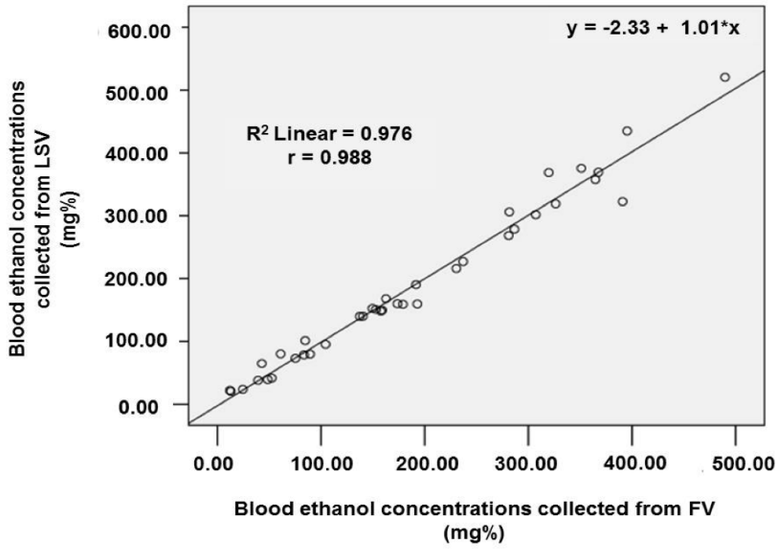

Figure 1 Correlation between blood ethanol concentrations collected from FV and LSV

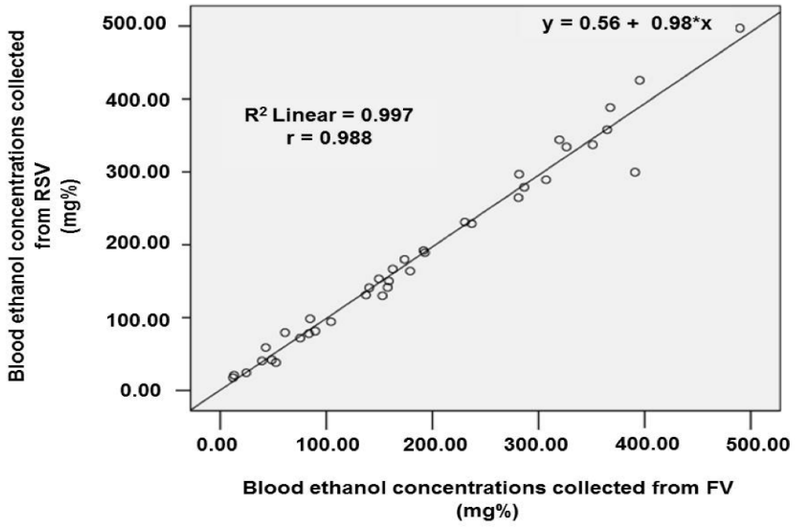

Figure 2 Correlation between blood ethanol concentrations collected from FV and RSV

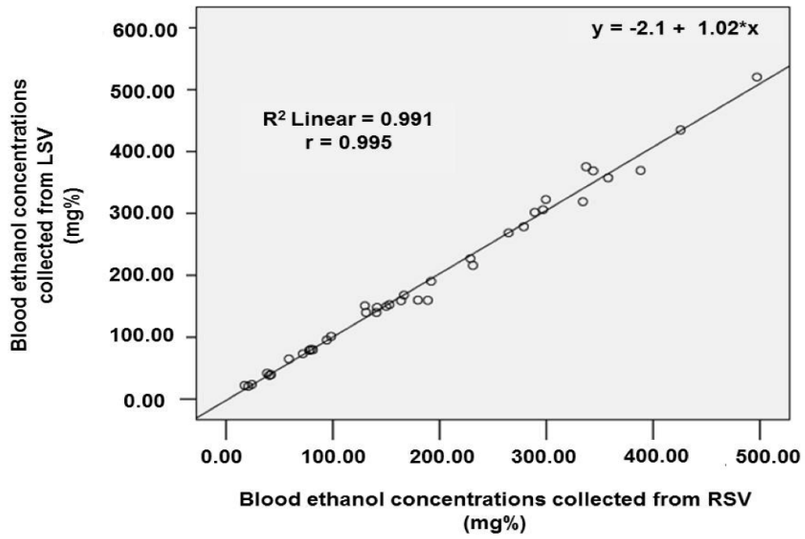

Figure 3 Correlation between blood ethanol concentrations collected from RSV and LSV

As using two-tailed paired t-test, it was shown that BAC in blood samples collected from LSV and RSVwere not significantly different from those collected from FV (p=0.915 and p=0.399, respectively).BAC in blood samples collected from LSV and RSV werealso not significantly different (p=0.269).

DISCUSSION

Result from this study was strongly consistent to the study of Sastre et al. [3] who demonstrated that BACs in subclavian blood were close to that found in femoral blood. However, in that study, they did not specify that the subclavian bloods were collected from the left or right side of subclavian vein. Since subclavian veins comprise left and right sides, this raises the issue whether either LSV or RSV can be used as the site of blood collection for ethanol determination. Thus, we aimed to compare the concentrations of ethanol in blood samples collected from left and right subclavian vein to those in samples collected from the femoral vein.

Because left subclavian vein is anatomically closer to the heart and stomach than the right one, impact of ethanol redistribution from the heart or other visceral organs is questioned. Normally, variation of postmortem ethanol concentration could be due to several mechanisms: the passive diffusion of ethanol through the stomach wall to the surrounding tissues/organs after death, rupture of the gastrointestinal tract causing contamination of ethanol from the gastric content, postmortem endogenous production from microbial contamination and decomposition of the body, reflux of gastric residue, life-saving heart massage/resuscitation, etc. [1, 9, 10]. These contaminations

of ethanol from the stomach explained the findings that ethanol concentrations in left cardiac blood were higher than those in the right cardiac blood and femoral blood because of the close anatomic apposition of the stomach and the left cardiac chamber [2]. In contrast, ethanol concentrations in subclavian blood were shown to be close to those in the femoral blood without the influence from putrefaction, reflux of gastric content in the airways, gastric ethanol concentration or cardiac resuscitation [3]. Despite not influenced by these factors, in this study, we included the subjects whose bodies were lack of putrefaction, open wounds or ruptured internal organs. Thus, if the different was found between BAC in LSV and RSV, the cause of difference was likely from ethanol redistribution/ diffusion from the heart or other visceral organs. However, we did not find the difference of BAC in LSV and RSV.

In conclusion, blood samples of 38 Thai corpses were analyzed for ethanol concentrations using headspace gas chromatography technique. The results showed that ethanol concentration in blood samples collected from left subclavian vein and right subclavian vein was not significantly different ($p = 0.269$) and the concentrations of ethanol in blood of both sides were not significantly different from those collected from femoral vein ($p = 0.915$ and $p = 0.399$, respectively). Ethanol concentration in blood collected from left and right subclavian veins was linearly correlated ($r = 0.995$, $p<0.001$) and both were linearly correlated to ethanol concentration in blood of femoral vein ($r = 0.988$, $p<0.001$ and $r = 0.988$, $p<0.001$, respectively). Therefore, both sides of subclavian vein can be used as an alternative site when femoral blood is not available for ethanol analysis.

REFERENCES

1. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in postmortem specimens: A review of the literature. Forensic Sci Int. 2007; 165:10-29
2. Pelissier-Alicot AL, Coste N, Bartoli C, Piercecchi-Marti MD, Sanvoisin A, Gouvernet J, et al. Comparison of ethanol concentrations in right cardiac blood, left cardiac blood and peripheral blood in a series of 30 cases. Forensic SciInt. 2006;156:35-9.
3. Sastre C, Baillif-Couniou V, Musarella F, Bartoli C, Mancini J, Piercecchi-Marti MD, et al. Can subclavian blood be equated with a peripheral blood sample? A series of 50 cases. Int J Legal Med. 2013; 127:379-84.
4. Tungtananuwat W, Lawanprasert S, Suwongthrom N. Comparative study of blood, vitreous humor and urine alcohol concentrations in Thai postmortem traffic accidental victims. Thai J Pharm Sci. 2000; 23(4): 165-172.
5. Honey D, Caylor C, Luthi R, Kerrigan S. Comparative alcohol concentrations in blood and vitreous fluid with illustrative case studies. J Anal Toxicol. 2005; 29(5): 365-9.

6. Jones AW, Holmgren P. Uncertainty in estimating blood ethanol concentrations by analysis of vitreous humour. J Clin Pathol. 2001; 54: 699-702.
7. Winek CL, Bauer J, Wahba WW, Collom WD. Blood versus synovial fluid ethanol concentrations in humans. J Anal Toxicol. 1993; 17: 233-5.
8. Sripong K, Wongchanapai W, Sasithornrojanachai S. Comparison of blood alcohol level between blood from superior sagittal sinus and femoral vein. J Forensic Physician Assoc of Thailand. 2008; 2(1): 10-5.
9. Pélissier-Alicot AL, Gaulier JM, Champsaur P, Marquet P. Mechanisms underlying postmortem redistribution of drugs: a review. J Anal Toxicol. 2003; 27(8):533-44.
10. Pounder DJ, Smith DR. Postmortem diffusion of alcohol from the stomach. Am J Forensic Med Pathol. 1995; 16:89-96.

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Abbreviation

Blood alcohol concentrations(BAC); Femoral vein (FV);Left subclavian vein (LSV); Right subclavian vein(RSV)