Comparative Study of Blood, Vitreous Humor and Urine Alcohol Concentrations in Thai Postmortem Traffic Accidental Victims

Wichian Tungtananuwat
Somsong Lawanprasert
Niti Suwongthrom

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การศึกษาเปรียบเทียบระหว่างความเข้มข้นของแอลกอฮอล์ในเลือดกับความเข้มข้นของแอลกอฮอล์ในรูฉนูนลูกนิ้วตาและปัสสาวะในคนไทยที่เสียชีวิตจากการพิจารณาเรื่องจากทางข้อมูล

วิเชียร ตั้งธนานุวัฒน์1, สมทรง ลาวัณ, ประเสริฐ 2 และนิติ สู่วงศ์ธรรม3

1 สถาบันนิติเวชวิทยา สำนักงานตำรวจแห่งชาติ กรุงเทพฯ 10330 ประเทศไทย
2 คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330 ประเทศไทย
3 ผู้เขียนที่สามารถติดต่อได้ E-mail address: lsomsong@chula.ac.th

บทคัดย่อ: การพิจารณาค่าความเข้มข้นของแอลกอฮอล์ (ในการวิจัยนี้หมายถึงเอทานอล) ในชีววัตถุอื่น มาจากข้อมูลค่าความเข้มข้นของแอลกอฮอล์ในเลือด ในกรณีที่เก็บตัวอย่างแอลกอฮอล์ในเลือดไม่ได้ หรือตัวอย่างแอลกอฮอล์ลูกนิ้วตา จะทำได้ด้วยการค่าความเข้มข้นของแอลกอฮอล์ในเลือดและในขั้นตอนนี้มีผลที่พิจารณาด้วย การวิจัยนี้เป็นการศึกษาเปรียบเทียบระหว่างความเข้มข้นของแอลกอฮอล์ในเลือดกับในรูฉนูนลูกนิ้วตาและปัสสาวะ โดยเก็บข้อมูลจากศพจากไทยที่เสียชีวิตเนื่องจากการพิจารณาจากทางจริงค่า BAC ของวิชัยส่งผลต่อความเข้มข้นของแอลกอฮอล์ในเลือด (BAC) มีค่าสัมประสิทธิ์บันจุดอย่างสูงสุดขั้นตอนของแอลกอฮอล์ในรูฉนูนลูกนิ้วตา (VHAC) และความเข้มข้นของแอลกอฮอล์ในปัสสาวะ (UAC) โดยมีค่าสัมประสิทธิ์บันจุด (r) เท่ากับ 0.932 และ 0.894 ตามลำดับ และมีผลการทดลองเป็นสัมประสิทธิ์บันจุดของ BAC สัมประสิทธิ์บันจุด VHAC = 1.02 VHAC + 8.13 (R = 0.86) และ BAC สัมประสิทธิ์บันจุด UAC = 24.30 (R = 0.799) การที่มีสัมประสิทธิ์บันจุดและมีความสัมพันธ์เชิงเส้นตรงระหว่าง BAC และ VHAC หรือ UAC นี้สัมประสิทธิ์บันจุดเป็นไปได้ในทางที่มีคำว่า VHAC และ UAC มีสัมประสิทธิ์บันจุด BAC ในกรณีที่มีความสัมประสิทธิ์บันจุดมากพอที่จะน่าจะใช้ในการพิจารณา BAC ได้

กุญแจคำ: ความเข้มข้นของแอลกอฮอล์ในเลือด, ความเข้มข้นของแอลกอฮอล์ในรูฉนูนลูกนิ้วตา, ความเข้มข้นของแอลกอฮอล์ในปัสสาวะ
Comparative Study of Blood, Vitreous Humor and Urine Alcohol Concentrations in Thai Postmortem Traffic Accidental Victims

Wichian Tungtananuwat1, Somsong Lawanprasert23 and Niti Suwongthorm1

1 Institute of Forensic Medicine, The Royal Thai Police Headquarters, Bangkok 10330, Thailand.
2 Faculty of Pharmaceutical Sciences, Chulalongkom University, Bangkok 10330, Thailand.
3 Corresponding author. E-mail address: lsomsong@chula.ac.th

ABSTRACT: To utilize alcohol (referring to ethanol) concentrations in other biological specimens to estimate a blood alcohol concentration (BAC) when suitable postmortem blood sample is unavailable or contaminated, a good correlation between alcohol concentrations in blood and in those biological specimens is needed. In this study, alcohol concentrations in blood, vitreous humor and urine were comparatively studied. Specimens were collected from 110 Thai postmortem traffic accidental victims and analyzed for ethanol by gas chromatography with headspace technique. The result showed a good correlation between BAC and vitreous humor alcohol concentration (VHAC) or urine alcohol concentration (UAC) with correlation coefficients (r) of 0.932 and 0.894, respectively. Simple linear regression analyses yielded the equations BAC = 1.02 VHAC + 8.13 (R² = 0.868) and BAC = 0.82 UAC + 24.30 (R² = 0.799). These strong correlations and linear relationships between BAC and VHAC or UAC suggest a possibility of utilizing VHAC and/or UAC to estimate BAC when proper blood samples are unavailable.

Key words: blood alcohol concentration, vitreous humor alcohol concentration, urine alcohol concentration

INTRODUCTION

Determination of postmortem alcohol (referring to ethanol) concentrations is one of the most frequent requests of forensic analysis. Blood is the usual specimen analyzed when relating alcohol concentration to the degree of intoxication and the medico-legal aspect [1]. However, when suitable blood sample is unavailable or contaminated, other biological specimens are needed to estimate a blood alcohol concentration (BAC). Several studies have been performed, mostly in western countries, regarding a relationship between alcohol concentrations in blood and other body fluids or tissues such as vitreous humor, urine, bile, cerebrospinal fluid, bone marrow and muscle [2-21]. Among these biological specimens, vitreous humor is reported the most suitable specimen to be used for estimating a BAC [2-14]. Even though urine is not a very good specimen for BAC estimation, it has been accepted as a BAC predictor for legal purpose [22]. Conversion factors, proposed for a prediction of BAC from vitreous humor alcohol concentration (VHAC) and urine alcohol concentration...
concentration (UAC), are varying among previous studies. The differences are probably resulted from several factors, such as differences in ethnic, body mass, drinking and dietary habits, criteria of specimen collection, analytical methods, etc. Therefore, to gain information for a specific population group, the aim of this study is to determine a relationship between alcohol concentrations in blood and vitreous humor as well as urine which are particularly collected from Thai cadavers that have not been reported before.

MATERIALS AND METHODS

Specimen collection

Specimens were collected from Thai traffic accidental victims whose cadavers were brought for autopsy at the Institute of Forensic Medicine, the Royal Thai Police Headquarters, Bangkok. All specimens (blood, vitreous humor and urine) were collected simultaneously at autopsy performing within 24 hours of death. Blood was obtained from neck vein. Vitreous humor was obtained by direct needle puncture of either eye while urine was obtained by direct needle puncture of the bladder. All specimens were drawn into closed-tight tubes containing sodium fluoride and potassium oxalate (1:1) and kept refrigerated until the time of analysis.

Determination of alcohol concentrations

A gas chromatography (Pye Unicam, PU 4500) equipped with a flame ionization detector and an automatic headspace injector (DANI, HSS 86.50) was used for the alcohol analysis. A column of 5 foot x 1/4 inch O.D. stainless steel packed with Porapak Q 80-100 mesh was utilized. The operating temperatures were as following: injection port, 205 °C, oven, 190 °C and detector, 205 °C. Nitrogen at a flow rate of 23 lb/inch² was used as carrier gas. A Shimadzu Model C-R6A Chromatopac was used to integrate the peaks.

The procedure of quantitative determination of alcohol concentrations in whole blood, vitreous humor and urine samples was adapted from the method previously described by Winek and Esposito in 1981 [5]. Briefly, each 10-ml bottle contained 200 μl of isopropanol internal standard solution (195 mg%), 200 μl of deionized water, 200 μl of whole blood or vitreous humor or urine and 1 g of sodium chloride. The bottles were then immediately capped with rubber stoppers and the content was mixed by gently swirling. The bottles were stored at room temperature for 15 minutes and then placed in the automatic headspace injector. The vapor samples were automatically injected into gas chromatography after the incubation time of 17 minutes at 50 °C. Duplicated bottles were prepared and injected for each unknown specimen. Five bottles of ethanol reference standard solution (158 mg%) were prepared and injected in the same manner as of the specimen. Alcohol concentrations in unknown specimens were calculated by comparing their peak area ratios to the mean peak area ratio of the ethanol reference standard.

Statistics

The strength of linear correlations between BAC and VHAC as well as between BAC and UAC were expressed by a correlation coefficient (r). Relationships between BAC versus VHAC, and BAC versus UAC were determined by fitting simple linear regression models to the experimental data utilizing a statistical software package of SPSS for Windows Release 7.5.1 Standard Version.

RESULTS

Of the 250 autopsy cases studied, there were 110 cases (accounted for 44 % of the total) whose blood, vitreous humor and urine specimens were correspondingly and positive for ethanol. Of these 110 ethanol positive cases of age between 16-60 years, 105 cases (95.5%) were male and 5 cases (4.5%) were female. The distribution of BAC and of the corresponding VHAC and UAC were
normally distributed (Figure 1). Ranges of BAC, VHAC and UAC were from 10 to 435 mg%, 16 to 387 mg% and 22 to 490 mg% , respectively. The means ± standard deviations of BAC, VHAC and UAC were 215 ± 90 mg%, 203 ± 82 mg% and 232 ± 98 mg%, respectively.

The distributions of BAC/VHAC ratio and BAC/UAC ratio were also normally distributed (Figure 2). Range of BAC/VHAC ratio and BAC/UAC ratio were from 0.51 to 1.53 and 0.43 to 2.08, respectively. The means ± standard deviations of BAC/VHAC and BAC/UAC were 1.06 ± 0.20 and 0.95 ± 0.26, respectively.

Figure 3 showed the scatter plots of BAC versus VHAC and BAC versus UAC. There was a strong positive linear correlation between BAC versus VHAC as shown by a correlation coefficient of 0.932 (P= 0.0001). Similar finding was found between BAC versus UAC with a correlation coefficient of 0.894 (P= 0.0001). Fitting a simple linear regression model to these data, two linear regression equations yielded: BAC = 1.02 VHAC ± 8.13 (R² = 0.868) and BAC = 0.82 UAC ± 24.30 (R² = 0.799). The 95% prediction interval for the regression model with VHAC as a predictor variable was ± 64.96 \sqrt{111/110} ± (VHAC - 203)/\sqrt{737878}. The regression model with UAC as a predictor variable contained a 95 % prediction interval of ± 80.27 \sqrt{111/110} + [(UAC - 232)/\sqrt{1044362}].

A multiple linear regression model was also fitted to the data. Both VHAC and UAC were entered as predictor variables in the model. The resulting equation was BAC = 0.78 VHAC + 0.21 UAC + 6.18 (R² = 0.936).

Figure 1. Display of the normal distributions of BAC (a); VHAC (b); and UAC (c).
(a) range 10 - 435 mg%; Mean ± SD. = 215 ± 90 mg%
(b) range 16 - 387 mg%; Mean ± SD. = 203 ± 82 mg%
(c) range 22 - 490 mg%; Mean ± SD. = 232 ± 98 mg%
Figure 2. Display of the normal distributions of BAC/VHAC ratio (a), and BAC/UAC ratio (b).
(a) range 0.51 - 1.53; Mean ± SD. = 1.06 ± 0.20
(b) range 0.43 - 2.08; Mean ± SD. = 0.95 ± 0.26

BAC = 1.02 VHAC + 8.13
(R^2 = 0.868) with 95% prediction interval of ± 64.96 \sqrt{(111/110) + [(VHAC - 203)^2/737878].

Figure 3. Display of scatter plots of BAC vs VHAC (a) and BAC vs UAC (b) along with the least squares regression line (middle line) and 95% prediction interval lines (upper and lower).
(a) BAC vs VHAC; r = 0.932 (P = 0.0001) with an equation of BAC = 1.02 VHAC + 8.13
(R^2 = 0.868) with 95% prediction interval of ± 64.96 \sqrt{(111/110) + [(VHAC - 203)^2/737878].
(b) BAC vs UAC; r = 0.894 (P = 0.0001) with an equation of BAC = 0.82 UAC + 24.30
(R^2 = 0.799) with 95% prediction interval of ± 80.27 \sqrt{(111/110) + [(UAC - 232)^2/1044362].
DISCUSSION AND CONCLUSION

Due to the common accepted association between a person's BAC and the degree of impairment, analysis of alcohol in blood/body fluids has been the most frequently requested in forensic toxicology [1]. Indeed, heavy drinking represents a major cause of accidents on roads, in workplaces/homes as well as a contributing factor in many suicides, violent crimes and other kinds of deviant behavior [23]. Consequently, a threshold alcohol concentration is limited in most countries and defined as an alcohol limit for driving under the influence of alcohol (DUI). The critical legal limit for DUI varies among countries ranging from 50-100 mg% [24]. Punishment for DUI includes fines, suspension of the driving license, and sometimes a period of mandatory imprisonment. Therefore, the highly reliable methods for measuring alcohol in blood and other body fluids are needed, particularly in the countries of which the punishment is severe. In Sweden, for example, a triplicate blood alcohol determination is made in all DUI investigations and the lower 99.9% confidence limit on the mean is the value used for prosecution [1].

In this study, we determine a relationship between alcohol concentrations in blood and vitreous humor as well as urine so as to find alternative biological samples when a suitable postmortem blood sample is unavailable or contaminated. Several studies have been performed since 1960, regarding a relationship between BAC and alcohol concentrations in other biological specimens [2-21]. Due to a strong correlation and a linear relationship between BAC and VHAC as well as its several advantageous properties, vitreous humor is reported the most suitable specimen to be used for estimating BAC from known VHAC or UAC, respectively. These serve vitreous humor as a good alternative sample when blood sample is not readily obtainable or obtainable but unsuitable for a meaningful analysis, such as the postmortem putrefaction. In fatalities, urine sample obtained is pooled bladder urine which has accumulated over an unknown time interval between last urination and death. Therefore, the bladder UAC does not necessarily reflect the BAC existing at the time of death. However, screening urine for ethanol has been accepted and even been proposed as a predictor of BAC in legal purposes in some countries [1]. As urine sample is usually collected for a routine forensic analysis and readily obtainable, we consider it is also interesting to study so as to use urine as another alternative sample for BAC prediction. We performed specimen collections in postmortem traffic accidental victims because these groups of people have been found mostly positive for an ethanol screening [25].

Utilizing a simple conversion factor of a mean of BAC/VHAC ratio or a mean of BAC/UAC ratio to estimate BAC from known VHAC or UAC, respectively is less meaningful. The means of BAC/VHAC ratios reported in different studies span a wide range from 0.73-1.05 [2-13,16]. Our results showed a mean BAC/VHAC ratio of 1.06 with ± 0.20 standard deviation. Our histogram of the BAC/VHAC ratio in Figure 2 (a) was similar to a study of Jollymore et al (1984) [7] which was unable to support the hypothesis of Coughlin (1983) of a possible bimodal distribution [6]. Also, the means of BAC/UAC ratios reported in different previous studies range from 0.80 to 0.86 [1,5,16] while our result showed a mean of BAC/UAC ratios of 0.95 with ± 0.26 standard deviation.

Simple linear regression analyses, with the BAC as a response variable and VHAC or UAC as a predictor variable, give regression equations which are more meaningful for an explanation of BAC prediction from VHAC or UAC. A good fit of the regression equations of BAC versus VHAC as well as BAC versus UAC was
obtained as shown by a rather high R² value of 0.868 and 0.799, respectively. The prediction interval provides a confident interval of BAC prediction with taking into account the uncertainty of the prediction for an individual subject. Even though a better fit was shown when a multiple linear regression model was fitted to the data, as shown by a higher R² of 0.936, the obtained equation did not necessarily represent the superior prediction equation. This was because of a multicollinearity which may occur due to an existence of a strong linear dependency between VHAC and UAC (r = 0.923; P= 0.0001) [26].

In summary, our present study on a sample group of 110 Thai cadavers demonstrated a strong linear relationship between BAC and VHAC or UAC. This suggests a possibility of utilizing VHAC and/or UAC to estimate BAC when proper blood samples are unavailable. Validation of the predicted BAC from the obtained regression equations with the observed BAC, utilizing other sample group of Thai specimens, is suggested exploring in a further study.

NOTATIONS

BAC = blood alcohol concentration
DUI = driving under the influence of alcohol
r = Pearson’s correlation coefficient
R² = coefficient of determination
UAC = urine alcohol concentration
VHAC = vitreous humor alcohol concentration

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REFERENCES


25. Institute of Forensic Medicine, The Royal Thai Police Headquarters. The determination of blood alcohol concentration of the Thai traffic accidental victims in Bangkok metropolitan area. 1986 (Unpublished data).