Correlation between Postmortem Ethanol Production and Low Molecular Weight Volatiles (LMWVs) in a Thai Population

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ABSTRACT

Objective: To determine the relationship between postmortem ethanol production and low molecular weight volatiles (LMWVs) in a Thai population.

Materials and Methods: A retrospective study was conducted on 157 Thai cadavers. Various subject data were recorded, including degree of postmortem changes (transition period group vs. decomposition group classified by the total body score [TBS]) and blood profiles of ethanol, acetaldehyde, and 1-propanol concentrations from femoral blood samples. Blood ethanol concentrations were diagnosed as postmortem production by vitreous humor analysis. Linear regression analysis was performed to determine the correlations between these three analytes and the TBS and between postmortem ethanol and the other two analytes.

Results: This study comprised 44 females and 113 males with a mean age at death of 47.79 years old. Postmortem ethanol, acetaldehyde, and 1-propanol concentrations escalated along with the increased TBS ($p<0.001$). These three analytes were significantly correlated with the TBS ($p<0.001$) and the correlations of determination (R^2) for postmortem ethanol and 1-propanol were better than for acetaldehyde ($R^2 = 0.488$ and 0.414 vs. 0.269). Acetaldehyde and 1-propanol were positively correlated with postmortem ethanol ($p<0.001$). The correlations between postmortem ethanol and acetaldehyde, 1-propanol, and the combination of these two analytes produced R^2 values of 0.413, 0.480, and 0.544, respectively.

Conclusion: Acetaldehyde and 1-propanol concentrations were significantly correlated with postmortem ethanol concentrations in Thai cadavers, and the combination of these two markers produced a better correlation with the postmortem ethanol concentration.

Keywords: Postmortem ethanol; acetaldehyde; 1-propanol; Thai population (Siriraj Med J 2023; 75: 299-305)

INTRODUCTION

Ethanol (alcohol) is the most common substance detected in forensic autopsy cases.[1] Ethanol plays an important role in many causes of death in medico-legal cases. In addition, a blood ethanol concentration beyond the legal limit for vehicle control is important for the diagnosis of driving under the influence of alcohol. In Thai legislation, Ministerial Regulations No. 16 B.E. 2537 (1994) and No. 21 B.E. 2560 (2017) by virtue of the provisions of Section 5 of the Road Traffic Act B.E. 2522 (1979) set the statutory cut-off point for blood ethanol concentration at 50 mg/dL for the general population

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https://doi.org/10.33192/smj.v75i4.260477

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and 20 mg/dL for people in some specific groups, for example, people who are under 20 years old. This cut-off may result in difficulty in blood ethanol interpretation, particularly in postmortem cases due to postmortem ethanol production from the time since death.

A previous study suggested that postmortem ethanol production commonly occurs in decomposed bodies, bodies retrieved from water, and bodies with extensive trauma because of the increasing risk of bacterial activities.[2] A review article suggested that blood ethanol concentrations less than 30 mg/dL in dead bodies were mainly caused by postmortem ethanol production.[1] The majority of postmortem ethanol blood concentrations have been reported to be not greater than 70 mg/dL.[2,3] There are only a few reports of postmortem ethanol production greater than 100 mg/dL.[3-6] Postmortem ethanol productions at 120 mg/dL and 160 mg/dL have been reported in decomposed bodies.[3,4] The diagnosis of postmortem ethanol production in these decomposed bodies was based on their vitreous humor (VH) ethanol concentrations that were less than 10–20 mg/dL, leading to inconsistent ratios of VH-to-blood ethanol concentrations (VH/B ratio).[3,4,7] Dead bodies with severe trauma can have a postmortem ethanol production of 180 mg/dL and 190 mg/dL.[5,6] Thus, the VH/B ratio is an important parameter for the interpretation of postmortem ethanol production.

Low molecular weight volatiles (LMWVs), such as acetaldehyde, 1-propanol, 2-propanol, and 1-butanol, were also reported to be useful for the diagnosis of postmortem ethanol production.[8-10] Ceciliason et al. and Boumba et al. indicated that 1-propanol was correlated with the degree of decomposition.[8-10] Chen et al. suggested that acetaldehyde was also positively correlated with postmortem ethanol production and this marker was more sensitive than 1-propanol.[9] Thus, 1-propanol and acetaldehyde are two potential markers to determine postmortem ethanol production.

The synthesis of LMWVs in dead bodies can vary depending on the postmortem interval (PMI), environmental factors, internal conditions of the dead bodies, and types of intestinal microbes.[11] There is still no current information about the association between postmortem ethanol production and LMWVs in Thai postmortem cases. Consequently, the aim of this study was to determine the correlation between postmortem ethanol production and LMWVs in a Thai population. This finding would be useful for the interpretation of postmortem ethanol production and the prediction of blood ethanol concentrations generated in postmortem periods, leading to the benefit of better understanding the states of dead bodies for legal procedures.

MATERIALS AND METHODS

Study design and data collection

A retrospective study was conducted of medico-legal cases sent for autopsy at the Department of Forensic Medicine, Siriraj Hospital, Mahidol University between January 1, 2021 and December 31, 2021. This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University (COA no. Si 061/2022, SIRB protocol No. 016/2565(IRB4)). The inclusion criteria were: Thai people who were 18 years old or over and their postmortem changes ranged from the transition period before decomposition to the decomposition period. Femoral blood and VH must be collected from each case in a sodium fluoride tube for the analysis of ethanol, acetaldehyde, and 1-propanol. The positive ethanol concentrations were considered as postmortem ethanol production according to two criteria[1,3,4,7]:

1. Blood ethanol concentrations were less than 30 mg/dL and VH ethanol concentrations were negative.

2. Blood ethanol concentrations were equal to or greater than 30 mg/dL and VH ethanol concentrations were less than 10 mg/dL.

Sex, age, underlying disease, cause of death, and postmortem changes were recorded for each case. The external findings of postmortem changes were classified into two groups based on two periods:

1. Transition period (before decomposition) group: presence of secondary flaccidity of rigor mortis and partial or total fixation of livor mortis without external signs of decomposition.

2. Decomposition group: presence of external signs of decomposition, including chromatic phase and gaseous phase (Table 1).

The decomposition group was scored by the total body (decomposition) score (TBS) adapted from previous works.[12-14] The scoring system based on the starting number of zero for the state of no decomposition was derived from Moffatt et al.[12] The body descriptions for the decomposition state were adapted from Megyesi et al.[13] and Gelderman et al.[14] Our TBS is described in Table 1.

The summation of all the TBS points from each area was calculated. For the decomposition group, this meant the scoring would be from 1 to 10 points. Next, decomposition group was categorized into three groups based on the degree of TBS: 1–3 points, 4–7 points, and 8–10 points, respectively.

Analyses of ethanol, acetaldehyde, and 1-propanol

Ethanol, acetaldehyde, and 1-propanol analyses were

TABLE 1. Developed TBS method for the body decomposition.

TBS	Points	Description
TBS_Head and Neck	0	No signs of decomposition
	1	Greenish discoloration of the face and neck
		Marbling of the face and neck
	2	Bloating of the face and neck
	3	Skin bleb and/or skin slippage of the face and neck
TBS_Torso (Trunk)	0	No signs of decomposition
	1	Greenish discoloration of the chest and/or abdomen
		Marbling of the upper trunk
	2	Bloating of the chest and abdomen
	3	Skin bleb and/or skin slippage of the chest and abdomen
TBS_Limb	0	No signs of decomposition
	1	Greenish discoloration of the upper limbs
		Marbling of the upper limbs
	2	Greenish discoloration of the lower limbs
		Marbling of the lower limbs
	3	Bloating of the upper and/or lower limbs (limb spreading)
	4	Skin bleb and/or skin slippage of the upper and/or lower limbs

performed by the method adapted from the previous study[15] using a headspace gas chromatography-flame ionization detector (HS-GC-FID) in an Agilent 7890A GC system. The GC was equipped with the RTX-BAC2 RESTEK capillary column (30 m × 0.32 mm × 1.2 µm). Helium gas was used as the carrier gas at a flow rate of 2 mL/min. GC introduction was carried out using split injection with a ratio of 10:1. The HS preparation was done using an Agilent G1888 HS with an oven temperature of 80 °C, transfer line temperature of 120 °C, and vial equilibration time of 5 minutes. The oven temperature was set at 47 °C. The FID temperature was set at 235 °C with the flow rates of hydrogen gas, air zero, and make-up gas (nitrogen) at 45, 450, and 8 mL/min, respectively. The analysis was performed in duplicate using isocratic elution with a total run time of 7.5 minutes.

Method validation was performed following the SWGTOX 2013 guidelines.[16] Selectivity and interference studies were conducted to ascertain there were no interference peaks at retention times of acetaldehyde, ethanol, and 1-propanol at 2.27, 3.36, and 5.93 minutes. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for ethanol, acetaldehyde, and 1-propanol were 1.5/2.5, 0.5/1, and 0.5/1 mg/dL, respectively. Calibration curves for ethanol were performed from 2.5 to 100 mg/dL (2.5, 5, 10, 20, 50, and 100 mg/dL). Calibration curves for acetaldehyde and 1-propanol were performed from 1 to 50 mg/dL (1, 2, 5, 10, 20, and 50 mg/dL). Calibration curves were generated using Agilent ChemStation Software® Version B.04.03 from back-calculated concentrations for each calibrator to achieve $R^2 > 0.99$ and acceptable accuracy. Three spiked QC samples for ethanol at 8, 25, and 80 mg/dL and for acetaldehyde/1-propanol at 3, 15, and 40 mg/dL were analyzed for assessing the accuracy and precision. The accuracy and precision for each QC were within acceptable criteria at ±15% accuracy and ±15% coefficient of variation (%CV).

Statistical analysis

Statistical analysis was performed using IBM SPSS® Statistics for Windows version 25. Descriptive statistics, including the mean, median and standard deviation (SD), were calculated. The Kruskal–Wallis H-test was performed for comparison of these three analytes and each TBS group. Linear regression analysis was performed for assessing the correlation between the TBS and each compound. Then, linear regression and multiple regression analyses were performed for assessing the correlation between ethanol and the other two compounds. Regression diagnostics and multicollinearity testing were performed for acetaldehyde and 1-propanol with the variance inflation factor (VIF) converged to 1.

RESULTS

According to two criteria for postmortem ethanol production stated above, there were 157 cases recruited in this study, comprising 44 females (28.03%) and 113 males (71.97%). The mean age at death was 47.79 ± 15.77 years old (range, 18–84 years old). The main causes of death were coronary artery disease (35.67%, 56/157), drowning (15.92%, 25/157), hanging (13.38%, 21/157), closed head injuries from falling (5.10%, 8/157), and cirrhosis (4.46%, 7/157), respectively. There were 34 cases (21.66%) that were in the transition period and 123 cases (78.34%) that presented with external signs of decomposition.

Overall, there were 47.13% (74/157) acetaldehyde-positive samples and 35.03% (55/157) 1-propanol-positive samples. There were a small number of positive cases for acetaldehyde (14.71%, 5/34), and no positive cases for 1-propanol in the transition period group; in contrast, there were 56.10% (69/123) acetaldehyde-positive cases and 44.72% (55/123) 1-propanol-positive cases in the decomposition group. The concentration ranges of ethanol, acetaldehyde, and 1-propanol in the decomposition group escalated with the increasing extent of decomposition. The comparison of ethanol, acetaldehyde, and 1-propanol between the three different TBS groups presented with significant differences for all three compounds ($p < 0.001$). The ranges and median concentrations of ethanol, acetaldehyde, and 1-propanol in the subjects classified by the postmortem changes are shown in Table 2.

In the decomposition group, all three analytes were positively correlated with the TBS ($p < 0.001$), but the coefficients of determination (R^2) for ethanol and 1-propanol were better than for acetaldehyde. The regression analysis results for these three analytes are shown in Fig 1 and Table 3.

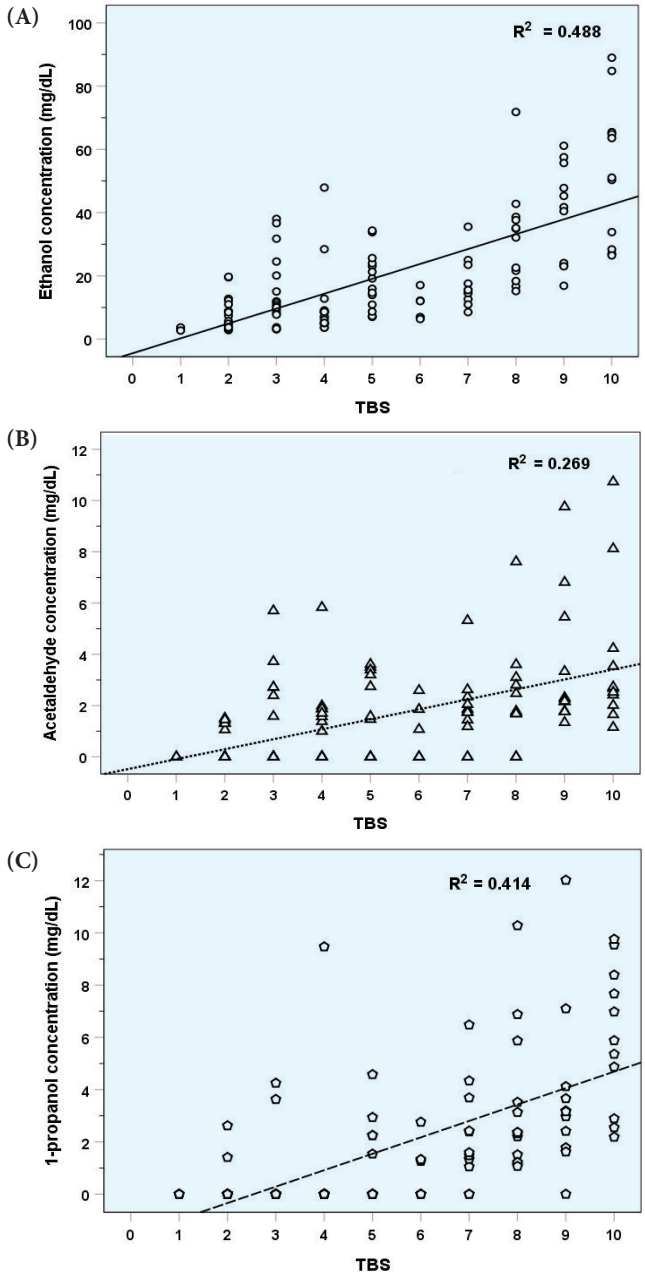


Fig 1. Regression analyses between TBS and (A) ethanol, (B) acetaldehyde, and (C) 1-propanol.

TABLE 2. Blood ethanol, acetaldehyde, and 1-propanol concentrations in the studied subjects.

Postmortem changes	Ethanol (mg/dL)		Acetaldehyde (mg/dL)		1-propanol (mg/dL)	
	Range	Median	Range	Median	Range	Median
Transition period group	2.71–22.78	6.56	ND–3.64	ND	ND	ND
Decomposition group	2.72–88.96	14.56	ND–10.73	1.34	ND–12.02	1.75
TBS 1–3 points	2.72–38.01	8.37	ND–5.70	ND	ND–4.25	ND
TBS 4–7 points	3.66–47.90	12.00	ND–5.83	1.18	ND–9.47	ND
TBS 8–10 points	15.18–88.96	38.14	ND–10.73	2.36	ND–12.02	3.18

*ND = Not detected (<LOD)

TABLE 3. Regression analyses for the correlation between all three analytes and TBS.

Regression equation	R	R^2	p-value
Ethanol = (4.704 × TBS) – 4.455	0.699	0.488	<0.001
Acetaldehyde = (0.389 × TBS) – 0.479	0.518	0.269	<0.001
1-propanol = (0.629 × TBS) – 1.598	0.643	0.414	<0.001

There were highly significant correlations between postmortem ethanol and acetaldehyde and 1-propanol ($p < 0.001$) with approximately equivalent R^2 values. The correlation between the combination of these two analytes and postmortem ethanol produced better results than using each marker alone. The regression curves and equations are shown in Fig 2 and Table 4, respectively.

DISCUSSION

Postmortem ethanol production can occur from the transition period to decomposition state in a cadaver because postmortem ethanol could be generated from several pathways and there are many substrates that are utilized in microbial activities.[11] This finding was consistent with a previous review that indicated the susceptibility to postmortem ethanol production in cadavers that had the postmortem intervals greater than 12-24 hours due to the invasion of intestinal bacteria to bloodstream.[17] Subjects in the transition period group also had the postmortem intervals greater than 12 hours because of the criteria of postmortem changes in this study. Therefore, subjects in the transition period group could present with some degree of postmortem ethanol production. In the decomposition group in the present study, ethanol concentrations increased following the increased scores of the TBS, and this finding was consistent with previous studies.[7,9] This finding indicated that ethanol concentrations in blood and VH samples obtained from postmortem

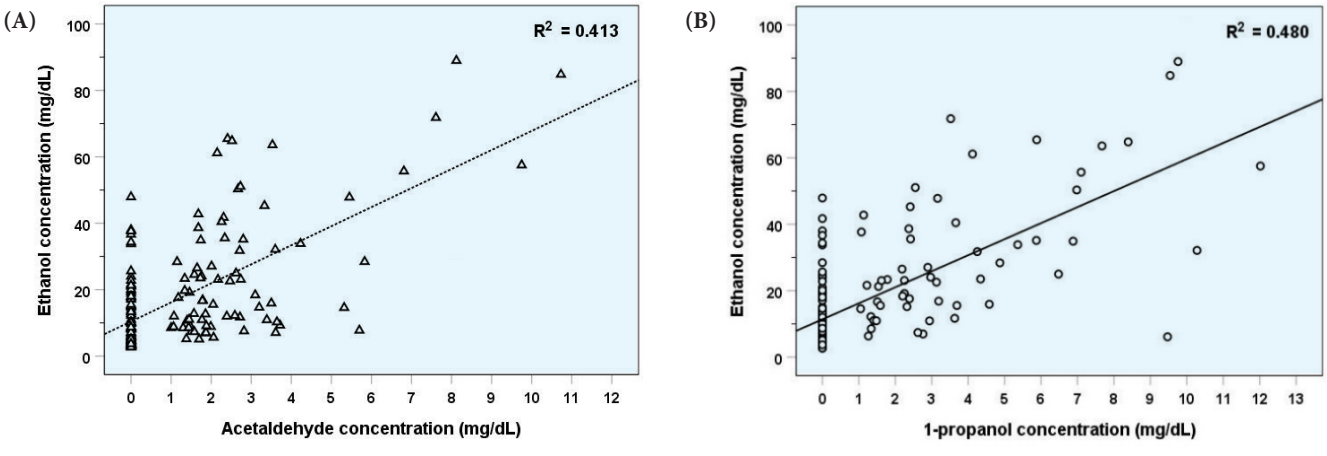


Fig 2. Regression analyses between ethanol and (A) acetaldehyde and (B) 1-propanol.

TABLE 4. Regression analyses for the correlation between postmortem ethanol and LMWVs.

Regression equation	R	R^2	p-value
Ethanol = (5.737 × acetaldehyde) + 10.425	0.643	0.413	<0.001
Ethanol = (4.826 × 1-propanol) + 11.379	0.693	0.480	<0.001
Ethanol = (2.981 × acetaldehyde) + (3.317 × 1-propanol) + 9.517	0.738	0.544	<0.001

cases were critical for the diagnosis of postmortem ethanol production using two criteria stated above. This study also showed that acetaldehyde and 1-propanol were positively correlated with the TBS. However, the R^2 values of postmortem ethanol and 1-propanol with the TBS were superior to acetaldehyde and this pattern was similar to in a previous study.[7] Although our R^2 values for postmortem ethanol and 1-propanol were relatively low (0.488 and 0.414), they were slightly better than in a previous study, which reported R^2 values of 0.16 and 0.29 for postmortem ethanol and 1-propanol, respectively.[7] This finding might have resulted from the difference in case recruitment because 78.34% of the cadavers in this study were decomposed cases, whereas the decomposed cases accounted for only 49% of the cadavers in the previous study.[7] Previous studies suggested that 1-propanol was a strong marker for the putrefactive state, whereas the other LMWVs could be variable[7,8,10,11], because 1-propanol was mainly generated from the amino acid pathway in bacteria and yeast and this production did not depend on the presence of glucose and carbohydrate metabolism.[11] Thus, 1-propanol produced a better correlation with the TBS than acetaldehyde and may be suitable for TBS evaluation in Thai postmortem cases.

Acetaldehyde (47.13%) was more commonly found than 1-propanol (35.03%) in the overall cases and this finding was consistent with previous studies.[8,10] Chen et al. found that 1-propanol was generated *in vitro* at a slower rate and lower amount than acetaldehyde and this explanation was congruent with our results that 1-propanol was only found in the decomposition group.[9] However, the acetaldehyde concentrations in this study were not as high as stated in the previous study because Chen et al. indicated that acetaldehyde beyond 14 mg/dL could be used as a marker for postmortem ethanol production[9], but the concentration range for acetaldehyde in this study was only ND–10.73 mg/dL.

Our study showed that acetaldehyde and 1-propanol were positively correlated with postmortem ethanol with equivalent R^2 values (R^2 = 0.413 and 0.480). When these two markers were combined for the regression analysis with postmortem ethanol, the R^2 value was improved compared with each marker alone (R^2 = 0.544). This finding was consistent with a previous study employing multiple markers (1-propanol, n-butanol, isobutanol, and methyl-butanol).[18] However, our R^2 was relatively moderate compared with the previous study that reported a high R^2 value (>0.7).[18] This result could be caused by differences in the data collection because the previous study derived the equation and R^2 from experiments using single bacteria in each experimental condition, whereas our study obtained the equation and R^2 from authentic cases. Although the previous study showed that their models from *C. perfringens* and *E. coli* were suitable for postmortem ethanol prediction, they estimated the postmortem ethanol concentrations with a standard error of <40% for approximately 63%–68% of the total cases.[18] This implied that postmortem ethanol production came from complex pathways and there were likely several microorganisms involved in this process. Furthermore, postmortem ethanol and LMWVs produced by microbial activities could vary due to the different substrates used and different types of microorganisms and this may be dependent on the cadaver condition.[11] Thus, the interpretation of postmortem ethanol concentrations using LMWVs should be carefully performed.

CONCLUSION

Acetaldehyde and 1-propanol were positively correlated with postmortem ethanol concentrations. The combination of these two markers in the regression analysis produced a better correlation with postmortem ethanol concentrations than employing each marker alone. Thus, postmortem ethanol concentration could be determined by using blood and VH ethanol concentrations coupled with acetaldehyde and 1-propanol concentrations that were correlated with blood ethanol concentrations.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Asst. Prof. Dr. Chulaluk Komoltri for her invaluable assistance and advice with the statistical analysis.

Conflict of Interest

None.

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