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Relationship of Blood and Urine Alcohol Levels in Postmortem Samples and Prevalence of Alcohol Level above Legal Limit in Hospital Kuala Lumpur

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Abstract: High blood alcohol content has been associated with fatal accident, traumatic death, and violent death. The question of alcohol concentration in the body is often raised in the court of law. It is important to estimate the level of alcohol which could cause impairment or lethality. One issue faced by pathologists when performing an autopsy is the inadequate blood sample to measure blood alcohol concentration (BLAC). Most often they are left with a urine sample to interpret the alcohol level in the body. Therefore it is important to understand the relationship between blood and urine alcohol concentrations. This retrospective cross-sectional study was conducted to interpret the relation between BLAC and urine alcohol concentration (UAC). A total of 473 postmortem cases with blood and/or urine samples were sent for alcohol analysis in the year 2016 at Hospital Kuala Lumpur. Total of 229 cases were analyzed for BLAC and UAC. About 2.76% of cases where urine alcohol was detected the blood alcohol was negative. There was a significant average difference between BLAC and UAC (t46 = -4.638, p < 0.001), however both were relatively strong and positively correlated (r = 0.609, p < 0.001). Regression formula could be represented using BLAC = 71.326 + 0.437 (UAC) with r = 0.609. When BLAC or UAS was detected over 50% of the cases were associated with a road traffic collision death. Prevalence of blood alcohol value above the legal limit of 80 mg/100ml was 24.6% amongst all the 142 motor vehicle accident (MVA) cases sent for BLAC analysis. The average ratio of UAC/BLAC for true positive of the determined prevalence was 1.29 ± 0.22 .

Keywords: Post mortem; Alcohol Concentration; Alcohol Ratio; Prevalence

1. INTRODUCTION

The terminology of alcohol throughout this manuscript is referring specifically to ethanol or ethyl alcohol. Alcohol level is always associated with fatal accidents, suicide, drowning, traumatic deaths, and other violent crimes. The legal blood alcohol concentration (BLAC) limit for driving in most of the countries such United Kingdom, United States, Canada and as Malaysia is 0.80 mg/ml or 80 mg/100 ml¹. It is difficult to resolve insurance claims if the person a fatal involved in accident was detected superseding the legal limit for driving. The postmortem interval, the condition of the body, the nature of the collected specimen for analysis, and the environmental conditions including temperature and humidity are important factors to be considered. Some investigators suggest measuring the water content of postmortem blood and if necessary correcting the concentration of ethanol to a mean value of 80% w/w equivalent to the fresh whole blood¹. Contemporary research has concentrated on developing several biochemical tests or markers of postmortem synthesis of ethanol. These include the urinary metabolites of serotonin and nonoxidative metabolites of ethanol e.g. phosphatidylethanol ethyl, glucuronide and fatty acid ethyl esters.

BLACs are regularly tendered as evidence in the criminal and civil trials. The forensic toxicologist is commonly asked to give an expert opinion on the alcohol concentrations measured in postmortem blood during routine work or court testimony. Thus, in order to interpret the detected BLAC correctly, it is suggested that several different specimens should be collected and analyzed for alcohol, such as stomach contents, bile, vitreous humor, cerebrospinal fluid, synovial fluid, inner ear fluid, chest or intra-abdominal fluid and/or urine samples in addition to blood samples especially if decomposition is present. These body fluids are isolated and preserved in different body cavities with firm tissue structures and are subject to less putrefactive changes due to

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bacterial propagation or alcohol diffusion. A good indication of putrefactive processes in tissue is the presence of other C_3 alcohols and especially n-propanol². In comparison with other body organs in badly decomposed bodies, muscletissue is likely the optimal specimen for alcohol analysis due to its low liability for postmortem production of alcohol².

2. LITERATURE REVIEW

In general, there is good traceability and detectability of the markers and ethanol in all matrices 3,4 . In forensic cases, blood and urinary ethanol concentration factors such as the time since last ingestion and multiple-dose ethanol ingestion of over a longer period of time provide an impression of recent consumption⁵. Preserving specimens with sodium fluoride after autopsy inhibits ethanol formation, however production in the body prior to sample collection is a confounding issue. Even though the postmortem levels of ethanol formed are generally low, this may be highly pertinent to cases where alcohol intake was forbidden, such as for pilots, drivers and in countries like Sweden with low legal alcohol limits for driving. Ethanol may be formed as a putrefactive product by a wide range of microorganisms. Ethanol production can be prevented by refrigeration of the body within 4 h after death⁶. Endogenous production does not generally exceed 0.3 g/l if samples are correctly stored. Right cardiac blood has higher ethanol content than in left cardiac blood because of postmortem hepatic glycogenolysis which produces glucose to the right heart via the hepatic veins and the inferior vena cava⁷. The case history, degree of putrefaction, and ethanol levels in different body fluids could be useful to determine whether detected ethanol has originated from the postmortem or antemortem⁶.

In forensic toxicology, femoral venous blood is conventionally considered as the reference medium for measurement of toxic substances. If femoral blood is not available during postmortem, toxicological analyses particularly the blood ethanol measurements could be carried out from subclavian vessels. Ethanol concentrations in subclavian blood were found to be close to those in peripheral blood (p > 0.05) and were not influenced by the degree of putrefaction (r=0.017), gastric ethanol concentration (r=-0.011), inhalation of gastric contents in the airways, or cardiac resuscitation attempts. It was also demonstrated that measurement of postmortem ethanol levels did not significantly differ between both medium of subclavian blood and femoral $blood^8$.

Ethanol can be produced from all the postmortem available substrates during the early stages of putrefaction⁹. Alcohol should be considered as endogenously produced when its concentration of more than 10 mg/100ml is detected in blood or chest fluid vet not detected in vitreous humor or urine2. The significance of low BLAC in autopsy specimens, which less than 30 mg/100 ml, is debatable without supporting evidence from analysis of ethanol in urine and vitreous humor. Gross intoxication caused by heavy drinking might be life threatening in several ways besides the direct ethanol toxicity on the depression of respiratory centers in the brain, which often occurs at BLAC over 400 mg/100 ml. Many drunk drivers have been apprehended with a BLAC over 400 mg/100 ml and a few have exceeded 500 mg/100 ml. The frequency distributions of BLAC in deaths with underlying cause due to acute alcohol were similar mean and median poisoning concentrations were determined at 360 mg/100 ml. During the time after discontinuation of drinking prior to death, the BLAC could be decreased appreciably depending on the alcohol elimination rate from the blood, whereby in heavy drinkers could exceed 20 - 30 $mg/100 ml per h^1$.

After drinking alcoholic beverages, the alcohol is absorbed from the gut into the portal vein where it is subsequently transported to the liver and then to the heart before distributing throughout all body fluids and tissues. The concentrations at equilibrium upon reaching various body organs and tissues depend on their relative water contents and the equilibration rate. The concentration of ethanol in arterial blood is higher than in venous blood at the time when alcohol is being absorbed from the gut. In the post absorptive phase, however, the venous blood contains a slightly higher concentration of ethanol compared to the arterial blood. Comparison between the concentrations of ethanol in different body fluids such as cardiac and femoral blood as well as urine and vitreous humor are virtually essential to make sure that correct diagnosis is given on whether a person was under the influence of alcohol at the particular time of death¹.

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The tubes used to collect and transport blood specimens to the laboratory should contain preservative such as sodium fluoride to ensure a final concentration of 1-2% w/v. Blood and urine specimens collected for determination of volatiles like ethanol should detain a small air-space to minimize its evaporation. The containers should also be made airtight with tamperproof seals and transported to the laboratory under refrigerated condition at about 4 - 8 °C. The average starting BLAC of 175 mg/100 ml drops to 161 mg/100 ml after 12 months storage. The BLAC at autopsy can be converted into the amount of alcohol absorbed and distributed in all body fluids during the time of death using the well-known Widmark equation provided that all the alcohol absorbed from the stomach upon time of death¹.

For various reasons, many people suspected of driving under the influence of alcohol (DUIA) have physiological sampling conducted after a period of time has lapsed after driving. The routine practice of sampling and measuring the BLAC and UAC, calculating urine/blood ratios (UAC/BLAC) and the changes in UAC between two successive voids furnishes useful information to support or challenge alleged drinking after driving. If the UAC/BLAC ratio for the first void was very close to or less than unity at the mean value of 1.04 ranging from 0.54– 1.21 and the UAC also increased by 0.21 g/L (range 0.02–0.57) between the two voids10.

3. OBJECTIVE

3.1 GENERAL OBJECTIVES:

i. To determine the relationship between BLAC and UAC of postmortem samples in Forensic Medicine Department of Hospital Kuala Lumpur.

ii. To determine the prevalence of blood alcohol level above legal driving limit of medicolegal cases in Forensic Medicine Department of Hospital Kuala Lumpur.

3.2 SPECIFIC OBJECTIVES:

i. To determine the difference between UAC and BLAC in postmortem samples

ii. To determine the BLAC at legal limit of 80 mg/100ml in relating to road traffic collision (RTC) death

iii. To determine the ratio of UAC/BLAC in road traffic collision (RTC) cases

4. METHODOLOGY

This was a retrospective cross-sectional study. All postmortem cases with blood and/or urine samples

sent for alcohol analysis in the year 2016 at Forensic Medicine Department of Hospital Kuala Lumpur. Exclusion criteria included decomposed body and age of under 16 years old. All the postmortem cases with blood and/or urine samples sent for alcohol analysis in the year 2016 were retrieved from Forensic Science Database. Blood samples were taken from the heart subclavian area while the urine was withdrawn from urinary bladder. Alcohol levels were recorded retrospectively using Microsoft Excel data collection sheet. Statistical tests. T-test and Pearson Correlation were used to analyze the results. Analysis was further conducted on the cases with road traffic collision deaths in relating the legal limit of UAC and BLAC. The range in ratio of UAC/BLAC was then determined from the true positive of the determined prevalence. No ethical approval was required as this was a retrospection study using postmortem data without any clinical intervention.

5. RESULTS AND DISCUSSION

A total of 473 post mortem cases were selected from year 2016. These cases met the inclusion criteria because the blood sample and / or urine sample had been sent for alcohol analysis to determine the respective blood alcohol concentration (BLAC) and urine alcohol concentration (UAC). Total of 229 cases were analyzed for BLAC and UAC. These were categorized by alcohol level detected or not detected in both blood and urine samples as shown in Table 1. Almost all the cases (47/48) that alcohol could be detected in both blood and urine at the same time. In 2.76% (5/181) of cases, alcohol was detected in urine when blood alcohol was not detected.

Table 1: Correlation of BLAC and UAC detection rate UACDetected

	UAC Detected	UAC Not Detected	Total
BLAC Detected	47	1	48
BLAC Not Detected	5	175	181
Total	52	176	229

A paired-sample T-test was conducted on the 47 cases where both BLAC and UAC were determined. There was a significant difference between BLAC and UAC (t46 = -4.638, p < 0.001), however both were relatively strong and positively correlated (r = 0.609, p < 0.001) as shown in the Statistics Table below.

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Paired Samples Test

		P	aired Diffe	erences		t df	df	Sig. (2- tailed)
	Mean	Std. Deviation	Std. Error	95% Confider of the Diff				
			Mean	Lower	Upper			
Pair 1 BLAC - UAC	-67.894	100.356	14.638	-97.359	-38.428	-4.638	46	.000

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	BLAC & UAC	47	.609	.000

On average, UAC was 67.89 mg/100ml higher than BLAC (95% CI [-97.36, -38.43]). The respective means were 247.40 \pm 125.41 mg/100ml and 179.51 \pm 89.99 mg/100ml as shown in the Statistic Table below. There were 44 cases out of 47 cases (93.6%) where UAC higher than BLAC and both parameters were well-correlated whereby the regression formula could be represented i.e. BLAC = 71.326 + 0.437 (UAC) with r = 0.609 and R2= 0.371 at p < 0.001 as shown in the

Statistic Table below

Paired Samples Statistics

	35	Mean	N	Std. Deviation	Std. Error Mean
D : 4	BLAC	179.51	47	89.990	13.126
Pair 1	UAC	247.40	47	125.414	18.294

Coefficientsa

	Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		В	Std. Error	Beta		
1	(Constant)	71.326	23.472		3.039	.004
	UAC	.437	.085	.609	5.156	.000

Subsequently, those cases were divided into RTC and non-RTC categories and further analyzed on the BLAC and UAC respectively. Over 50% of the cases where BLAC or UAC were detected relating to the road traffic collision death as shown in the Table 2 and 3.

Table 2: BLAC detection rate based on RTC and non-RTC cases

	RTC	Non-RTC	Total
BLAC Detected	41	40	81
BLAC Not Detected	101	290	391
otal	142	330	472

Table 3: UAC detection rate based on RTC and non-RTCcases

	RTC	Non-RTC	Total
UAC Detected	28	24	52
UAC Not Detected	57	121	178
Total	85	145	230

Taking into consideration of the legal blood alcohol limit for driving in Malaysia was set at 80 mg/100ml.

Prevalence of blood alcohol value above the legal limit of 80 mg/100ml was 24.6% (35/142) amongst all 142 RTC cases sent for BLAC analysis. A total of 35 out of 60 cases analyzed (58.3%) based on concentration > 80 mg/100ml were related to the road traffic collision death. In addition, there were only 6 cases (28.6%) not related to road traffic collision death from a total of 21 analyzed cases where concentration < 80 mg/100ml with alcohol level detectable.

Table 4: Alcohol concentration related to RTC death

	RTC	Non-RTC	Total
> 80 mg/100ml	35	25	60
1 - 80 mg/100ml	6	15	21
Total	41	40	81

The ratio of UAC/BLAC were further analyzed within the true positive (35 cases) and false negative (6 cases) based on the legal limit. The average ratio of UAC/BLAC for true positive was 1.29 ± 0.22 while for true negative was 1.95 ± 0.03 . The ratio value for true positive cases indicated that the alcohol has partially excreted during the death incident while false negative cases indicated relatively high UAC/BLAC ratio and hence higher excretion rate. The limitation of this study was those brought in dead cases through emergency department might involve massive blood loss or transfusion and affecting the interpretation of alcohol levels.

6. CONCLUSION

A total of 473 post mortem cases were selected based on inclusion criteria from year 2016. Total of 229 cases where both blood alcohol concentration (BLAC) and urine alcohol concentration (UAC) were analyzed. There was a significant average difference between BLAC and UAC (t46 = -4.638, p < 0.001), however both were relatively strong and positively correlated (r = 0.609, p < 0.001). Regression formula could be represented using BLAC = 71.326 + 0.437 (UAC) with r = 0.609. Mean of UAC (247.40 ± 125.41 mg/100ml) was higher than that of BLAC ($179.51 \pm 89.99 \text{ mg}/100 \text{ml}$). Over 50% of the cases where BLAC or UAC were detected relating to the road traffic collision death. Prevalence of blood alcohol value above legal limit set at 80 mg/100ml was 24.6% amongst the all the 142 road traffic collision cases sent for BLAC analysis. About 58.3% of the cases analyzed based on concentration > 80 mg/100ml were related with the

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road traffic collision death. The average ratio of UAC/BLAC for true positive of the determined prevalence was 1.29 ± 0.22 .

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