

Evaluation of Ethanol and N-propanol in Victims

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ABSTRACT

Background: Blood alcohol concentration is a major factor in forensic analysis in criminal and civil litigation. Along with ethanol, other volatile compounds such as n-propanol are produced as products of putrefaction. The aim of this study is the evaluation of ethanol and n-propanol in postmortem blood.

Methods: A case-control study was done on 300 blood samples obtained from cadavers that were referred to Legal Medicine Organization (LMO) of Tehran, Iran, over a year (2009-2010). Blood ethanol and n-propanol concentrations were analyzed in duplicate by HSGC. Data on cadavers were taken from the information sheets and were analyzed by SPSS software.

Results: In this study, mean n-propanol level in blood was 8.311 mg/dl in putrefied and 1.58 mg/dl in non-putrefied victims. Interval times between death and autopsy in putrefied and non-putrefied victims were 3.5 and 1.25 days. Results show that n-propanol concentrations increase 1.23 mg/dl/day after death. In 99% of putrefied victims, ethanol level was more than 10 mg/dl. There was a high correlation between ethanol and n-propanol concentrations. By increasing 1mg/dl of n-propanol concentration, ethanol levels increased 2.5 and 1.4 mg/dl in putrefied and non-putrefied victims, respectively.

Conclusion: N-propanol produced in putrefied victims with ethanol. Production of ethanol increased by rising n-propanol levels. Therefore, we might use n-propanol as a putrefaction factor in postmortem blood to distinguish endogenous and exogenous ethanol production.

Keywords: Blood, Ethanol, N-propanol, Putrefaction

INTRODUCTION

Blood alcohol concentration is an important factor in forensic analysis in the criminal and civil litigation. The interpretation of the analytical results obtained from autopsy materials has difficulties because of production of ethanol due to putrefaction, evaporation of ethanol, and lack of homogeneity of blood samples.

Studies on ethanol production due to putrefaction phenomenon are controversial. Several researches have reported an increase in ethanol levels due to production by bacteria, yeasts, and fungi (1). Alcohol production may occur either in the intact body between death and autopsy or in body fluids, especially blood collected at autopsy (2).

Immediately after death, endogenous microorganisms of body distribute quickly into the body fluids and tissues. This phenomenon leads to some differences in measurement of ethanol concentration after death (3,4). High ambient temperatures after death, ante mortem hyperglycemia, septicemia, and sever trauma with wound contamination provide particularly suitable conditions for ethanol synthesis (5).

Postmortem ethanol synthesis was first discussed in 1936. In 1978, Corry composed a comprehensive review of microbiological research on postmortem ethanol synthesis (6,8).

Interpretation of postmortem ethanol results is very important because it is often difficult to distinguish between postmortem

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ethanol production and ante mortem alcohol ingestion (4,6)

During putrefaction, other volatile compounds, such as n-propanol, isopropanol, butanol, and so on are produced along with ethanol (8,10). The existence of these volatiles can be used as a marker of postmortem ethanol synthesis because these compounds do not exist naturally in living organisms at the detection limits of routine ethanol analysis (9,11).

METHOD AND MATERIALS

A case-control study was done on 300 blood samples obtained from cadavers that were referred to Legal Medicine Organization (LMO) of Tehran, Iran during one year starting in March 2009. 150 samples were n-propanol positive and the rest were n-propanol negative.

Peripheral blood samples were collected from victims via aseptic technique. Sodium fluoride was added, and the samples were stored in refrigerator at 4 °C.

Chemicals

Ethanol and n-propanol used as calibrator and isobutanol in a aqueous $(\text{NH}_4)_2 \text{SO}_4$ solution as internal standard were purchased from Merck Chemical Company (Germany) in high purity and used without further putrefaction.

Instrumentation

Analytic separation was achieved using Agilent 6890N headspace gas chromatography (HS-GC). Headspace vials were purchased from Agilent Company (USA). The column used here was DB-ALC1 (30m \times 320 μm \times 1.8 μm). The GC was fitted with an Agilent headspace GC injection system that allowed for automated sample pretreatment and injection.

The sample volume was set to 1000 μlit and got incubated at 60°C for 15 min prior to injection. The syringe was heated to 60°C. Loop and transfer lines were set at 140°C. Identification and quantification of analytes were accomplished using a flame ionization

detector (FID) and the GC injector and detector temperature were set at 150°C and 300°C

Several studies have been conducted on postmortem synthesis of ethanol in order to clarify the significance of other putrefactive products (8,11,14). The aim of this study was to evaluate n-propanol production, as a putrefactive factor in postmortem blood and its effect on the interpretation of alcohol analysis results and the quantitative relationship between n-propanol and ethanol in putrefied and non-putrefied victims.

respectively. The GC oven was held at 35 °C for 5 min. Helium was used as the GC carrier gas at a flow rate of 4.9ml/min and 18.55 PSI pressure. The gases required to operate the FID were hydrogen and compressed air with 35 and 250 ml/min flow rates, respectively. The Agilent system software (Chemstation) integrated all peaks in a chromatogram. Peak area was used for the quantification of each analyte of interest from a chromatogram. The retention times for ethanol and other volatiles were established following the analysis of a prepared blood calibrator.

Analytical parameters

The limit of detection (LOD) and the limit of quantification (LOQ) were determined for the detection of ethanol and n-propanol. LOD and LOQ were 0.1 and 1mg/dl for ethanol and 0.15 mg/dl and 1.5 mg/dl for n-propanol, respectively. Data such as time and place of death, and autopsy findings were collected from information sheets and were analyzed by SPSS software. Inclusion criteria were the samples from healthy and putrefied cadavers, whereas exclusion criteria were cases with a history of alcohol consumption or peritonitis.

Statistical analysis

Mean analysis comparison was performed on n-propanol concentrations in putrefied and non-putrefied groups. Linear regression analysis was utilized for evaluating the effect of time after death, controlled for sex and age, on n-propanol concentrations while logistic regression analysis was used for assessing the effect of putrefaction on n-

propanol concentrations controlled for age and sex.

RESULTS

The 150 blood samples obtained from n-propanol positive cadavers, 128 men (85.33%) and 22 women (14.66%), were analyzed during this one-year study (2009–2010). The mean age for putrefied cadavers was 37.9 ± 1.45 years (Mean \pm SD).

Of the 150 blood samples obtained from non-putrefied cadavers, 120 (80%) were men and 30 (20%) were women. The mean age in this group was 36.4 ± 1.1 years. The mean concentrations of n-propanol in putrefied and non-putrefied samples were 8.311 ± 1.05 mg/dl and 1.58 ± 0.379 mg/dl, respectively ($P = 0.0001$).

The mean interval time between death and autopsy in putrefied cases was 3.5 days (CI 0.95% = 3–4 days) while minimum and maximum days were 0.75 day and 14 days, respectively. The mean interval time in non-putrefied cases was 1.25 days (CI 0.95% = 1.15–1.38 days). Table 1 summarizes mean time, standard deviation (SD), and standard error of measurement (SE) in the two groups. The distribution of the place of death according to the situation of cadavers is shown in Table 2.

Table 3 demonstrates the linear regression of n-propanol concentrations versus interval time after death by controlling sex, age, place of death, and situation of cadavers. Results showed that in all cadavers, the concentration of n-propanol increased 1.23 mg/dl each day after death controlling the age and sex, whereas in putrefied cadavers, n-propanol concentration is 4.58 mg/dl higher than non-putrefied cadavers.

The odd ratio of putrefaction and production of n-propanol are shown in Table 4. Statistical analysis (logistic regression) shows that putrefaction might produce production of n-propanol in blood 300 times more than the non-putrefied cadavers.

In putrefied specimens, ethanol was detected in all of the samples and the mean concentration of ethanol was 81.42 ± 5.6 mg/dl. In non-putrefied specimens, however, the mean concentration of ethanol was equal to

23.96 ± 3.22 mg/dl. In 54.2% of cadavers, ethanol was not detected and in 57.9% of those with ethanol, ethanol concentration was less than 10 mg/dl. Table 5 shows the coefficients for linear regression of ethanol concentration on n-propane concentrations by controlling sex, age, and place of cadavers.

Results indicated that by a 1 mg/dl increase in n-propanol concentration, ethanol increased to 2.68 mg/dl. In putrefied cadavers, an appropriate correlation was seen between ethanol and n-propanol concentrations and by increasing 1 mg/dl in n-propanol concentration, ethanol increased to 2.5 mg/dl.

In non-putrefied samples, on the other hand, for each 1 mg/dl increase in n-propanol concentration, ethanol concentration increased as much as 1.4 mg/dl. Distribution of n-propanol situation according to ethanol situation is shown in Table 6.

DISCUSSION

Alcohol is a major CNS depressant and over-consumption of alcoholic beverages always plays an important role in fatal accidents, death due to trauma, drowning, suicide, and many crimes due to violence as documented by police reports, accident, and emergency department records (15,17). In forensic and legal medical practice, distinguishing antemortem ingestion from postmortem synthesis of ethanol is sometimes vital (2).

Accurate interpretation of blood ethanol concentration at the time of death presents a difficult task since the origin of detected ethanol in postmortem cases may vary (2,6,7). It has been reported that ethanol could be produced postmortem in variable and non-predictable amounts, as a function of the type and number of microorganisms present either in corpses or specimens collected at autopsy (2,18). Many forensic pathologists look on n-propanol as a good index of putrefaction and postmortem ethanol production by microorganisms (18,19).

One assumption is that the postmortem ethanol concentration is about 20 times higher than the n-propanol production (20). Postmortem ethanol production in corpses was

first reported in 1936 by Nicloux and Wagner (21).

A good indication of putrefactive processes in tissue is the presence of other C₃ alcohols, especially n-propanol. Detection of n-propanol in blood or tissue can distinguish between cases in which ethanol was formed postmortem or those in which it was present antemortem (1,2,22). The ante mortem ethanol production in the intra abdominal body fluid of victim might have been caused by microorganisms (11).

Many researchers have focused their attention on n-propanol as an indicator of putrefaction, but the quantitative relationship between the amount of n-propanol detected and the amount of ethanol produced in postmortem blood has not been strong. Other studies have shown that the ratio of ethanol concentrations to n-propanol is 10:1 in muscle and less than 20:1 in blood (8,23).

Morya and Hashimoto (2004) and Vassiliki (2007) considered n-propanol as the volatile most correlated with microbial postmortem ethanol production (2,9).

N-propanol was detected along with ethanol, in the intra-abdominal body fluid, urine, and gastric content of living persons suffering from peritonitis, thus supporting the idea that n-propanol could monitor microbial activity (11).

In our investigation, n-propanol concentration in putrefied and non-putrefied corpses was significantly different ($P=0.0001$) and these results corroborated the results from other studies (8,11,24). Results showed that in 0.7% of putrefied cadavers, n-propanol was not detected while it was found in 24.4% of non-putrefied samples. The existence of n-propanol in non-putrefied specimens might be due to storage of the samples in nonstandard conditions.

Collection of samples should be performed as early as possible after death in order to minimize the possibility of microbial growth in the corpse. The proper handling of the collected samples should include the immediate addition of proper amounts of the preservatives and, finally, storage of the sample at a temperature close to 0°C (5,25).

Here the relationship between interval time after death and n-propanol concentration was studied. Our results are consistent with most of the studies performed by Kupice and Huffine and it is concluded that by increasing this time, n-propanol concentration increases ($P=0.001$) (CI=0.53-1.93) (20).

In putrefied specimens, the mean interval time between death and autopsy was 3.5 days, whereas in non-putrefied samples, this was 1.25 days. Our analysis showed that the n-propanol concentration increased 1.23 mg/dl/day in two groups. Moriga et al studied 29 brain tissues of drowned victims and found a significant correlation between putrefaction and n-propanol concentration (9).

In our study, results show that sex and age do not have a significant effect on n-propanol production and this is supported by other studies (1,9,11). Also, it was revealed that the number of putrefied cadavers in humid places is more than non-putrefied cadavers (17.2% vs. 4.6%). Therefore, humidity might play a role in the progression of putrefaction process after death, but according to our study, this progression does not have a significant effect on the concentration of n-propanol ($P=0.92$).

Also, the results showed that putrefaction can cause an increase in n-propanol concentration 300 times more than non-putrefied cases. But it does not mean that the existence of n-propanol is certainly due to putrefaction.

Schuberth (2007) demonstrated that during the microbial synthesis of ethanol, other low-molecular volatiles are generated in blood and tissues that include higher aliphatic alcohols (isoamyl alcohol, n-propanol, and n-butanol) as well as other organic acids (15,18,26). Other researchers reported finding n-propanol as a product of putrefaction in postmortem blood in concentrations about 0.03-0.07 g/l (1,24,26,18).

The ratio of ethanol to n-propanol concentration has previously been determined in order to verify the existence of postmortem ethanol and n-propanol production. Moriya and Hashimoto showed that endogenous ethanol concentration to n-propanol is less than 10:1 in muscles and less than 20:1 in blood (9).

Lewis et al and Skopp (2004) showed that the quantitative relationship between volatiles production in postmortem specimens still remains obscure (5,13). Felby and Nielsen also reported a quantitative relationship between postmortem production of ethanol and n-propanol. They found that ethanol concentration increased 0.001% with a 0.0·01% increase in the n-propanol concentration in 62 postmortem cases (27).

In our study, by increasing 1mg/dl in n-propanol concentration, ethanol concentration increased about 2.5 and 1.4mg/dl in putrefied and non-putrefied cases, respectively.

Further attempts to show other indicators of postmortem ethanol formation have led to the identification of other bio-molecules and the realization that n-propanol are not a specific marker indicative of postmortem ethanol production. We suggest utilization of other markers such as ethyl glucuronide (a minor metabolite of ethanol), fatty acid ethyl esters, and methylesters in the interpretation of postmortem ethanol analysis results to differentiate between endogenous and exogenous ethanol.

The presence or absence of ethanol cannot be ascertained with a high degree of accuracy, precision, and selectivity using C3 alcohols such as n-propanol. Due to endogenous ethanol production in the absence of n-propanol, using other specific markers is necessary.

Also, other unconventional samples (such as brain) could be analyzed qualitatively and quantitatively to achieve reliable results in postmortem ethanol analysis and interpretation of results.

REFERENCES

- O'Neal CL and Poklis A. Postmortem production of ethanol and factors that influence interpretation. A critical review. *Am J Forensic Med Pathol.* 1996;17: 8-20.
- Kallirroe Z, Vassiliki A. Insights into the origin of postmortem ethanol. *Int J of toxicol.* 2005; 24:69-77.
- Gulle JP, Deveaux M. Judicial expertise in blood alcohol determination and toxicological analysis. *Ann Biol Clin.* 2003; 61: 269-274.
- Butzbach D.M. The influence of putrefaction and sample storage on post-mortem toxicology results. *Forensic Sci Med Pathol.* 2010; 6: 35-45.
- Skopp G. Preanalytic aspects in postmortem toxicology. *Forensic Sci Int.* 2004;142: 75-100.
- Corry JE. Possible sources of ethanol ante- and postmortem: its relationship to the biochemistry and microbiology of decomposition. *J Appl Bacteriol.* 1978; 44: 1-56
- Johnson RD, Lewis RJ, Blank DL. Accurate assignment of ethanol origin in postmortem urine: Liquid chromatography-mass spectrometric determination of serotonin metabolites. *J chromatogr B.* 2004; 805: 223-234.
- Gudrun H, Ritva K. A study of ethylglucuronide in postmortem blood as a marker of ante mortem ingestion of alcohol. *Forensic Sci Int.* 2007; 165:41-5.
- Moriga F, Hashimoto. Postmortem production of ethanol and propanol in the brain of drowned persons. *American Journal F Medical Pathol.* 2004; 25:131-133.
- Moffat AC. Clarke's analysis of drugs and poisons. The pharmaceutical press. London. 2004.
- Moryia, F and Ishizu H. Can microorganisms produce alcohol in body cavities of a living persons? A case report. *J Forensic Sci.* 1994; 39: 883-888.
- vassiliki A, Kallirroe S. Biochemical pathways generating postmortem volatile compounds co-detected during forensic ethanol analysis. *Forensic Sci Int.* 2008; 174:133-151.
- Lewis RJ, Johnson RD, Angier MK. Ethanol formation in unadulterated postmortem tissue. *Forensic Sci Int.* 2004;146:17-24
- Amick G, Habben KH. Inhibition of ethanol production by *saccharomyces cerevisiae* in human blood by NaF. *J Forensic Sci.* 1997; 42:690-692.
- Bilban M, Skibin L. Presence Of alcohol in suicide victims. *Forensic Sci In.* 2005; 147:S9-S12.
- Fredrik C, Kugelberg, Alan wayne Jones. Interpreting results of ethanol analysis in

- postmortem specimens A review of the literature .Forensic Science International. 2007; 165:10-29.
17. Martins DE, Braga A. Alcohol distribution in different postmortem. Human and Experimental Toxicology.2006; 25: 93-97.
 18. 18- Yajama d,Motani H,Kamei K. Ethanol production by candida albicans in postmortem human blood samples. Effects of blood glucose levels and dilution. For Sci Int.2006; 164:116-201.
 19. 19- Toskos M. Neogenesis of ethanol and fusel oils in putrefing blood . Forensic Pathology review.2006; 4: 205-259.
 20. 20-Canfield Dv, Kupiec VT, Huffine E. Postmortem alcohol Production in fetal aircraft accident. J Forensic Sci. 1993; 38:914-917.
 21. 21-Nicloux M Wagner. Neoformation alcool ethlique dan le cadaver humain en voie de putrefaction. Compte rendu des seances de biologie paris 121:975-978.
 22. 22-Athanselis S, Stefandio M. 2005. Interpretation of postmortem alcohol concentrations. Forensic Sci Int.1936; 149:289-291.
 23. 23-Nanikawa K, Ameno Y, Hashimoto and Hamda K. Medicolegal studies on alcohol detected in dead bodies- alcohol levels in skeletal muscle. Forensic Sci Int.1982; 20 :133-140.
 24. 24- Jonson RD, Russell J, Mike K. Formation of ethanol in postmortem tissues. Office of Aerospace Medicine Washington. 2004 ;1-10
 25. 25- Olsent, Hearn WL. Stability of ethanol in postmortem blood and vitreous humor in long-term refrigerated storage J Anal Toxicol.2003; 27: 517-9.
 26. 26- Schuberth J. Volatile compounds detected in blood land drunk drivers by headspace-capillary gas chromatography .Biological Mass Spectrometry.2007; 2:699-702.
 27. 27- Felby S, Nielsen E. Postmortem blood alcohol concentration. Blutalkohol.1993; 30: 244-250.
 28. 28- O'Block R. Considerations for the analysis of forensic samples following extended exposure to the environment. The Forensic Examiner Articles. May 19: 2010.http://WWW.Acfei.com/about_acfei/aa/