Importance of Sampling Sites for Postmortem Evaluation of Ethyl Alcohol
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Abstract
Detection of ethyl alcohol and its origin in postmortem specimens is essential in terms of medico-legal aspects. It is a complicated process to determine whether alcohol has been taken in the ante-mortem period and/or originates from postmortem endogenous production.

In this study, considering sampling sites and storage conditions, we aimed to develop an approach for postmortem ethyl alcohol investigations. Samples were collected from 32 cases. Blood specimens drained from the femoral vein and the vena cava inferior were put into well covered PET tubes with anti-coagulants and urine samples were put into well covered PET tubes without anti-coagulants and preserved at +4°C, analyzed with enzymatic immunoassay within five days of specimen collection.

Scene investigations, sampling and sampling sites, specimen handling, preserving specimens and preparations before analyses are highly important for an accurate and scientific evaluation of postmortem ethyl alcohol. There were significant differences in ethyl alcohol concentrations between blood from the femoral vein and vena cava inferior and urine. Femoral vein and urine specimens seemed to be more reliable than vena cava inferior specimens.

Keywords: Postmortem ethyl alcohol; Forensic toxicology; Sampling sites

Introduction
The determination of ethyl alcohol and its origin in postmortem specimens are essential for medicolegal purposes [1-4]. It is well known that researching whether the determined alcohol origin belongs to the ante-mortem period or not is difficult due to the many kinds of both endogenous and exogenous factors in the postmortem period [5-8]. Blood ethanol concentrations in decomposed bodies may show drinking during life and/or endogenous production after death [8-12].

In this study, we aimed to develop an approach for the evaluation of postmortem ethanol based on the data about sampling sites and storage conditions.

Material and Methods
We investigated the effects of different causes of death, handling medical history, postmortem interval, gastric contents, trauma to the abdominal cavity and thoracic organs and decomposition stages on postmortem ethanol concentrations in different sampling sites. We also compared ethanol concentrations in the vena cava inferior closer to the heart, the femoral vein and urine and determined whether urine and central and peripheral blood alcohol concentrations collected from different sites were correlated.

Blood specimens from the femoral vein and vena cava inferior and urine specimens were collected from a series of 32 medicolegal autopsies performed in Morgue Department of Council of Forensic Medicine, Izmir, in Turkey. All cases were examined; scene investigations, histopathological studies and toxicological analyses were performed and medical records, external and internal findings, and history of events were reviewed. Blood and urine samples were aspirated by disposable sterile syringes as in the following: first, femoral venous blood was aspirated under direct visualization. Second, the vena cava inferior closer to the heart was punctured and a sample of blood was aspirated. Third, the bladder was punctured under direct visualization and urine was aspirated. Blood samples were put into well covered (purple lid) 9 mL Polyethylene Terephthalate (PET) tubes which containing EDTA. Urine samples were put into well covered (red lid) 9 mL PET tubes. Sodium Fluoride (NAF) or any other preservative was not used for preventing bias on immunoassay method and also samples were analyzed immediately at +4°C. Trichloroacetic acid (6% solution) was used for preanalytic process. Ethanol was measured with enzymatic immunoassay (912 Hitachi auto-analyzer) by using DRI ethyl alcohol assay, calibrators and controls. Sensitivity of the method was high for ethanol levels ranging between 10 mg/dL and 600 mg/dL.

Data were analyzed with SPSS for Windows 11.0. Non parametric test (Friedman Variance Analyze) was used to test the difference between the specimens and “t” test (Wilcoxon test and Bonferroni Correction) was used to determine which specimen was different. The study was approved by Ethics Committee of Dokuz Eylul University and Council of Forensic Medicine.

Results
The mean postmortem vena cava inferior ethanol concentration [VCEC] was 51.1 ± 66.7 mg/dL, ranging from 0 to 253 mg/dL, the mean femoral blood ethanol concentration [FBEC] was 39.8 ± 50.4 mg/dL, ranging between 0 and 195 mg/dL and the mean urine ethanol concentration was 21.1 ± 28.1 mg/dL, ranging between 0 and 131 mg/dL.

There was a marked increase in ethanol concentration in vena cava inferior blood samples compared with femoral vein blood samples in all cases (ante-mortem alcohol ingestion history prior to death or

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decomposition) ("t" test, p=0.001). [VCEC] and [FVEC] were found significantly and strongly correlated (r=0.88, p<0.005).

The effects of different causes of death, handling medical history, postmortem interval and gastric contents on the mean [VCEC], [FVEC] and [UEC] are shown in Figures 1,2.

Of 32 cases in the study, 7 were mildly decomposed and 8 were markedly decomposed. Figure 3 shows the effect of decomposition on the mean blood and urine ethanol concentrations.

Effects of decomposition on the mean blood and urine ethanol concentrations were found statistically significant (p=0.013). [VCEC] was found significantly higher than [FVEC] and [UEC] ("t" test, p values respectively 0.018, 0.012).

Discussion and Conclusion

In fact, femoral vein and urine specimens seemed to be more reliable than vena cava inferior specimens since ethanol concentrations closer to the heart, significantly increased compared to the peripheric vein blood and urine ethanol concentrations. This can be explained by diffusion of ethanol from the intact stomach into the vena cava inferior blood in the chest cavity during the interval between death and autopsy, postmortem redistribution, ingestion of a large quantity of ethanol shortly before death and postmortem endogenous production of ethanol. There have other been studies showing the site-dependence of postmortem ethanol levels in blood due to postmortem redistribution, consistent with the results of the present study [3,6,9,13-15].

In addition, urine sampling was found to be a reliable and accurate alternative to blood sampling for alcohol determination in this study consistent with other reports [2,16,17].

In addition to considering sampling sites, we thought that postmortem formation of ethanol may have been due to the presence of bacteria in tubes and an absence of sodium fluoride as a preservative added to the tubes [2,7,17,18]. Also the method of enzymatic immuno assay could be an appropriate way to determine the levels of ethanol [19-20].

Considering the factors such as cost and the time, we have reached to a conclusion that selecting specimen from different sample sites, the appreciate volume of the specimen, preserving samples (at + 4°C) in the well covered tubes within maximum one week and analyzing by the enzymatic method will be a good practice. We suggest that for a reliable estimation of premortem ethanol levels, femoral venous blood and urine could be used for ethanol analysis in cadavers. A close cooperation between the pathologist, forensic medicine specialist and toxicologist is also needed.

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