A comparative study on production of ethyl and methyl alcohol in postmortem specimens (blood, brain tissue and muscle)

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ABSTRACT

This study was designed to determine the amounts of ethyl and methyl alcohol in postmortem tissues, whether due to consumption or as fermentation products, over the period between death, collection of samples and analyses. Three variables were considered throughout the storage, namely: temperature, time and environment. The main objective of the study is to misinterpretation of results when the detected ethyl alcohol was due to causes other than actual consumption. Such misinterpretation would bear on legal consequences in Muslim courts as alcohol consumption is absolutely forbidden in Islam.

Key words: brain, muscle, ethanol, methanol, fermentation, storage, GC-HS/MS.

INTRODUCTION

Ethanol production in postmortem tissue samples was dealt with in several research publications. The microbial formation of ethanol in postmortem specimens is the most significant problem encountered when evaluating ethanol results. The postmortem formation of ethanol was first reported in 1936 [1]. The first studies to light up the mechanism of postmortem ethanol production were carried out in the early 1970s [2, 3]. Many species of bacteria, yeast and fungi produce ethanol and other volatile organic compounds as a byproduct of their metabolism [4-12]. They may be introduced from the environment in favorable circumstances, particularly in violent deaths. With a speed relative to environmental factors, endogenous and or exogenous microbes start consuming glucose and other nutrients present in the dead body and produce ethanol and or other organic volatiles as metabolic byproducts. Under optimal conditions, substantial concentrations of ethanol may be formed within hours of death [13]. The relatively short time required for microbes to begin producing ethanol complicates the interpretation of a positive ethanol result in postmortem specimens. The potential for postmortem ethanol formation is disquieting when considering that a positive ethanol result may help discern the cause of an aviation accident. Therefore, the preservation of biological specimens to minimize postmortem ethanol formation has been a priority for nearly 30 years. Storage temperature and duration play an important role in the microbial formation of ethanol [13-15]. This study is designed to observe the formation of alcohol in three group of

postmortem bodies, bodies with high blood alcohol concentration (BAC), low BAC and group of no alcohol consumption (BAC zero). The types of containers used, storage temperatures and duration of storage were all taken in consideration.

MATERIALS AND METHODS

Study design

Seven autopsy cases were selected, and separated into 3 groups. Four cases were negative for alcohol, the other three were positive, two of them showed high blood alcohol concentration (BAC). Negative cases were selected to observe postmortem production of alcohol (ethanol) and its increasing rate. The positive cases were used to see the mixed effects of alcohol degradation and/or production due to micro-organisms and environmental effects.

Specimen collection

Blood, brain and muscle tissues from each of the 3 groups were collected and portioned into different containers:
Type A: Plastic containers (Sterlin®) filed with N2 gas (anaerobic conditions).
Type B: 20 ml headspace glass vials with aluminum caps (aerobic conditions).
Samples were further subdivided into four groups, stored respectively at room temperature (RT, 26 °C), refrigerator (RF, 8 °C), out door and/or freezer (- 40 °C).
Analyses were carried out after 24, 48, 72 h respectively.

Sample preparation

0.1 ml of the blood sample or 100 mg of the tissues were placed into a 20 ml headspace vial and mixed with 1 ml t-butanol in water as internal standard. All samples were analyzed by GC-headspace.

Instrument

Headspace GC model 6890 N (Agilent-Technologies) equipped with a FID and a capillary column HP-DP1 for determination of ethyl and methyl alcohol and coupled with a capillary column HP- 5MS to a MSD for the identification of unknown volatiles.

RESULTS AND DISCUSSION

Evaluation and validation of the analytical method

Linearity tests for ethanol and methanol were carried out using 6 levels to cover the range between 5 and 300 mg/dl.

The calibration curves were linear (r= 0.999 and 0.998) for ethanol and methanol respectively.The precision test: two ethanol levels were selected (50 and 200 mg/dl) to determine the repeatability and reproducibility. The results revealed a CV% range between 0.344-1.17%. The LOD was measured to be < 3.5 mg/dl.

Results and toxicological assessment of this study

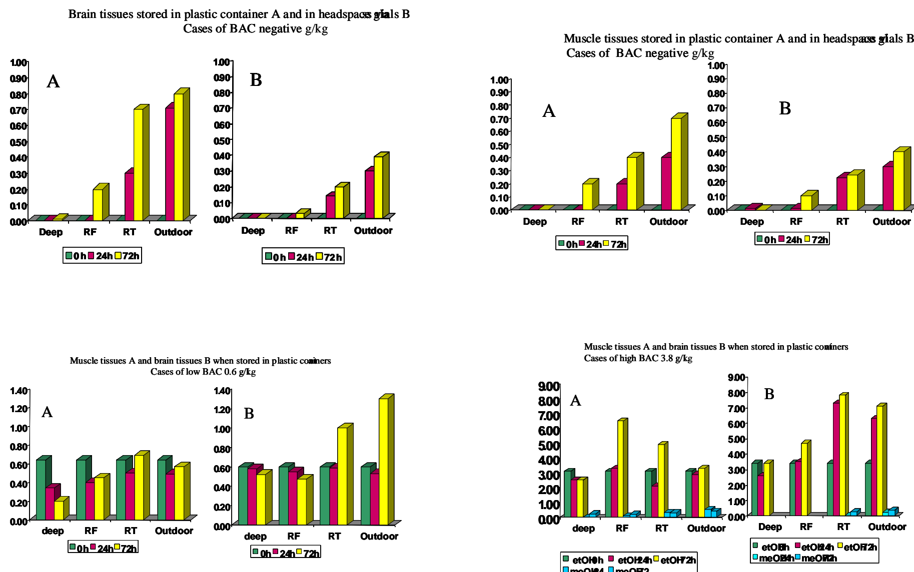
Result (1): Alcohol negative samples

Ethanol was not detected for 48 h when the samples were stored in a refrigerator at 8 ºC and for 72 h under aerobic conditions (glass vials). We observed the production of ethanol (0.2 g/kg) in the samples stored under nitrogen in plastic containers for 72 h under RF conditions. Ethanol even increased to 0.8 g/kg when the samples were stored at room temperature (RT) or at out door temperatures (ODT). No ethanol was detected in both systems when stored in a freezer at -40 °C.

Result (2): Alcohol positive samples

In the positive cases we sometimes observed a loss of ethanol, especially when stored in plastic containers in the freezer, or an important increase especially when stored at RT or ODT. In one positive case with a BAC 3.5 g/kg we observed a peak concentration of 7.8 g/kg in the brain tissue after 72 h at RT. Methanol was detected in all samples at low concentrations. Other fermentation products including alcohols, volatiles and sulfates derivatives were detected and identified using the headspace GC/MSD.

The following diagrams show an overview of alcohol production in a negative alcohol autopsy and the changes in ethanol concentration in the positive cases during storage.



CONCLUSIONS

Ethyl alcohol may be detected in the postmortem material even if there is no history of alcohol consumption. On the other hand, a loss of ethanol can be observed during storage in plastic containers or because of microbiological degradation. Our results show the necessity to standardize the sample collection and storage procedures for the determination of alcohol in postmortem toxicology. Till then we recommend to analyze different tissues taken during the autopsy and to store them in the freezer in glass vials. If the alcohol concentrations in different samples from a certain case show a high variability, great caution must be exercised when interpreting of results.

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