Detection of Alcohol in Different Post-Mortem Samples

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Abstract:

Alcohol detection in post-mortem cases becomes more important at the present time due to the increase in the number of alcohol related fatalities in the world. Toxicological analysis can give a good idea about the presence and levels of alcohol in different post-mortem samples. Drug and blood chemical level data for more than 200 drugs including alcohol is published [1]. This data is used to differentiate between the therapeutic, toxic and lethal drug concentrations mainly in the blood. However, the results are difficult to interpret when compared with this data because the latter does not mention the site of the post-mortem collection of the blood sample. In general, the preferable sample for alcohol detection in forensic cases is the femoral blood but this sample may be difficult to obtain in many situations (e.g. stabbing, sever haemorrhage and trauma). Interpretation of alcohol results in blood samples obtained from body sites other than the femoral vein represents a practical problem. In this study, about 66 post-mortem fluid samples representing femoral blood, cardiac blood, urine, vitreous humour, bile and stomach contents were analysed and findings were compared. Correlation between alcohol concentrations in the femoral blood and other samples was made by regression study. It was found that values of the minimum and maximum concentrations were respectively: 4mg/dl, 365 mg/dl for the femoral blood (FB), 16mg/dl and 323mg/dl for the cardiac blood (CB), 4mg/dl and 346 mg/dl for the vitreous humour (VH), 77mg/dl and 920 mg/dl for the urine (U), 65 mg/dl and 241 mg/dl for the bile and 40mg/dl and
490mg/dl for the stomach contents. There were significant correlations between CB and FB and VH and FB alcohol levels. The mean FB/CB ethanol ratio was 0.56, \( r^2 = 0.65 \) and \( p = 0.005 \), the mean FB/VH ethanol ratio was 0.80, \( r^2 = 0.85 \) and \( p = 0.009 \). There were no significant correlations between the femoral blood and the urine, bile and stomach contents where the mean FB/U ethanol ratio was 0.23, \( r^2 = 0.003 \) and \( p = 0.91 \), the mean FB/Bile ethanol ratio was 0.37, \( r^2 = 0.096 \) and \( p = 0.55 \) and the mean FB/Stomach ethanol ratio was 0.58, \( r^2 = 0.048 \) and \( p = 0.52 \). These results suggest that vitreous humour is the sample of choice for the interpretation of alcohol results in post-mortem cases when femoral blood sample is not available.
Introduction:

Ethanol (alcohol) is a central nervous system depressant. Alcohol is the most commonly found drug in post-mortem cases. According to National Statistics Online, alcohol-related death in UK increased from 6.9 per 100,000 populations in 1991 to 12.9 in 2005. Alcohol-related death per 100,000 cases is higher in male than female being 17.9 and 8.3 respectively. The highest age group in both male and female is 55-74 (National Statistics, 2006). The toxic blood level for ethanol is 80 mg/dl or 107 mg/dl in urine in UK and USA based on the legal driving limit. Ethanol is taken orally and most of its metabolism (90%) occurs in the liver while the remaining (10%) occurs in other extra-hepatic tissues such as stomach, intestine, kidneys and lungs. Alcohol absorption takes place in the stomach and small intestines. Bioavailability of ethanol after oral administration is around 80% which increases by increasing ethanol uptake (dose dependent). Blood ethanol levels greater than 300mg/100mls are usually associated with fatalities [1]. Blood ethanol levels of up to 190mg/100mls may occur due to post-mortem bacterial metabolism depending on the degree of body decomposition or disruption and may not be indicative of ethanol consumption prior to death [2]. For example, Candida albicans and some other micro-organisms are known to produce ethanol in post-mortem blood samples by fermenting glucose present in the sample when it is stored at the room temperature. For this reason, sodium fluoride (NaF) is added to the post-mortem blood samples to prevent ethanol production [3].

Presence of ethanol in vitreous humour and urine is a good indicator of alcohol consumption prior to death and its absence maybe an indicator of an artefactual source in the matching blood sample.
In 2003 Jones found that urine alcohol concentration (UAC)/blood alcohol concentration (BAC) ratio for the new and casual users was about 1.25:1, based on the average water content of whole blood (80%) and urine (100%) [5], [4] and [6]. Finding a mean UAC/BAC ratio of 1.30:1 or more indicates that the person had reached the post absorptive phase of ethanol metabolism at the time of death [5]. In the post absorptive phase the urine-to-blood ethanol ratio is usually greater than 1.2:1 but less than 1.3:1[5]. Femoral blood ethanol concentration (BAC) ratio to vitreous humour alcohol concentration (VHAC) in post-mortem cases was studied [7]. It was found that blood/vitreous ethanol ratio is 0.94 which indicates that the vitreous humour ethanol concentration almost indicates the ethanol level in the femoral blood. However, another study of more than 350 cases found that VHAC/BAC ratios was 1.1-1.5, bile alcohol concentration/BAC was 0.9- 1.4 and urine alcohol concentration/BAG was 1- 2 [8]. In many cases this ratio might be different due to the source (site of collection) of blood sample, body condition, post-mortem interval, and time relapse since last dirking and death, post-mortem changes and the media where the deceased is found [9]. In post-mortem cases where there is no urine sample, the blood/vitreous humour alcohol ratio can also be safely used to assess whether or not the deceased died during the absorption phase [10]. A study of 295 alcohol positive cases has found that BAC/VHAC ratios from 1.01-2.20 are more commonly found when alcohol level is >100mg/dl [11]. Blood ethanol production by bacteria in post-mortem blood was reported in animal and human [13], [12], [14], [15] and [2]. As mentioned earlier, levels of up to 190mg/dl may occur due to post-mortem bacterial metabolism depending on the degree of body decomposition or disruption and may not be indicative of ethanol consumption prior to death [2]. The presence of ethanol in vitreous humour and urine is a good indicator of alcohol consumption and its absence maybe an indicator of an artefact.
The major metabolite of ethanol is acetaldehyde. A recent study has found that increased alcohol consumption leads to increased acetaldehyde level in the blood and this induce sedation and impairment of both memory and movement effects [16].

Generally speaking, the preferable sample for alcohol detection in forensic cases is the femoral blood [19]. However, in many post-mortem situations femoral blood sample is not available or not suitable for toxicological analysis or interpretation. For instance, in deaths due to stabbing, severe haemorrhage, trauma and burning a femoral blood sample is difficult to obtain and in cases of fluid or blood transfusion a blood sample taken at the post-mortem may not be suitable for analysis or interpretation of results. This would represent an important practical problem and therefore, in such situations finding an alternative sample may become a necessity. In this study, about 66 post-mortem fluid samples representing, cardiac blood, urine, vitreous humour, bile and stomach contents were collected from 11 forensic fatalities with alcohol and drugs of abuse history and toxicological results were compared with those of the femoral blood samples from the same cases.

**Materials and method:**

Post-mortem samples were collected at autopsy from 11 subjects in whom alcohol was implicated in the medical history or the cause of death. Ethical requirements were fulfilled. The samples were provided by Professor Michael Tosokos University of Berlin, Germany. Postmortem samples collected from each subject were femoral blood, cardiac blood, vitreous humor, bile, urine and stomach content. All cases were screened for volatile solvent (Ethanol, Acetone, Methanol and Isopropanol) by Agilent 6890 series GC with auto-sampler (HP, UK). Working internal standard used C (n-propanol 25 mg/100mls). Controls used are LGC-Promochem Aqueous Ethanol Controls Bi-
Level (80 and 200 mg/100mls), Biorad Serum Volatile Control level 1 and 2 (B1 and B2) and Solution B (Ethanol 100mg/100mls and Methanol 100mg/100mls). Samples are diluted in water containing n-propanol as internal standard and injected onto a polar GC capillary column or ZB-WAX 7EM-G007-17 (15m x 0.32MM I.D., film thickness0.50µm). Ethanol analyses were carried out in duplicate. The oven programme is 80⁰C, rum=n time 3min, inlet temperature and detector is 250⁰C and the injection volume is 1 µl. Samples were spin down in the Abbot ultracentrifuge for 10 minutes at 10800 rpm to obtain cell-free sample for analysis. In 5ml plastic vials 50 µl standard, sample or control is added according to our work sheet. 500 µl of standard C (working internal standard) added to all tubes then vortex mix is done for 5second. All samples were centrifuged for 5 minutes at 3500rpm. 200 µl of centrifuged samples were transferred to GC vials and cap and read. Helium was used as carrier gas. An analytical method for ethanol was validated before the assay was applied to the postmortem samples. All statistical analysis was made by SPSS programme, version 14 provided by University of Leicester.

**Aim of study:**

To correlate the mean alcohol concentration values of the femoral blood with those of the other fluids samples involved in this study and to find which sample can be used for alcohol detection as an alternative to the femoral blood.

**Results:**

Detail of the cases is given in Table 1. Maximum, minimum, median and mean values are shown in Table 2. There was a significant correlation between FB and CB ethanol concentrations. The mean FB/CB ethanol ratio was 0.56 with $r^2=0.65$ and p=0.005 (figure1). A significant correlation was found between FB and VH ethanol
concentrations where the mean FB/VH ethanol ratio was 0.80 with $r^2=0.85$ and $p=0.009$ (figure 2). However, the correlations between the urine (U), bile and the stomach contents (SC) on one side and the femoral blood (FB) on the other side were not significant. The mean FB/U ethanol ration was 0.23 with $r^2=0.003$ and $p=0.91$ (figure 3). The mean FB/Bile ethanol ratio was 0.37 with $r^2=0.096$ and $p=0.55$ (figure 4). The mean FB/SC ethanol ratio was 0.58 with $r^2=0.048$ and $p=0.52$ (figure 5).

**Discussion:**

Drug concentrations were not normally distributed because pharmacological response generally represents a logarithmic relation to substance concentration [17]. Medians are therefore more appropriate for describing these distributions. The values of the median ethanol concentration for the samples studied are shown in Table 2. The results of the alcohol concentrations in the vitreous humour found in this study support the observation made previously by Honey, 2005 and Kraut, 1992 [11], [8] and they are much closer to the results obtained by Sylvester, et al 1992[7]. Our findings confirm that vitreous humour alcohol concentration is the nearest to and most representative of the femoral blood concentration in the ante mortem stage. Study of the median alcohol concentration values (Table 2 and Figure 6) showed that the values of the vitreous humour and cardiac blood samples are the closest to those of the femoral blood samples. In our view, vitreous fluid sample is preferable to that of the cardiac blood for alcohol analysis. Firstly to avoid any possible post-mortem contamination or false result as the cardiac blood is very near to the stomach and some workers have reported a possible post-mortem diffusion of alcohol from the stomach content to the central blood [18]. Secondly, a vitreous humour sample is more easily obtained that the cardiac blood, which may not be available anyhow, for example in cases of severe haemorrhage. And
thirdly, the vitreous fluid is preserved and protected inside the eye socket and less likely to be lost due to trauma than the cardiac blood.

**Conclusion:**

This study suggests that in post-mortem cases where femoral blood sample is unavailable or unsuitable for analysis cardiac blood and preferably vitreous fluid samples can be safely used as alternative to the femoral blood sample for assessing ante mortem body alcohol concentration or level. However, more studies are required to confirm this conclusion.

**Acknowledgement:**

We are thankful to Professor Michael Tsokos, University of Berlin, Germany for providing the post-mortem samples.
Table 1: Alcohol cases details

<table>
<thead>
<tr>
<th>CASE</th>
<th>Age (year)</th>
<th>Gender</th>
<th>Medical history</th>
<th>Post-mortem findings</th>
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<tr>
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<td>M</td>
<td>Alcohol abuse</td>
<td>Brain and lung oedema</td>
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<tr>
<td>2</td>
<td>61</td>
<td>M</td>
<td>Alcohol abuse</td>
<td>Fatty liver</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>M</td>
<td>Alcohol abuse</td>
<td>Brain and lung oedema</td>
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<td>M</td>
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<td>Brain and lung oedema</td>
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<tr>
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<td>28</td>
<td>F</td>
<td>Alcohol abuse</td>
<td>No pathological finding</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>M</td>
<td>Alcohol abuse</td>
<td>Fatty liver</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>M</td>
<td>Alcohol abuse</td>
<td>Brain and lung oedema and fatty liver</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>M</td>
<td>Alcohol abuse</td>
<td>Brain and lung oedema</td>
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<td>M</td>
<td>Alcohol abuse</td>
<td>Brain and lung oedema</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>M</td>
<td>Alcohol abuse</td>
<td>Liver cirrhosis.</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
<td>M</td>
<td>Alcohol abuse</td>
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Table 2: Alcohol concentration in different post-mortem fluid samples (mg/dl)

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<tr>
<th>CASE NO</th>
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<th>VH</th>
<th>U</th>
<th>BILE</th>
<th>SC</th>
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<td>217</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>198</td>
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</table>

Minimum Value (mg/dl) | 4   | 16  | 4   | 77  | 65   | 40  |
Maximum Value (mg/dl) | 365 | 323 | 346 | 920 | 241  | 490 |
Mean Value (mg/dl)    | 131 | 98  | 131 | 332 | 166  | 188 |
Median Value (mg/dl)  | 134 | 20  | 46  | 252 | 183  | 168 |

NA= Not available, FB=femoral blood, CB=cardiac blood, VH= vitreous humour, U=urine and SC= stomach contents.
Figure 1: Regression study for femoral and cardiac blood ethanol concentration.
**Figure 2**: Regression study for femoral and vitreous humour ethanol concentration.
Figure 3: Regression study for femoral and urine ethanol concentration.
Figure 4: Regression study for femoral and bile ethanol concentration.
Figure 5: Regression study for femoral and stomach ethanol concentration.
**Figure 6**: Median alcohol concentrations in different samples

FB = femoral blood, CB = cardiac blood, VH = vitreous humour, U = urine, SC = stomach contents.
References:


