Study on the relationship between the concentration of ethanol in the blood, urine and the vitreous humour

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Abstract: Ethanol (ethilic alcohol) is widely available, being the most commonly used drug worldwide and often involved in forensic cases. It therefore becomes essential to analyze and correctly interpret the blood alcohol concentration (BAC) in samples collected during autopsy. The purpose of our study was to establish a relationship between the concentration of ethanol in vitreous humor, urine and blood, which should lead to a more accurate assessment of the BAC, in cases where direct measurement is difficult because of putrefaction, severe trauma, embalming etc. The study was conducted at the Institute of Forensic Medicine Iasi between December 2010 and August 2012 on a total of 202 forensic cases in which samples of blood, urine and vitreous humor were collected for toxicological examination. We found a strong correlation between the concentration of ethanol in blood and vitreous humor and a weaker correlation between the concentration of ethanol in blood and urine. We also identified a method for calculation of blood alcohol concentration at the time of death from blood alcohol concentration at the time of autopsy.

Key Words: ethilic alcohol, blood, vitreous humour, urine, autopsy.

Determining the cause of death in forensic practice is one of the most important and difficult task of a forensic pathologist. The toxicological exam from the proper collection of samples, to analyzing and correctly interpreting them often plays an important role in this process, given that the toxic substances may determine or favour death.

Ethanol, the psychoactive constituent of alcoholic beverages, is widely available, being the most commonly used drug worldwide and often involved in forensic cases. It therefore becomes essential to analyze and correctly interpret the blood alcohol concentration (BAC) in samples collected during autopsy. The challenge is all the more important as the interpretation of postmortem BAC may come across difficulties due to lack of homogeneity in blood samples, bacterial postmortem alcohol production, diffusion of alcohol from stomach contents or contaminated airways. Also, post-mortem BAC can be influenced by changes in hematocrit due to refill volume replacement therapy by administering blood products, volume expander or other resuscitation maneuvers which may result in dilution of intravascular concentration of alcohol.

The ingested alcohol is rapidly absorbed in the digestive tract, with varying rates at different levels. Because ethanol is soluble in water, its concentration in...
different compartments of the body directly correlates with the water content of each tissue or fluid. Therefore, biological products with a high content of water, such as blood, urine and the vitreous humor have much higher concentrations of ethanol versus parenchymal organs (e.g., liver). For this reason, the literature recommends routine collection of blood, urine and vitreous humor samples in order to perform the toxicological examination.

Blood is the "golden" biological product for the determination of post-mortem alcohol concentration because it directly correlates with the degree of intoxication achieved during life and thus with the triggered pathophysiological effects.

Urine is the second important biological product in post-mortem toxicological analysis, being the main route of elimination of toxics. However, urine alcohol concentration (UAC) is correlated with the intravascular level of ethanol only if urine is formed after the ingestion of ethanol. The alcohol concentration in urine is generally smaller than that in the blood until the BAC peaks (absorption phase), later becoming bigger as the BAC decreases (excretion phase). The ratio between the concentration of ethanol in blood and in urine varies in a very wide range, between 0.21 and 2.66 [1], with an average accepted value of 1.28. In fact, UAC reflects an average of BAC for as long as it was produced, and this average is influenced by many factors. Under these conditions, an independent value of UAC may not reflect or predict the BAC, but, in conjunction with the value of BAC, it may point to the alcohol metabolizing phase in the body. But the level of UAC may as well be influenced by decay. Although, in general, it is considered that the urine is less likely to be influenced by the effects of postmortem production of alcohol than blood, there are studies indicating that large amounts of alcohol can be produced when glucose (in persons suffering from diabetes) and a microorganism (Candida albicans) are found in the urine.

Vitreous humor is one of the biological products most widely used in forensic toxicology. The vitreous is particularly useful in cases where the body is severely damaged, affected by putrefaction or where the toxicological exam is done after embalming the body and BAC determination becomes irrelevant. The isolated location of the eyeball makes the vitreous humor not be exposed to bacterial contamination and alcohol diffusion from gastric and pulmonary levels. The intact eyeball is relatively avascular and isolated from other tissues and fluids, so that vitreous humor is a sterile product, useful for quantitative determinations. The level of alcohol in the vitreous humor (VAC) is not influenced by the formation of alcohol during putrefaction and the electrolyte and metabolites concentrations of drugs in this environment remain stable post-mortem for a longer period of time than in blood. Due to these features, the quantitative determination of VAC is an excellent way to interpret the value of alcohol, as a measure of quality control. Given the high concentration of vitreous water, the concentration of ethanol in this environment is proportionally higher than in blood, when they are in balance, the report referred to in the literature is VAC / BAC = 1.2: 1 [2-4]. The balance between the BAC and VAC is about 1-2 hours delayed. The VAC thus gives us an opportunity to look back and provide information on the BAC about 1-2 h before the time of death. In the phase of absorption, the VAC is lower than BAC. If the person stops drinking alcohol, the blood alcohol level will continue to rise as absorption continues, then it is kept in plateau for a while and then it decreases. The alcohol in vitreous humor, which is metabolized more slowly than in blood, continues to grow when the intravascular alcohol remains in plateau. Subsequently, this also reaches a maximum which lasts for a certain period, followed by decrease. When balancing the concentrations, the VAC is higher than BAC due to more elevated water level. The ratio 1.2: 1 between the two concentrations remains constant as the VAC and the BAC decrease simultaneously.

**OBJECTIVES**

The purpose of this study was to establish a relationship between the concentration of ethanol in vitreous humor, urine and blood, which should lead to a more accurate assessment of the BAC, in cases where its direct measurement is difficult because of putrefaction, severe trauma, embalming etc.

We also followed if the official method of alcohol dosage in Romania (Cordebard) can be applied for qualitative and quantitative analysis of alcohol in the vitreous in order for these determinations to be available (from technical and financial perspective) to all forensic services in the country.

**MATERIAL AND METHOD**

The study was conducted at the Institute of Forensic Medicine Iasi between December 2010 and August 2012 on a total of 202 forensic cases in which samples of blood, urine and vitreous humor were collected for toxicological examination. Also, demographic data regarding the victim (age, sex), postmortem interval (PMI) and the time of death were collected. In the event that there was a previous hospitalization period, it was intended that this period be less than 24 hours.

Blood samples were collected before opening the body, mainly from the peripheral vessels (femoral vein - of choice) so as to avoid possible artifacts caused by contamination of the blood with peritoneal fluid, gastric contents etc. as a consequence of postmortem increase of biological membranes and vascular walls permeability. This was possible in 188 cases; in 14 cases liver blood was collected.
Urine samples were collected in 39 cases, directly from the bladder through an incision. Vitreous humor was collected in 86 cases, by puncturing the external angle of both eyeballs with a thin needle [5-8]. Of the 86 cases in which the vitreous humor harvesting succeeded, 1 case was excluded because the value of the BAC was zero; of the 39 cases in which urine was collected, 3 were excluded as the BAC was also zero.

The study group consisted of 163 men and 39 women, with an age average of 49.9 ± 17.4 years (Table 1).

The samples were analyzed by Cordebard method (modified by D. Banciu and I. Drocu). This method is based on the oxidation of ethanol which was isolated by distillation to the acetic acid with a nitrochromic mixture (composed of an aqueous solution of potassium dichromate and nitric acid), followed by iodometric retitration of the dichromate excess (with the potassium iodide solution 2% freshly prepared).

To reduce the risk of errors, equal amounts of each sample were used for toxicological analysis. The effects of environmental conditions on the concentration of ethanol in various fluids were studied using weather reports in METAR format. This type of report is used by pilots prior to takeoff and by meteorologists to obtain weather forecasts. Information (including the setting of hourly temperature, humidity, atmospheric pressure, rainfall, dew point and other parameters) was collected from publicly available data of Iasi International Airport (International Civil Aviation Organization - LRIA) located 8 km NE of the city centre (27°38'E longitude, latitude 47°10’N, altitude 102 m).

Analysis of the raw data from METAR system was preferred over data from other weather stations as they mostly present aggregated data. METAR data validation was performed by comparison with data from two other weather stations in the surroundings of Iasi (Adamachi and Miroslava). Relatively constant values were seen in all 3 recording points (i.e. March 2012 - Table 2).

The data obtained was analyzed with the SPSS system, version 19. The linear regression allowed us to identify the parameters that influence the BAC. In view of the statistical analysis the cases in which toxicology results were negative were eliminated.

### RESULTS AND DISCUSSION

The statistical analysis revealed a strong correlation between the concentration of ethanol in urine and vitreous humor and blood.

In the relation UAC-BAC, the Pearson correlation coefficient is $r = 0.905$ (36 cases - Fig. 1), while, in the relation VAC-BAC, this coefficient is $r = 0.887$ (85 cases - Fig. 2).

The VAC-BAC ratio on the 85 cases in which both blood and vitreous humor were collected, was 1.21 (SD 0.98) with an average of 1.11.

During data analysis, two extreme values were observed in which, most likely, the BAC value was very low, possibly mistaken. By excluding these two cases, the average relation VAC / BAC became 1.07 (SD 0.299), with the same average value of 1.11 (Table 3).

Our results can be placed within the limits of the ratio VAC/BAC identified by other studies quoted in the literature. In one of the largest studies on this topic (706

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Weather station/ Decade</th>
<th>Average METAR (LRIA)</th>
<th>Adamachi</th>
<th>Miroslava</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average temperature [°C]</td>
<td>1-10</td>
<td>-1.9</td>
<td>-1.8</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>5.2</td>
<td>5.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>9.1</td>
<td>8.8</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>4.3</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Relative humidity [%]</td>
<td>1-10</td>
<td>78</td>
<td>73</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>72</td>
<td>63</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>63</td>
<td>59</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>71</td>
<td>65</td>
<td>71</td>
</tr>
</tbody>
</table>
forensic cases), Jones and Holmgren have identified an average VAC/ BAC of 1.19, with a standard deviation of 0.285 [9]. DiMaio [2] and Molina [4] identified a VAC/ BAC ratio of 1.2, while Spitz [3] provides values ranging between 1.12 and 1.2. Coe and Sherman placed this report between 0.9 and 1.38, with an average of 1.2. Other studies provide larger intervals, such as 0.71 to 3.71, with an average of 1.29 [10]. The literature review by Kugelber [11] in 2007 mentioned an average reference interval for the VAC / BAC ratio of 1.15-1.20.

Obtaining a VAC/ BAC ratio within the limits provided by the literature allows us to consider that the Cordesbard method can be applied in VAC determination; we must mention that for a correct determination of BAC and VAC equal amounts of the two specimens must be analyzed.

In the current forensic practice, it is necessary to identify a method of calculating BAC from alternative biological sample in cases where blood samples are not available or are corrupted by postmortem processes, trauma, or embalming [12, 13].

Data analysis by linear regression allowed the examination of the influence of two types of parameters on the concentration of alcohol in blood, urine and vitreous humor:

- Individual parameters: age, postmortem interval (PMI), blood collection site, sex.
- Environmental factors: temperature (minimum value, maximum, average- measured in degrees Celsius), atmospheric pressure (average values measured in mmHg), dew point average (measured in degrees Celsius), average relative humidity, average rainfall, apparent temperature (temperature-humidity index- THI based on the relationships identified by Carl Schoen [14]). Only those values (minimum, maximum and/ or average) within the postmortem interval registered for each case (in order to have a correct reference of postmortem processes) were considered and included in the statistical processing.

In the 85 cases where it was possible to determine the alcohol concentration in both blood and vitreous humor, there was a strong dependence of VAC to BAC (p <0.000001) and a moderate dependence of BAC to PMI (p = 0.011). For all other parameters, individual or environmental, statistical significance was much lower, which is why they were not taken into account for further analysis. Applying linear regression, using the selected parameters, the following formula is obtained:

\[
VAC \ [g/l] = 0.901 \times BAC \ [g/l] - 0.0105 \times PMI \ [h] + 0.5585 (1)
\]

with p <0.000001 (BAC), p = 0.011 (PMI), p = 0.0004 (constant); Linear model R2 = 0.815.

Analysis of the alcohol concentration in the vitreous humor and urine (36 cases) showed a strong dependence of the VAC to UAC (p <0.00001) and a moderate dependence of the VAC to the average postmortem temperature (p= 0.016), maximum postmortem temperature (p = 0.014) and the mean postmortem atmospheric pressure (p = 0.005). All other parameters, including PMI had a lower significance. Applying linear regression using the selected parameters, the following formula resulted:

\[
VAC [g/l] = 0.795 \times UAC [g/l] + 0.108 \times Taverage[°C] - 0.094 \times Tmax[°C] + 0.046 \times Paverage[mmHg] 34.479 (2)
\]
Equation 2 identified by us to calculate the VAC from UAC and environmental factors (temperature and atmospheric pressure), was developed from a small number of cases, suggesting the need to extend the research on a larger number of cases. Also, the use of environmental factors should be approached cautiously, since outdoor weather data do not always correspond to the environment where the body is placed till the autopsy is performed.

Inverse linear regression allowed the identification of the following relationship between BAC, VAC and PMI:

$$\text{BAC}[\text{g/l}] = 0.903 \times \text{VAC}[\text{g/l}] + 0.0084 \times \text{PMI} [\text{h}] - 0.150$$ (3)

In the above relation, the $p$ constant is not statistically significant ($p = 0.357$) leading to a high degree of uncertainty regarding the results and influencing the possible use of the PMI (statistically significant $p = 0.042$) to estimate the overall effect the PMI on the BAC (with an increase of 0.0084 g/hour).

The relationship between the BAC, UAC, temperature and pressure obtained by the linear regression is:

$$\text{BAC}[\text{g/l}] = 0.856 \times \text{UAC}[\text{g/l}] + 0.054 \times T[°C] - 0.031 \times T_{\text{max}} [°C] + 0.024 \times P_{\text{average}} [\text{mmHg}] - 18.110$$ (4)

Linear regression in the above equation denotes the same influence of the atmospheric pressure and temperature on the urinary excretion process; only the UAC ($p < 0.00001$) and the temperature ($p = 0.021$) coefficients are significant, others are borderline insignificant ($p = 0.084 - 0.087$).

In most cases, the calculation of the VAC from UAC and/ or BAC is not necessary because the reference in terms of somatic and psychological effects of the ethanol is the BAC [15, 16]. However, as showed above, the accurate assessment of the postmortem BAC is sometimes difficult because of the influence of various factors [17-19].

Literature states [11] that the advantage of the VAC over the BAC is the fact that the vitreous is less exposed to bacterial contamination, especially in cases where the body was subjected to processes of decomposition or severe traumatic injuries.

Studies in the literature correlate mainly VAC with BAC, but also UAC with BAC, based on the results of toxicological analysis performed at the time of autopsy which allows approximation with some margin of error, the value of the alcohol at the time of death which is relevant from the forensic perspective [5]. However, in some cases vitreous humor can not be analyzed (such as trauma to the eyes, degradation of the eye due to putrefaction, etc.).

If these situations overlap the possible elements of influencing the real value of the BAC (e.g. decomposition, severe trauma, embalming), toxicology has limited value because the possible changes of the BAC due to the previously mentioned factors can not be verified.

For these situations in which the vitreous humor can not be collected and the BAC is suspected to be altered by factors that acted postmortem on the body we propose the application of the equation 1 identified in this study for estimating the VAC at the time of death based on the BAC at the time of autopsy and the postmortem interval. Considering that the concentration of the ethanol in the vitreous humor is constant, we estimate this value as the one at the time of death or about 1-2 hours prior to death. Further, considering the VAC as a descriptor of the BAC at the time of death, by applying the theoretical VAC/ BAC ratio we obtain the following relationship:

$$\text{VAC}[\text{g/l}] = (1,12-1,2) \times \text{BAC0}[\text{g/l}]$$.

By applying this ratio in equation 1 we can estimate the blood alcohol concentration at the time of death as being included in the range:

$$0,0804 \times \text{BAC1} - 0,009 \times \text{PMI} (\text{h}) + 0,498 / 0,75 \times \text{BAC1} - 0,008 \times \text{PMI} (\text{h}) + 0,465$$ (5)

where $\text{BAC1}$ is the value of the blood alcohol concentration at the time of autopsy.

Assuming constant VAC (equation 5), according to the equations (1) and (2) we can estimate the influence of various parameters on the concentration of ethanol (Table 4). The last column shows conditions (individual

Table 4. The influence of the measured parameters on BAC and UAC.

<table>
<thead>
<tr>
<th>Measured parameters</th>
<th>Influence factors</th>
<th>The sense of influence</th>
<th>Amount</th>
<th>Circumstances of validity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC [g/l]</td>
<td>Postmortem interval</td>
<td>increase</td>
<td>$+ 0.012$ g/l/h</td>
<td>PMI $= 26.9 \pm 13.1$ h</td>
</tr>
<tr>
<td>UAC [g/l]</td>
<td>Average temperature*</td>
<td>decrease</td>
<td>$- 0.136$ g/l/°C</td>
<td>$T_{\text{average}} = 10.7 \pm 11.8$ °C</td>
</tr>
<tr>
<td></td>
<td>Maximum temperature*</td>
<td>increase</td>
<td>$+ 0.118$ g/l/°C</td>
<td>$T_{\text{max}} = 17.4 \pm 13.5$ °C</td>
</tr>
<tr>
<td></td>
<td>Average atmospheric pressure*</td>
<td>decrease</td>
<td>$- 0.058$ g/l/mmHg</td>
<td>$P_{\text{average}} = 764.2 \pm 5.7$ mmHg</td>
</tr>
</tbody>
</table>

*p in PMI
and environmental) in which measurements were made and represents the limit of validity of the detected influences.

**CONCLUSIONS**

Our study demonstrated a strong correlation between the concentration of ethanol in blood, urine and vitreous humor. VAC correlates with BAC stronger than UAC.

The VAC/ BAC ratio determined in our study using the Cordebard method for determining the concentration of ethanol in blood and vitreous humor falls within the ranges provided in the literature. This allows us to conclude that the Cordebard method (cheap and accessible) can be applied to determine the VAC, a proper comparison between BAC and VAC requiring the analysis of equal amounts of biological samples.

Also, our study allowed the identification of a method for calculation of BAC at the time of death from BAC at the time of autopsy. This method could be useful in situations where BAC is suspected of distortion due to postmortem factors (decomposition, severe trauma, embalming) and the vitreous humor can not be collected.

In line with similar studies in the literature our study supports the idea that harvesting as many biological samples as possible is mandatory for a good quality toxicological analysis and for a proper interpretation of its results.

**References**

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