Liver Total Protein in Relation to Cause of Death in Man

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A population study carried out in England showed that human ADH activity levels in livers from patients dying from sudden traumatic accidents are higher than those dying from acute death. Also, the acute-illness patients have ADH levels higher than those dying with a long-standing illness, while the cancer patients have the lowest enzyme activity levels (1). These differences, however, are independent of age or sex effect.

Assuming that the liver is the main organ to be affected by any general ill process in man, the reported finding for ADH deserves further investigation. The main question to answer is whether the liver proteins as a whole, show variation among the diseases’ categories which parallel those observed for ADH, or is the ADH finding unique. In the present paper the association between liver protein determinations and cause of death is investigated in a sample of Brazilians, in parallel with alcohol dehydrogenase activity levels. The effects of age, race, and sex are also examined in relation to both protein and ADH levels.

MATERIAL AND METHODS

Human livers. Liver samples were obtained during routine necropsies at the Instituto Médico Legal Nina Rodrigues, in Salvador, capital of the State of Bahia, Brazil. This institute is a state organization entitled to autopsy and to issue death certificates to anyone whose death occurred without medical assistance. The liver samples were refrigerated in the necropsy room, and during transportation to the Laboratório de Genética Médica in the same city. Upon arrival in the laboratory the samples were stored at about –10°C for subsequent study since there is no evidence of ADH instability after death (1).
Alcohol dehydrogenase assay. A few days later a fragment of tissue was cut from the liver samples without thawing. The tissue fragment was washed three times in 0.05 M phosphate buffer, pH 7.0, and dried in filter paper before having it's weight adjusted to 1 g. Two milliliters of the phosphate buffer was added to the 1 g tissue fragment and homogenization was carried out in a Silverson homogenizer. The homogenates were filtered in surgical cloth and the filtrate was spun at 1000g for 30 min at 4°C. The enzyme activity was measured in 1 ml of the supernatant, by the method described by von Wartburg et al. (2). The reaction mixture contained 1.6 mM ethanol, and 1.6 mM NAD in 3.3 M pyrophosphate buffer, pH 8.8. A temperature-controlled (28°C) Gilford II register spectrophotometer was used to follow NAD reduction at 366 nm. ADH activities are expressed as ΔOD₃₆₆ per gram of tissue per minute at pH 8.8.

Protein determination. The liver homogenates prepared for ADH assay were used. Total protein was determined by the method of Bucher (3), and the results expressed in milligrams per gram of tissue.

Study protocol. During the liver sample collection the routine autopsy numbers were used for specimen identification. Information on age, race, sex, and cause of death were obtained later from the autopsy records in the Institute's files. Race was classified in three groups: white, mulattoes, and blacks, as generally used in Bahia, (4). In some records the information on age and/or race was missing.

Statistical analysis. Cause of death was grouped into four categories: sudden traumatic accident, acute illness (nonmalignant), chronic illness (nonmalignant), and cancer, (1). Because in the cancer group there were four individuals only, it was not considered for statistical analysis. The average levels of ADH activity were compared among the remaining three groups of diseases by a single-classification analysis of variance with unequal sample size (5). From its characteristics the sudden traumatic death group was taken as representative of a healthy group to allow investigation of biological variables. Thus, the effect of sex, age, and race were studied within the accidental sudden-death group only. Age was grouped into two major classes: from 10 to 50 years old, and older than 50. The unpaired t test for mean differences was used to compare the results between the sexes and between the age groups. The effect of race was studied by unequal sample size analysis of variance.

The same statistical procedure was carried out for the analysis of protein levels.

The correlation coefficient between ADH and protein levels was estimated within each disease group.

RESULTS

Excluding the four cases of cancer, a total of 343 liver homogenates were tested. The mean levels and significance tests for ADH activity and
<table>
<thead>
<tr>
<th>Cause of death</th>
<th>ADH activity (ΔOD₆₅₀/g tissue/min)</th>
<th>Protein determination (mg/g tissue)</th>
<th>ANOVA</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden traumatic accident</td>
<td>152 9.9 ± 6.71</td>
<td>152 83.6 ± 30.71</td>
<td>$F = 21.09$</td>
<td>$F = 4.43$</td>
</tr>
<tr>
<td>Acute illness (nonmalignant)</td>
<td>78 7.8 ± 6.97</td>
<td>78 76.7 ± 33.15</td>
<td>$P &lt; 0.005$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>Chronic illness (nonmalignant)</td>
<td>113 5.3 ± 3.93</td>
<td>113 73.1 ± 31.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA, analysis of variance.
*SD, standard deviation.
<table>
<thead>
<tr>
<th></th>
<th>ADH activity (Δ ODₕₒₑₓ/g tissue/min)</th>
<th>Significance tests</th>
<th>Protein determination (mg/g tissue)</th>
<th>Significance tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>t</td>
<td>n</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>120</td>
<td>9.6 ± 6.29</td>
<td>1.04</td>
<td>120</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>11.0 ± 8.13</td>
<td>&gt;0.05</td>
<td>32</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–50 years</td>
<td>109</td>
<td>10.3 ± 6.67</td>
<td>0.41</td>
<td>109</td>
</tr>
<tr>
<td>&gt; 50 years</td>
<td>28</td>
<td>9.7 ± 6.93</td>
<td>&gt;0.05</td>
<td>28</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>28</td>
<td>10.7 ± 6.97</td>
<td>-0.08</td>
<td>28</td>
</tr>
<tr>
<td>Mulatto</td>
<td>87</td>
<td>10.3 ± 7.15</td>
<td>&gt;0.05</td>
<td>87</td>
</tr>
<tr>
<td>Black</td>
<td>14</td>
<td>9.8 ± 6.35</td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

* 15 individuals without information for age.
* 23 individuals without information for race.
for protein determination in the three diagnostic categories are shown in Table 1. On both ADH activity and protein levels, there is a significant effect of the cause of death: the longer the disease process, the lower the ADH activity, and the lower the total protein determination ($F(2,340) = 21.09; P < 0.05$ for ADH; $F(2,340) = 4.43; P < 0.05$ for protein).

The variables, age, race, and sex have no effect at all on the ADH activity levels on the protein determination, as shown by the mean levels and significance tests in Table 2.

There is a consistent and positive correlation between the ADH activity and the protein determination within each disease group (Table 3). Note that the ADH activity levels were estimated in gram of tissue, independent of the protein concentration, just to avoid pseudocorrelation between ADH and protein levels.

**DISCUSSION**

The lowering of protein levels in liver tissue are generally attributed to greater protein lost from inner cell compartments, or to lowering of protein synthesis. Greater protein lost in tissue, could be caused by an increment of catabolism, by cell membrane damage, or by cell necrosis. On the other hand, lower protein synthesis could be a consequence of low amino acid intake (external cause) as has been specifically shown for ADH (6) or be due to protein synthesis impairment itself (internal cause) (7). Besides that, a sick organism becomes more likely to have its protein synthesis influenced by other factors related to the disease itself, such as virus and hormones (8), or related to the treatment, such as antibiotics (9). Thus, from an overall view of the process involved in the maintenance of protein level in the liver, one may conclude that for those individuals whose death was preceded by a disease, either acute or chronic, there are several ways by which the liver protein can be lowered. Within the human organism complexity one may speculate that as disease progresses the
lowering of liver protein and the disease itself become mutually collaborative in aggravating patients' health. This collaborative ill effect may explain the lowest protein and ADH activity in liver from chronic patients. Finally, to be aware of these facts is of major importance to physicians for availing laboratory tests in the chronically ill patients, as well as to geneticists using postmortem liver samples for population study of genetic markers.

SUMMARY

Human liver samples obtained from 343 autopsies at the Instituto Médico Legal Nina Rodrigues in Salvador, capital of Bahia State, Brazil, were studied for protein determination in parallel with ADH activity levels. There is no effect of age, race, or sex on the level of protein concentration, or on ADH levels. However, cause of death was found to be an important factor on total liver protein concentration in a similar manner as previously reported for ADH. Livers from patients dying from sudden traumatic accident had higher levels of protein concentration than those dying from acute illness. Also the acute illness group had higher protein concentration than those patients dying of chronic illness. Similar types of association were also confirmed for ADH activity levels in the present data. The possible mechanisms responsible for lowering liver protein during diseases are discussed.

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REFERENCES