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PAPER

PATHOLOGY/BIOLOGY

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Basal Renal Tubular Epithelial Cell Vacuolization and Alcoholic Ketoacidosis

ABSTRACT: Subnuclear renal tubular epithelial cell vacuolization is a marker for diabetic ketoacidosis. Whether it is because of hyperglycemia or of ketoacidosis is unclear. To examine the effect of ketoacidosis on renal cells in isolation, five cases of lethal alcoholic ketoacidosis without hyperglycemia were examined (vitreous humor β-hydroxybutyrate: 6.42–8.75 mM, mean 7.66 mM; and glucose: 0.1–4.2 mM, mean 1.46 mM). Microscopic examination of the kidneys revealed basal vacuoles in three cases (60%). Seven control cases with acute alcohol toxicity without ketoacidosis (blood alcohol: 0.18–0.43%, mean 0.31%; and β-hydroxybutyrate: 0.12–0.42 mM, mean 0.21 mM) did not have these changes. In this study, basal epithelial vacuolization was found only in cases with significant ketoacidosis. Although the numbers are small, the finding of basal renal tubular epithelial vacuolization in normoglycemic cases with elevated β-hydroxybutyrate levels provide further evidence that disordered lipid metabolism may be involved in the pathogenesis of this phenomenon.

KEYWORDS: forensic science, alcoholic ketoacidosis, intoxication, Armanni–Ebstein phenomenon, diabetes mellitus, alcoholism

The term ''Armanni–Ebstein phenomenon,'' first described in 1877 by Luciano Armanni, has been used to refer to subnuclear vacuolization of renal tubular epithelial cells, which when severe enough may present macroscopically as renal cortical pallor. First recognized in poorly controlled diabetic states, it was initially thought to be a direct effect of hyperglycemia causing cytoplasmic glycogen accumulation (1–5). However, there is now evidence that the intracellular vacuoles contain lipid (6–8). A recent study also failed to demonstrate a significant correlation between the degree of hyperglycemia and these morphological changes, suggesting a more complicated etiology possibly involving the effect of ketoacidosis or lipiduria on tubular cells (9). Although this subnuclear vacuolization has been reported in cases of lethal hypothermia, it appears that in at least some of these cases it may be related to underlying diabetic ketoacidosis, that is, basal epithelial vacuolization in these cases may be merely a marker of diabetic metabolic derangement rather than to hypothermia *per se* (10,11). To examine the effect of ketoacidosis on renal cells in isolation, without concomitant hyperglycemia, a series of deaths because of alcoholic ketoacidosis were reviewed. A control group consisted of cases with significantly elevated blood alcohol levels, but no evidence of ketoacidosis.

Materials and Methods

Case files over a 7-year period from 2004 to 2010 at Forensic Science SA, Adelaide, South Australia, were retrospectively reviewed for all cases of nontraumatic deaths where alcohol was listed as a direct cause of death. Cases where deaths were attributed to alcoholic ketoacidosis or where acute alcohol intoxication had played a role were then selected. All cases had full coronial and police investigations with complete forensic autopsies. Alcoholic ketoacidosis was diagnosed when the vitreous humor β-hydroxybutyrate was ≥5 mM with normoglycemia or hypoglycemia (vitreous glucose ≤ 11 mM), in conjunction with a history of alcohol abuse and/or autopsy findings suggestive of chronic alcoholism. Alcohol toxicity was taken as a blood alcohol level of >0.15%. Cases where vitreous humor biochemistry and full toxicology had not been performed were excluded. Case files were summarized and all available microscopic slides of the kidneys were then blindly reviewed for basal epithelial vacuolization; there were no histologic sampling differences between the alcoholic ketoacidosis and the acute alcohol toxicity group, with one section per kidney being reviewed. Cases where the kidneys were too autolysed for accurate assessment were also excluded.

Results

A total of 26 cases were identified, consisting of 12 deaths because of alcoholic ketoacidosis, and 14 because of acute alcohol toxicity. Of the 12 cases of alcoholic ketoacidosis, six were excluded because of incomplete vitreous humor biochemical evaluations, and one was excluded because of insufficiently raised β-hydroxybutyrate (<5 mM). Of the 14 deaths where alcohol toxicity had contributed to the lethal episode, five were excluded because of lack of vitreous biochemistry, and two were excluded because of insufficiently raised blood alcohol concentrations (<0.15%).

All five cases where death was because of alcoholic ketoacidosis had a history of chronic alcohol abuse, two with hepatomegaly, four with steatosis and periportal fibrosis, and one with splenomegaly. The age range was from 51 to 72 years (mean 61 years) and all were men. Vitreous humor β-hydroxybutyrate levels ranged from 6.42 to 8.75 mM (mean 7.66 mM) with no elevation in glucose levels (range 0.10–4.20 mM; mean 1.46 mM). In one case,

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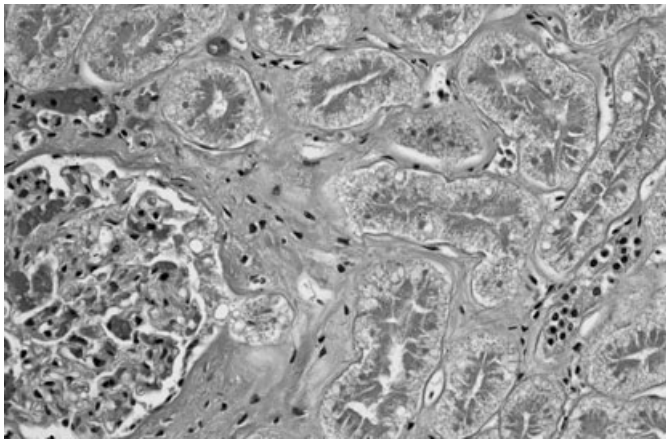

FIG. 1—*Typical vacuolization in the basal portions of renal tubular epithelial cells in a case of normoglycemic alcoholic ketoacidosis. Mild autolytic change is present (hematoxylin and eosin × 280).*

the blood alcohol concentration was 0.20%, and alcohol was not detected in the others. The postmortem interval in which samples were drawn ranged from 4 to 8 days, with a mean of 5.6 days. No cases showed evidence of significant putrefaction. Microscopic examination of the kidneys revealed basal epithelial vacuolization in three cases (Fig. 1). There was no history of diabetes mellitus in any of these cases.

A total of seven cases with acute alcohol toxicity without ketoacidosis were studied; six had a history of chronic alcohol abuse with hepatomegaly at autopsy in four cases, marked cirrhosis in one case, early cirrhotic changes in four cases, mild steatosis in one case, and splenomegaly in four cases. The ages ranged from 37 to 68 years (mean 51.1 years). The male to female ratio was 3:4. In one case, there was a medical history of insulin-dependent diabetes mellitus. Blood alcohol concentrations ranged from 0.18 to 0.43% (mean 0.31%). β-hydroxybutyrate levels ranged from 0.12 to 0.42 mM (mean 0.21 mM). Vitreous glucose was tested in three cases and ranged from 0.1 to 1.3 mM (mean 0.5 mM). The postmortem interval in which samples were withdrawn ranged from 2 to 6 days, with a mean of 3.9 days. No cases showed evidence of significant putrefaction. None of the cases exhibited basal epithelial vacuolization in the kidneys (Fig. 2).

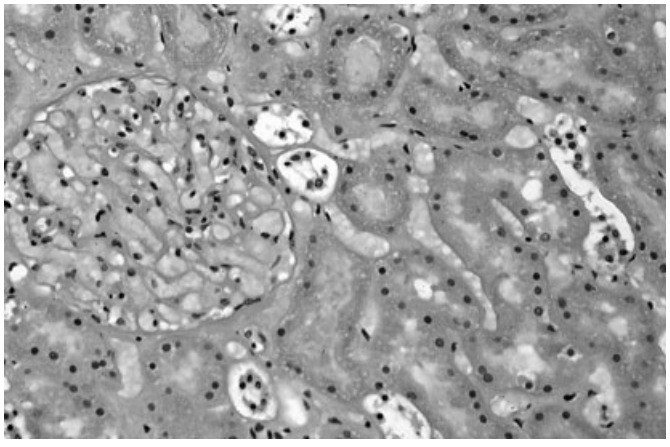

FIG. 2—*Normal renal tubular epithelial cells in a case of acute alcoholic toxicity (blood alcohol = 0.43%) with no evidence of vacuolization (hematoxylin and eosin × 280).*

Discussion

Ethanol is a commonly used substance, the excessive consumption of which can have a variety of effects on the body with forensic repercussions. Ethanol readily crosses the blood–brain barrier into the cerebral extracellular fluid and has a direct depressant effect on neurons. Chronic excessive alcohol intake is associated with many significant conditions including malnutrition, cirrhosis with liver failure, esophageal varices, chronic pancreatitis, and cardiomyopathy (12,13). Alcohol is also frequently an indirect cause of death with excessive alcohol consumption associated with aspiration of gastric contents and a variety of unnatural deaths that include drowning, falls, burns, and other forms of trauma (13).

Alcoholic ketoacidosis has been reported in 7–10% of alcoholic patients suffering sudden death (14). β-hydroxybutyrate levels >2.5 mM are considered lethal (15), and a typical episode involves binge drinking with subsequent reduced caloric intake (16). Ethanol levels are frequently low or absent (<10 mg/dL) at autopsy, as the victims have often stopped drinking after the onset of symptoms (17,18). Alcoholic ketoacidosis accounts for up to 25% of cases of ketoacidosis (19), occurring once for every four cases of diabetic ketoacidosis (17). It results from low protein and carbohydrate stores, and/or alcoholic liver disease with subsequent hepatic glycogen depletion, a situation that causes a fall in blood glucose concentrations with suppression of insulin secretion (14,19). Volume depletion caused by vomiting, decreased fluid intake, and/or diaphoresis can lead to hypotension and a sympathetic response, further decreasing insulin production and increasing catecholamines, cortisol, growth hormone, and glucagon levels (14,18). Starvation and hypothermia may also lead to an elevation in these hormone levels, which in conjunction with the direct affect of ethanol, promotes lipolysis, and increases the supply of fatty acids to the liver (14). When this exceeds the rate of oxidation, a surplus of acetyl-CoA results and is converted to acetoacetate (20). In addition, ketogenesis is favored by the inhibition of gluconeogenesis and decreased pyruvate levels. Subsequent accumulation results, as clearance of ketoacids is also impaired by volume depletion and low plasma insulin (17).

In the present series, typical basal epithelial vacuolization in renal tubular epithelial cells was found in three of the five cases (60%) of alcoholic ketoacidosis that fulfilled the criteria for the study. In addition, these so-called Armanni–Ebstein changes were present only in cases where there was significant ketoacidosis and were not related to blood ethanol levels. While vitreous glucose levels decline after death, in our experience markedly elevated levels can still be detected for many days after death (10), and thus, the glucose levels in the three cases with basal vacuolizations were not considered to have been significantly raised prior to death. In addition, there was no history of diabetes mellitus in any of these victims. Although the numbers are small, the detection of renal tubular epithelial vacuolization in cases where β-hydroxybutyrate levels were elevated in the absence of hyperglycemia may provide further evidence for the role of disordered lipid metabolism in the pathogenesis of this phenomenon.

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