JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, November 2011, Vol. 56, No. 6
doi: 10.1111/j.1556-4029.2011.01865.x
Available online at: onlinelibrary.wiley.com

PAPER

PATHOLOGY/BIOLOGY

Chong Zhou[1]; John D. Gilbert,[2] *F.R.C.P.A.*; and Roger W. Byard,[1] *M.D.*

How Useful is Basal Renal Tubular Epithelial Cell Vacuolization as a Marker for Significant Hyperglycemia at Autopsy?

ABSTRACT: Basal vacuolization of renal tubular epithelial cells (so-called Armanni–Ebstein phenomenon) has been attributed to hyperglycemia causing accumulation of cytoplasmic glycogen. Review of 34 autopsy cases with significant hyperglycemia (vitreous glucose ≥15 mmol/L/270 mg/dL) was undertaken to determine whether there was any significant association between the degree of hyperglycemia and the severity of this morphological change (graded as 0, 1+, 2+, and 3+). No association was demonstrated. Review of the subgroup of 14 cases with terminal hyperglycemia without ketoacidosis was then undertaken to assess the effect of hyperglycemia in isolation on renal tubular epithelial cells. Vitreous glucose levels in these 14 cases ranged from 17 to 49.7 mmol/L (306–894.6 mg/dL) with a mean of 26.25 mmol/L (472.5 mg/dL) and β-hydroxybutyrate levels ranged from 0.02 to 2.55 mmol/L (0.36–45.9 mg/dL) with a mean 0.79 mmol/L (14.22 mg/dL). Not one of the latter cases displayed basal vacuolization. No relationship between basal vacuolization of renal tubular epithelial cells at autopsy and terminal hyperglycemia could, therefore, be demonstrated.

KEYWORDS: forensic science, diabetes mellitus, hyperglycemia, Armanni–Ebstein, lipid, ketoacidosis

The term "Armanni–Ebstein phenomenon" has been used to refer to basal vacuolization of renal tubular epithelial cells and has been strongly associated with poorly controlled diabetic states. It has been asserted that hyperglycemia with subsequent glucosuria leads to accumulation of cytoplasmic glycogen and that the observed vacuolization represents a type of "glycogen nephropathy" (1–5). Although there has been evidence put forward to support lipid, rather than glycogen accumulation within affected cells (6–8), the precise pathogenesis remains unclear. To further investigate the association between terminal hyperglycemia and renal tubular epithelial cells basal vacuolization, the following study was undertaken.

Materials and Methods

Case files over a 7-year period from January 2004 to February 2010 at Forensic Science SA, Adelaide, South Australia, were retrospectively reviewed for cases where death was attributed, or significantly contributed to, by hyperglycemia. Hyperglycemia was assessed by routine biochemical analysis of samples of vitreous humor taken at autopsy in a series of patients who had presented with histories of diabetes mellitus. Significant hyperglycemia was recorded when the vitreous glucose was ≥15 mmol/L (270 mg/dL). Case files were reviewed, and histologic sections of kidney were then blindly assessed for the presence of basal vacuolization of tubular epithelial cells, the so-called Armanni–Ebstein phenomenon.

[1]The University of Adelaide Medical School, Frome Road, Adelaide, SA, 5005, Australia.
[2]Forensic Science SA, 21 Divett Place, Adelaide, SA 5000, Australia.

Received 20 March 2010; and in revised form 13 Sept. 2010; accepted 16 Oct. 2010.

Cases were graded histologically as 0 if there was no evidence of vacuolization, 1+ if there was focal mild change, 2+ if there were focal severe or diffuse mild changes, and 3+ if there were moderate to marked diffuse changes (Fig. 1). Cases where the kidneys were found to be too autolyzed/putrefied for accurate identification of lesions were excluded from the study. Vitreous glucose levels were then plotted against their respective histologic grade.

To study the effects of glucose in isolation, all cases with associated ketoacidosis, taken as a vitreous β-hydroxybutyrate ≥5 mmol/L (90 mg/dL), were then excluded.

Results

A total of 55 cases with terminal hyperglycemia were identified, 14 were excluded as a result of insufficiently raised vitreous glucose levels (vitreous glucose <15 mmol/L or 270 mg/dL), one case was excluded as β-hydroxybutyrate levels were not tested for, and six cases were excluded as the extent of autolysis/putrefaction impeded accurate identification of basal vacuolizations, leaving a total of 34 cases. Plotting vitreous glucose levels against the respective histologic grade in these 34 cases showed no significant association between the degree of hyperglycemia and the severity of these lesions (Fig. 2).

Deaths in the 34 cases were caused by diabetic ketoacidosis ($N = 20$), ischemic heart disease ($N = 6$), hyperglycemic hyperosmolar nonketotic coma ($N = 2$), mixed drug toxicity ($N = 2$), trauma ($N = 2$), pulmonary thromboembolism ($N = 1$), and aspiration pneumonia ($N = 1$). All victims were found dead or in extremis with no cause of death being determinable prior to autopsy.

Twenty of the 34 cases had concurrent ketoacidosis (vitreous β-hydroxybutyrate ≥5 mmol/L or 90 mg/dL) and were subsequently excluded, resulting in a total of 14 cases with terminal

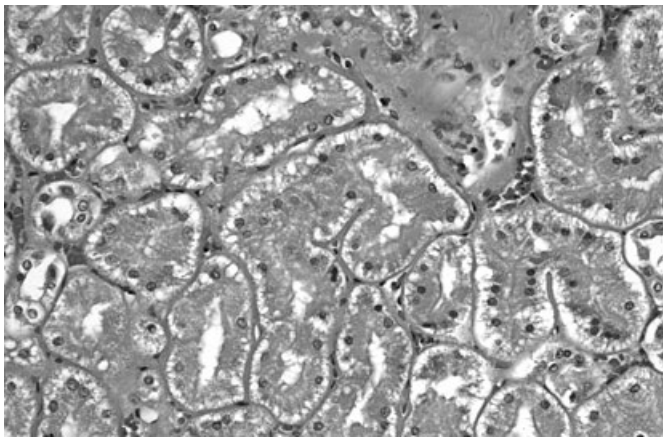

FIG. 1—*Typical basal renal tubular epithelial cell vacuolization of renal tubular epithelium showing diffuse basal subnuclear vacuolization (Grade 3) (hematoxylin and eosin × 120).*

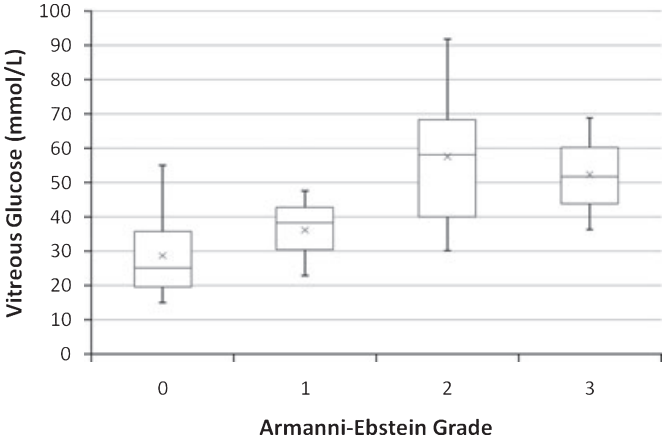

FIG. 2—*Plotting vitreous humor glucose levels against the grade of basal renal tubular epithelial cell vacuolization in 34 cases of hyperglycemia where vitreous glucose ≥15 mmol/L (270 mg/dL). In 20 cases, the vitreous β-hydroxybutyrate was ≥5 mmol/L (90 mg/dL). (Standard box and whisker plot: whiskers = maximum and minimum data values, upper limit = 75th percentile, lower limit = 25th percentile, center line = median,* x *= mean.)*

hyperglycemia in isolation. The age range was 14–60 years (mean 44.9 years) with a male to female ratio of 2.5:1. Four victims had type 1 diabetes, eight had type 2, and the nature of the diabetes was uncertain in two cases. The postmortem interval ranged from 1 to 6 days, with an average of 3 days. Vitreous glucose levels ranged from 17 to 49.7 mmol/L (306–894.6 mg/dL) with a mean of 26.25 mmol/L (472.5 mg/dL), and β-hydroxybutyrate levels ranged from 0.02 to 2.55 mmol/L (0.36–45.9 mg/dL) with a mean of 0.79 mmol/L (14.2 mg/dL). None of these cases displayed basal vacuolization of the renal tubular epithelial cells.

Discussion

Diabetes mellitus is one of the most common endocrine disorders, affecting almost 6% of the world's population (9). Type 1 diabetes mellitus constitutes the minority of cases (5–10%) and is characterized by early onset in childhood or adolescence. It is because of an autoimmune process with T-cell-mediated destruction of pancreatic β-cells and an absolute insulin deficiency. The clinical onset is often abrupt, involving hyperglycemia and ketosis once more than 90% of β-cells have been destroyed (10). Most diabetic patients have type 2 disease, which tends to manifest later in life. The pathogenesis is multifactorial, not related to an autoimmune process, but instead involves the interplay of multiple environmental and genetic factors resulting in insulin resistance and β-cell dysfunction owing to functional exhaustion (9,10).

Both type 1 and type 2 diabetes mellitus can cause significant hyperglycemia as lack of insulin or insulin resistance leads to decreased glucose uptake and utilization by most cells of the body, with increased hepatic glucose output because of accelerated glycogenolysis and gluconeogenesis (11). Insulin deficiency and/or resistance also causes increased lipolysis, with breakdown of triglycerides and increased proteolysis, delivering more free fatty acids and amino acids to the liver as substrates for gluconeogenesis. Consequently, plasma glucose levels rise leading to hyperglycemia and impaired renal function (12). Lethal hyperglycemia may occur in isolation, as in the present cases, with death resulting from hyperosmolar nonketotic coma and/or dehydration (12,13).

At autopsy, diabetes mellitus may be identified by elevated vitreous glucose and ketone levels. Multiorgan involvement is characteristic, and there may be ischemia of the lower extremities with gangrene, diabetic lipoatrophy, and evidence of subcutaneous insulin injection. The heart may be hypertrophied with coronary atherosclerosis and ischemic damage. The liver may also be enlarged with evidence of diabetic steatohepatitis (14). The kidneys may show evidence of diabetic nephropathy and microangiopathy, although histologic changes may be masked by putrefactive changes that may be accelerated in the presence of hyperglycemia.

Basal vacuolization of renal tubular epithelial cells in diabetes mellitus may be first suspected macroscopically when diffuse cortical pallor is noted on cut section (15). There is some discrepancy in the literature regarding the principal site of involvement, with the terminal straight portion of the proximal convoluted tubule (2) and the thick ascending limb of the loop of Henle being proposed (16). While Ritchie and Waugh localized these changes to the outer medulla, extending to the innermost cortex, but never appearing in the middle or outer cortex (2), Kock and Vestergaard noted Armanni–Ebstein changes throughout the cortex and the outer medulla, involving the proximal convoluted tubules, distal convoluted tubules, and the ascending limbs of the loop of Henle (3).

In addition to the dispute over the principal location of these so-called Armanni–Ebstein lesions, there is also disagreement regarding their composition and pathogenesis. Certain researchers have attributed the vacuolization to cytoplasmic glycogen accumulation (2,17–19); for this reason, Armanni–Ebstein lesions have been referred to as representing a type of "glycogen nephrosis" (1). It was proposed that these changes correlated with marked glucosuria and thus reflected excessive reabsorption of glucose in the antemortem period immediately before death (3). Thus, the nature and location of Armanni–Ebstein lesions remain somewhat controversial.

Evidence to support hyperglycemia as the major factor in the formation of these vacuoles holds that the affected portions of the renal tubules are normally exposed to little or no filtered glucose and therefore have reduced ability to handle this carbohydrate (2). The epithelial cells of the affected segments in diabetic kidneys also show abnormal concentrations of enzymes involved in glucose metabolism and thus may have a greater capacity for glycogenesis leading to glycogen accumulation (2,16). This may also be facilitated by both acidosis and dehydration that may cause excessive glycogenesis or defective glycogenolysis in tubular cells (2). Quantitative studies of antemortem blood glucose with associated tubular vacuolization have also been conducted, with Smith and Glickman reporting the presence of Armanni–Ebstein changes in patients with

blood glucose levels above 200 mg/dL in the 72-h period prior to death (5). In a study of alloxan-treated diabetic rats, Curtis et al. concluded that the appearance of these lesions "depended solely" upon the terminal blood glucose level; Armanni–Ebstein changes were invariably present with terminal levels above 350 mg/dL and consistently absent below 300 mg/dL (4).

Histologic evidence of glycogen accumulation has also been demonstrated with periodic acid-Schiff staining for glycogen in some (2,18), but not all studies (3). Electron microscopy has also on occasion demonstrated minute diffusely distributed granules characteristic of glycogen particles within the cytoplasm of affected cells (19).

However, more recent studies have suggested that the cytoplasmic vacuoles contain accumulated triglycerides rather than glycogen, with strong staining for lipids (6–8). The proposed pathogenesis for triglyceride accumulation is lipiduria as opposed to glucosuria, with the increased glomerular permeability in diabetic kidneys facilitating the filtration of lipids, which are then reabsorbed within the proximal tubules and synthesized into triglycerides in the mitochondria (8).

The current study was undertaken to further clarify the possible role of hyperglycemia in the pathogenesis of basal vacuolizations in renal tubular epithelial cells. A series of 34 cases with hyperglycemia were assessed for the degree of basal vacuolization; however, no correlation could be demonstrated between the level of glucose and the histologic grade of the morphological change. In addition, the cases where there was a significantly elevated vitreous glucose level (≥15 mmol/L) with a low β-hydroxybutyrate level were then identified so that the effect of elevated glucose in isolation could be observed on renal tubular epithelial cells. None of these cases showed basal vacuolization. These findings suggest that the pathogenesis of basal vacuolization of renal tubular epithelial cells in diabetes mellitus is not related simply to elevation of glucose levels, but to more complicated metabolic factors possibly involving either the effect of ketoacidosis or lipiduria on tubular cells.

References

1. Bamri-Ezzine S, Ao ZJ, Londoño I, Gingras D, Bendayan M. Apoptosis of tubular epithelial cells in glycogen nephrosis during diabetes. Lab Invest 2003;83:1069–80.
2. Ritchie S, Waugh D. The pathology of Armanni–Ebstein diabetic nephropathy. Am J Pathol 1957;33:1035–57.
3. Kock KF, Vestergaard V. Armanni–Ebstein lesions of the kidney: diagnostic of death in diabetic coma? Forensic Sci Int 1994;67:169–74.
4. Curtis GW, Robbins SL, Glickman I. Studies on glycogen nephrosis in alloxan-treated diabetic rats. J Exp Med 1947;85:373–9.
5. Smith ME, Glickman P. Diabetic vacuolation of the iris pigment epithelium. Am J Ophthalmol 1975;79:875–7.
6. Thomsen JL, Hansen TP. Lipids in the proximal tubules of the kidney in diabetic coma. Am J Forensic Med Pathol 2000;21:416–8.
7. Nielsen H, Thomsen JL, Kristensen IB, Ottosen PD. Accumulation of triglycerides in the proximal tubule of the kidney in diabetic coma. Pathology 2003;35:305–10.
8. Thomsen JL, Kristensen IB, Ottosen PD. The histological demonstration of lipids in the proximal renal tubules of patients with diabetic coma. Forensic Sci Med Pathol 2006;2:249–52.
9. Adeghate E, Schattner P, Dunn E. An update on the etiology and epidemiology of diabetes mellitus. Ann NY Acad Sci 2006;1084:1–29.
10. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, 8th edn. Philadelphia, PA: Elsevier Saunders, 2010.
11. Sherwood L. Human physiology: from cells to systems, 6th edn. Belmont, CA: Thomson Brooks/Cole, 2007.
12. Cydulka RK, Maloney GE Jr. Diabetes mellitus and disorders of glucose homeostasis. In: Marx JA, Hockberger RS, Walls RM, Adams JG, Barsan WG, Biros MH, Danzl DF, Gausche-Hill M, Ling LJ, Newton EJ, editors. Rosen's emergency medicine: concepts and clinical practice, 7th edn. St. Louis, MO: Mosby, 2009;1633–49.
13. Hamblin PS, Topliss DJ, Chosich N, Lording DW, Stockigt JR. Deaths associated with diabetic ketoacidosis and hyperosmolar coma. 1973–1988. Med J Aust 1990;152:327–8.
14. Ludwig J. Handbook of autopsy practice, 3rd edn. Clifton, NJ: Humana Press Inc., 2002.
15. Zhou C, Gilbert JD, Byard RW. Early diagnosis of Armanni–Ebstein phenomenon at autopsy. Forensic Sci Med Pathol 2010;6:133–4.
16. Rasch R, Østerby R. No influence of an aldose reductase inhibitor on glycogen deposition in tubules from streptozotocin diabetic rats. J Diabetes Complicat 1989;3:198–201.
17. Rasch R, Gøtzsche O. Regression of glycogen nephrosis in experimental diabetes after pancreatic islet transplantation. APMIS 1998;96:749–54.
18. Kumari K, Murthy PSR, Sahib MK. Monoaminoguanidine prevents sorbitol accumulation, nonenzymatic protein glycosylation and development of kidney lesions in diabetic rats. Experientia 1991;47:252–4.
19. Reyes AA, Kissane J, Klahr S. A high cholesterol diet ameliorates renal tubular lesions in diabetic rats. Proc Soc Exp Biol Med 1990;194:177–85.

Additional information and reprint requests:
Roger W. Byard, M.B.B.S., M.D.
Discipline of Pathology
Level 3 Medical School North Building
The University of Adelaide
Frome Road
Adelaide
SA 5005
Australia
E-mail: roger.byard@sa.gov.au