Liver Pathology in First Presentation Diabetic Ketoacidosis at Autopsy

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ABSTRACT

Diabetes mellitus is an enormous health burden on developed and developing nations. Eight percent of people in the United States are stated to have diabetes mellitus and 79 million people have impaired glucose tolerance. Sudden death from diabetic ketoacidosis (DKA) is common and nonalcoholic fatty liver disease (NAFLD) is a frequent finding in patients with diabetes mellitus and impaired glucose tolerance. Diabetic ketoacidosis accounts for around 1% of autopsy cases in our units and 25% of these cases did not have a previous diagnosis of diabetes mellitus. We have analyzed for the presence of NAFLD in 16 patients dying on first presentation of DKA. Some degree of NAFLD was present in all cases, with all but one case having some degree of steatosis and some degree of fibrosis was present in 14 out of 16 cases, though none were cirrhotic. Inflammation was present in nine of 13 cases and glycogenated nuclei in five of 13 cases. NAFLD can be well established in patients dying of DKA who were not known to be diabetic before death. The pathology shares features with alcoholic liver disease. They should not be mistakenly diagnosed as dying of other causes of ketoacidosis based upon the liver pathology present. *Acad Forensic Pathol.* 2016 6(2): 271-280

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This article does not contain any studies conducted with animals or on living human subjects

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INTRODUCTION

Diabetes mellitus (DM) is a common disorder and a major cause of morbidity and mortality (1). It is reported that 8% of the U.S. population has DM, with one third reported to be unaware they are diabetic and 1.9 million people are diagnosed with DM each year. It is also reported that 79 million people have impaired glucose tolerance, also known as being prediabetic. Worldwide, it is estimated there are 346 million people with DM, with significant numbers in China and India (1, 2). Thus, it is a global problem with a significant proportion of people at autopsy having DM or being prediabetic. Diabetes mellitus affects multiple organ systems and is associated with increasing obesity and one complication is nonalcoholic fatty liver disease (NAFLD). The prevalence of NAFLD varies between countries and ethnic groups. Its frequency is as low as 4.5% in non-diabetic Africans and as high as 99% in patients undergoing bariatric surgery. In the U.S. general population its incidence is probably over 25% and in some parts of China it is as high as 51% (3). Nonalcoholic fatty liver disease represents a spectrum of disorders that have by definition the presence of at least 5% hepatic steatosis (fatty liver) in individuals who consume less than 20 g of ethanol per week. The term nonalcoholic steatohepatitis (NASH) is used to describe a histological subset of NAFLD (4). NAFLD may show all the changes associated with alcoholic liver disease: steatosis, steatohepatitis, and steatofibrosis, even though the features of steatohepatitis, hepatocyte ballooning, Mallory-Denk bodies, and neutrophilic infiltration are often less prominent than they are in alcohol-related injury (5). Scoring systems have been developed for histologic examination of NAFLD (6-8).

Ketoacidosis is seen in a number of situations, most notably in diabetic and alcoholic ketoacidosis and is a well-recognized immediate cause of death (9-14). Without carbohydrates, due to insulin insufficiency or an absent intake, fats become the main energy source. Large quantities of fatty acid become available and are converted to ketone bodies. The ketone bodies acetoacetic acid, β -hydroxybutyrate, and acetone can be measured at autopsy. They provide the basis for the diagnosis of ketoacidosis (15-18). Postmortem metab-

olism of glucose makes determination of glucose at the time of death problematic. A high blood glucose concentration may represent diabetes mellitus, but may also result from resuscitation. In an analysis of 1000 cases by Coe, there was a peripheral blood glucose concentration above 27.8 mmol/L (500 mg/dL) in 103 nondiabetics with 87 recorded as due to resuscitation (19). Samples from the right side of the heart and the inferior vena cava will usually have a higher glucose concentration because of glycogenolysis in the liver and diffusion of the glucose into the blood vessels. In the cases of high blood glucose in non-diabetic patients, none had a raised vitreous glucose concentration. Coe found that a vitreous glucose above 11.1 mmol/L (200 mg/dL) (indicated diabetes) (19). Other biochemical tests that can be used to determine diabetes at autopsy include hemoglobin A_{1c} (HbA_{1c}) and fructosamine (20, 21). In hypothermia, vitreous glucose can be elevated from normal postmortem values, with Coe finding levels of 3.3-10.0 mmol/L (59.4 – 180 mg/dL) in over half of cases of hypothermia (19). Thus the main test of value to diagnose hyperglycemia at autopsy is vitreous glucose. A low vitreous glucose, however, does not indicate antemortem hypoglycemia as glucose concentrations quickly fall postmortem. Occasional cases may be encountered with a low vitreous glucose where HbA_{1c} is raised, confirming a diagnosis of diabetic ketoacidosis (DKA).

Deaths from DKA can be identified by a history of DM along with the biochemical markers of ketoacidosis, combined with a raised vitreous glucose concentration and/or where there is a raised HbA_{1c}. It may also be supplemented with pathological findings (e.g., diabetic nephropathy). The Armanni-Ebstein lesion and basal (subnuclear) vacuolation (**Image 1**) of the renal tubules are useful morphological changes associated with ketoacidosis, but may be seen in alcoholic ketoacidosis, starvation, and hypothermia, as well as DM (22-28). Basal (subnuclear) vacuolation (with fat in the vacuoles) has often been called the Armanni-Ebstein lesion of the kidney, though actually Armanni and Ebstein described a different lesion (29-31). In 2010 Byard pointed out that a clue to the diagnosis is the macroscopic appearance of the cortex with pallor present (32). Parai and colleagues have reported the same appearance in the kidney in alcoholic ketoacidosis (28).

As NAFLD is very common, we have examined the pathology of the liver in persons at autopsy that were shown to have diabetic ketoacidosis and were not previously known to have diabetes mellitus. The cause of death in these cases might be mistaken for other causes of ketoacidosis, particularly alcoholic ketoacidosis, based upon their liver pathology and where postmortem glucose concentrations are not available.

METHODS

Cases of ketoacidosis were identified in the files of the Toronto and Ottawa units of the Ontario Forensic Pathology Service in a five-year period from 2009 to 2013. Deaths with ketoacidosis in the immediate cause of death were identified. Ketone bodies were measured along with either vitreous glucose and/or HbA_{1c} to establish the presence of diabetes mellitus. Acetone was measured in each case. Routine β -hy-

Image 1: Basal vacuolation of proximal renal tubules (Osmium stain, x200).

droxybutyrate was introduced during the period of the study and not measured in all cases. Biographical data were collected, including age, sex, date of death, and height and weight (from which the body mass index was calculated) in each case.

Liver histopathology was examined in each case. Each liver section was stained using hematoxylin and eosin and a Masson trichrome. Each liver was scored based on the method of Kleiner et al. (5), but not all features were scored because the material was autopsy histology, not biopsy specimens. The following were scored:

- i) Degree of steatosis (**Image 2**) 0-3, with 0 = 5%, 1 = 5-33%, 2 = 33-66%, 3 = > 66%.
- ii) Location of steatosis 0-3; 0 = zone 3, 1 = zone 1, 2 = azonal, 3 = panacinar.
- iii) Fibrosis 0-4, 0 = none, 1 = persinusoidal or periportal (**Image 3**), 2 = periportal and portal/periportal, 3 = bridging fibrosis (**Images 2 and 4**), 4 = cirrhosis.
- iv) Lobular inflammation (**Image 5**) 0-3, 0 = none, 1 = < 2 foci per 200 x field, 2 = 2-4 foci per 200 x field, 3 = > 4 foci per 200 x field.
- v) Portal inflammation, 0 = none to minimal, 1 = greater than minimal.
- vi) Presence of glycogenated nuclei (**Image 6**), 0 = none to rare, 1 = many.

Image 2: Grade 3 panacinar steatosis (with bridging fibrosis) (H&E, x50).

RESULTS

During this period, a total of 9332 autopsies were performed in the study group. In 151 deaths, ketoacidosis was given in the immediate cause of death, which constituted 1.6% of all deaths. Twenty-one of the 151 cases were excluded because ketoacidosis was not the principal cause of death or was excluded because the exact cause of ketoacidosis was not determined. Of the remaining 130 cases, 82 cases were confirmed as cases of diabetic ketoacidosis, representing 0.9% of all deaths; 48 deaths were due to alcoholic ketoacidosis (AKA), which constituted 0.5% of all deaths.

Of the 82 cases of DKA cases, four were excluded because there was insufficient data for further analysis. Twenty of the 78 (25.6 %) DKA deaths did not have a prior history of diabetes mellitus. Thirteen were male and seven, female. The age range of these first presentation patients was 30-68 years, with a mean of 50 years and a median of 51 years. The range of body mass index (BMI) in these cases was available in 19 cases and was 16-40.4 kg/m², with a mean of 26.6 kg/m² and median of 25.4 kg/m². A history of ethanol use was present in seven cases.

Eighteen cases from Toronto were analyzed for liver pathology. Two cases could not be scored at all because

Image 3: Perivenular fibrosis (Masson trichrome, x200).

of decomposition changes. Three cases could only be assessed for steatosis and fibrosis but not for inflammation or the presence or absence of glycogenated nuclei. The results are shown in **Table 1**. Microscopic examination identified some degree of steatosis in all but one case and was grade 2/3 for degree in seven of 16 cases and grade 2/3 for location in 12 of 16 cases. Fibrosis was present in 14 of 16 cases and was grade 2/3 in ten of 16 cases. None of the cases were cirrhotic. Portal and/or lobular inflammation was present nine of 13 cases. Glycogenated nuclei were seen in five of 13 cases.

DISCUSSION

Ketoacidosis constituted 1.6% of cases in our autopsy practice, with nearly 1% being cases of diabetic ketoacidosis. In a study from Maryland examining deaths from diabetic ketoacidosis, DKA deaths constituted just over 1.5% of all autopsies performed (13). The first presentation of diabetes mellitus as fatal ketoacidosis has been recognized in previous studies. In this study it constituted approximately 25% of all cases of DKA (20 of 78 cases). In the study from Maryland,

Image 4: Bridging fibrosis (and steatosis) (Masson trichrome, x100).

nearly 35% of cases of DKA were not known to be diabetic (13). In a study of 42 deaths from North Carolina, six cases did not have a previous diagnosis of diabetes (14%), with five being cases of DKA and one case hyperosmolar nonketotic coma (9).

The mean age of previously undiagnosed diabetics in our study was 50 years with a median of 51 years and a range of 30-68 years. In the Maryland study, the mean for previously diagnosed diabetics was 40.8 years (range 10-74), with undiagnosed diabetics

having a mean age of 42.6 years (range 14-70) and a median age of 43.5 years. Death during the first presentation of diabetic ketoacidosis forms a significant proportion of ketoacidosis deaths.

CONCLUSION

The prevalence of NAFLD is high in many different populations in many countries around the world and is associated with risk factors such as obesity, diabetes mellitus, and dyslipidemia (3). Advanced fibrosis is

Image 5: Lobar inflammation (H&E, x400).

the most significant predictor of mortality in NAFLD (33). This study has shown that significant degrees of nonalcoholic fatty liver disease are already present in a high proportion of these patients dying on first presentation of diabetes mellitus, with significant degrees

of fibrosis already established. These patients were generally not obese and had a median BMI of 25.4 kg/m². The presence of fatty liver disease with fibrosis cannot be assumed to be related to excess alcohol consumption at autopsy in people dying of ketoacidosis.

Image 6: Glycogenated nuclei (Hematoxylin, x400).

Table 1: Liver Histopathology Findings

Age/ Sex	Body Mass Index	Steatosis Degree	Steatosis Location	Fibrosis	Portal Inflammation	Lobular Inflammation	Glycogenated Nuclei
53F	20.4	0	0	1	0	0	0
35M	24.1	1	0	0	0	2	0
49F	37.4	1	2	2	0	0	0
30M	26.4	2	2	2	N/A	N/A	N/A
51M	28.2	3	3	3	1	1	1
47F	37.9	3	2	2	1	1	1
33M	29.3	1	2	1	0	0	1
41M	N/A	1	3	2	N/A	N/A	N/A
60F	17.4	3	3	3	1	1	0
55M	25.4	1	1	1	N/A	N/A	N/A
46F	30.2	1	2	0	0	1	0
51M	29.6	2	3	2	1	1	0
63M	27.9	2	3	2	0	0	0
60F	21	1	0	2	0	1	1
63F	16	1	2	1	0	1	0
53M	40.4	2	2	2	0	1	1

REFERENCES

- 1) Maitra A. The endocrine system. In: Kumar V, Abbas AK, Aster JC, editors. Robbins and Cotran pathologic basis of disease. 9th ed. Philadelphia: Elsevier; 2015. p. 1105-1119.
- 2) Danaei G, Finucane MM, Lu Y, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*. 2011 Jul 2; 378(9785):31-40. PMID: 21705069. [http://dx.doi.org/10.1016/s0140-6736\(11\)60679-x](http://dx.doi.org/10.1016/s0140-6736(11)60679-x).
- 3) Sayiner M, Koenig A, Henry L, Younossi ZM. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin Liver Dis*. 2016 May; 20(2):205-14. PMID: 27063264. <http://dx.doi.org/10.1016/j.cld.2015.10.001>.
- 4) Kleiner DE, Makhlof HR. Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. *Clin Liver Dis*. 2016 May; 20(2):293-312. PMID: 27063270. <http://dx.doi.org/10.1016/j.cld.2015.10.011>.
- 5) Theise D. Liver and gallbladder. In: Kumar V, Abbas AK, Aster JC, editors. Robbins and Cotran pathologic basis of disease. 9th ed. Philadelphia: Elsevier; 2015. p. 845-7.
- 6) Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005 Jun; 41(6):1313-21. PMID: 15915461. <http://dx.doi.org/10.1002/hep.20701>.
- 7) Brunt EM, Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. *World J Gastroenterol*. 2010 Nov 14; 16(42):5286-96. PMID: 21072891. PMCID: PMC2980677. <http://dx.doi.org/10.3748/wjg.v16.i42.5286>.
- 8) Tiniakos DG. Nonalcoholic fatty liver disease/nonalcoholic steatohepatitis: histological diagnostic criteria and scoring systems. *Eur J Gastroenterol Hepatol*. 2010 Jun; 22(6):643-50. PMID: 19478676. <http://dx.doi.org/10.1097/meg.0b013e32832ca0cb>.
- 9) Bell RA, Howard D, Norman M, et al. Medical examiner cases associated with previously undiagnosed diabetes mellitus, North Carolina, 1994. *South Med J*. 1998 Feb; 91(2):151-4. PMID: 9496867. <http://dx.doi.org/10.1097/00007611-199802000-00006>.
- 10) Thomsen JL, Felby S, Theilde P, Nielsen E. Alcoholic ketoacidosis as a cause of death in forensic cases. *Forensic Sci Int*. 1995 Oct 30; 75(2-3):163-71. PMID: 8586340. [http://dx.doi.org/10.1016/0379-0738\(95\)01783-6](http://dx.doi.org/10.1016/0379-0738(95)01783-6).
- 11) Pounder DJ, Stevenson RJ, Taylor KK. Alcoholic ketoacidosis at autopsy. *J Forensic Sci*. 1998 Jul; 43(4):812-6. PMID: 9670504. <http://dx.doi.org/10.1520/jfs14311j>.
- 12) Brinkmann B, Fechner G, Karger B, DuChesne A. Ketoacidosis and lactic acidosis--frequent causes of death in chronic alcoholics? *Int J Legal Med*. 1998; 111(3):115-9. PMID: 9587792. <http://dx.doi.org/10.1007/s004140050130>.
- 13) Ali Z, Levine B, Ripple M, Fowler DR. Diabetic ketoacidosis: a silent death. *Am J Forensic Med Pathol*. 2012 Sep; 33(3):189-93. PMID: 22835961. <http://dx.doi.org/10.1097/paf.0b013e31825192e7>.

- 14) Palmiere C, Augsburger M. The postmortem diagnosis of alcoholic ketoacidosis. *Alcohol Alcohol*. 2014 May-Jun; 49(3):271-81. PMID: 24334515. <http://dx.doi.org/10.1093/alcac/agt177>.
- 15) Guyton AC, Hall JE. Textbook of medical physiology. 10th ed. Philadelphia: Saunders; 2000. 1064 p.
- 16) Williamson DH, Mellanby J, Krebs HA. Enzymatic determination of D(-)- β -hydroxybutyric acid and acetoacetic acid in blood. *Biochem J*. 1962 Jan; 82: 90-6. <http://dx.doi.org/10.1042/bj0820090>.
- 17) Denmark LN. The investigation of beta-hydroxybutyrate as a marker for sudden death due to hypoglycemia in alcoholics. *Forensic Sci Int*. 1993 Dec; 62(3):225-32. PMID: 8307532. [http://dx.doi.org/10.1016/0379-0738\(93\)90211-r](http://dx.doi.org/10.1016/0379-0738(93)90211-r).
- 18) Iten PX, Meier M. Beta-hydroxybutyric acid--an indicator for an alcoholic ketoacidosis as cause of death in deceased alcohol abusers. *J Forensic Sci*. 2000 May; 45(3):624-32. PMID: 10855969. <http://dx.doi.org/10.1520/jfs14739j>.
- 19) Coe JI. Postmortem chemistry update. Emphasis on forensic application. *Am J Forensic Med Pathol*. 1993 Jun; 14(2):91-117. PMID: 8328447. <http://dx.doi.org/10.1097/00000433-199306000-00001>.
- 20) John WG, Scott KW, Hawcroft DM. Glycated haemoglobin and glycated protein and glucose concentrations in necropsy blood samples. *J Clin Pathol*. 1988 Apr; 41(4):415-8. PMID: 3366927. PMID: PMC1141467. <http://dx.doi.org/10.1136/jcp.41.4.415>.
- 21) Valenzuela A. Postmortem diagnosis of diabetes mellitus. Quantitation of fructosamine and glycated hemoglobin. *Forensic Sci Int*. 1988 Sep; 38(3-4):203-8. PMID: 3192143. [http://dx.doi.org/10.1016/0379-0738\(88\)90167-3](http://dx.doi.org/10.1016/0379-0738(88)90167-3).
- 22) Kock KF, Vestergaard V. Armanni-Ebstein lesions of the kidney: diagnostic of death in diabetic coma? *Forensic Sci Int*. 1994 Aug 10; 67(3):169-74. PMID: 7959473. [http://dx.doi.org/10.1016/0379-0738\(94\)90087-6](http://dx.doi.org/10.1016/0379-0738(94)90087-6).
- 23) Preuss J, Dettmeyer R, Lignitz E, Madea B. Fatty degeneration in renal tubule epithelium in accidental hypothermia victims. *Forensic Sci Int*. 2004 May 10; 141(2-3):131-5. PMID: 1506295. <http://dx.doi.org/10.1016/j.forsciint.2003.12.017>.
- 24) Nielson H, Thomsen JL, Kristensen IB, Ottosen PD. Accumulation of triglycerides in the proximal tubule of the kidney in diabetic coma. *Pathology*. 2003 Aug; 35(4):305-10. PMID: 12959765. <http://dx.doi.org/10.1080/00313020307540>.
- 25) Thomsen JL, Hansen TP. Lipids in the proximal tubules of the kidney in diabetic coma. *Am J Forensic Med Pathol*. 2000 Dec; 21(4):416-8. PMID: 11111809. <http://dx.doi.org/10.1097/00000433-200012000-00024>.
- 26) Zhou C, Byard RW. Basal renal tubular epithelial cell vacuolization and alcoholic ketoacidosis. *J Forensic Sci*. 2012 Jan; 57(1):126-8. PMID: 22074296. <http://dx.doi.org/10.1111/j.1556-4029.2011.01957.x>.
- 27) Milroy CM, Parai JL. Armanni-Ebstein lesion, ketoacidosis and starvation in a child. *Forensic Sci Med Pathol*. 2011 Jun; 7(2):213-6. PMID: 21213071. <http://dx.doi.org/10.1007/s12024-010-9213-6>.
- 28) Parai JL, Kodikara S, Milroy CM, Pollanen MS. Alcoholism and the Armanni-Ebstein lesion. *Forensic Sci Med Pathol*. 2012 Mar; 8(1): 19-22. PMID: 21805374. <http://dx.doi.org/10.1007/s12024-011-9264-3>.
- 29) Armanni L. [Fünf Autopsien mit histologischen Untersuchungen und klinischer Epicrise]. In: Cantani A., editor. Diabetes mellitus. Berlin: Vierzehnte Vorlesung; 1877. p. 315-29. German.
- 30) Ebstein W. [Weiteres über diabetes mellitus, insbesondere über die complication deselber mit Typhus abdominalis]. *Deutsches Arch Klin Med*. 1882; 30:1-44. German.
- 31) Zhou C, Yool AJ, Nolan J, Byard RW. Armanni-Ebstein lesions: a need for clarification. *J Forensic Sci*. 2013 Jan; 58 Suppl 1:S94-8. PMID: 22971043. <http://dx.doi.org/10.1111/j.1556-4029.2012.02274.x>.
- 32) Zhou C, Gilbert JD, Byard RW. Early diagnosis of Armanni-Ebstein phenomenon at autopsy. *Forensic Sci Med Pathol*. 2010 Jun; 6(2): 133-4. PMID: 20084554. <http://dx.doi.org/10.1007/s12024-009-9140-6>.
- 33) Brunt EM, Kleiner DE, Wilson LA, et al. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology*. 2011 Mar; 53(3):810-20. PMID: 21319198. PMID: PMC3079483. <http://dx.doi.org/10.1002/hep.24127>.