Original Article
Necrotic epithelial cells in proximal renal tubules of 2nd trimester fetuses: is this "acute tubular necrosis"?

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Abstract: The aim of this study is to describe the occurrence of necrotic tubular cells in kidneys of non-macerated fetuses. Methods: Description of histology and immunostaining results using C9 immunostain of proximal tubular epithelium of kidneys from 30 consecutive non-macerated fetuses' autopsies. Results: the gestational age ranged from 13 to 22 weeks. The mean gestational age was 18.6 weeks; the cause of death was acute chorioamnionitis in 13 cases (43.3%), termination of pregnancy for fetal anomalies in 13 (43.3%) and other causes in 4 (13.3%). Histology of the kidneys revealed vacuolation of proximal tubule epithelial cells (100%), dilatation of tubules (93.4%) and tubular cell necrosis (53.4%). C9 immunostaining was positive in 24 cases (80%) and was seen in all gestational ages. Conclusions: These results indicate that tubular cell necrosis is not an uncommon finding in the kidneys of 2nd trimester fetuses and may represent acute tubular necrosis (ATN). C9 is a helpful marker in confirming this diagnosis. Future studies may further explore this preliminary observation.

Keywords: Fetus, C9, autopsy, hypoxic-ischemic lesion, kidney, tubular necrosis

Introduction

Tubular dilatation, vacuolation and deeply eosinophilic cells, representing necrotic cells, in the epithelium lining the proximal renal tubules are classic features of Acute Tubular Necrosis (ATN) [1]. Although tubular cell necrosis is not a constant feature in the clinical setting of this condition, which is now termed Acute Kidney Injury (AKI) [2], it is frequently observed in post mortem examinations of children and adult patients where it is interpreted as part of the hemodynamic failure that culminates in death.

In our daily practice in perinatal pathology, we frequently identify the above described changes in non-macerated fetuses. We are not aware that these features have been described in the English language medical literature.

C9 is part of the membrane attack complex (C5b-9) of the complement system and is a reliable marker of complement deposition which occurs in necrotic cells of different tissues and organs [3-6]. Therefore, immunostaining with anti-C9 antibody is recognized as highly specific and its use has proven to be useful in demonstrating necrosis in different tissues, such as acute myocardial infarction [4] and hypoxic-ischemic brain lesions [6].

The aim of our study was to describe the occurrence of epithelial cell necrosis in the proximal renal tubules of 30 consecutive autopsies of the second trimester fetuses, confirmed with C9 immunostaining, and discuss its potential links to ATN.

Material and methods

The reports, slides and tissue blocks of 30 consecutive post mortem examinations of non-macerated fetuses performed in a 12 months period with appropriate consent for research were retrieved from the files. The main criterion for inclusion was a non-macerated fetus. Induction of labour was the method used in cases of termination of pregnancy.

Haematoxylin and Eosin (H&E)-stained slides containing both kidneys were reviewed using a Nikon Eclipse 80i light microscope with

Table 1. Histological and C9 immunostaining findings in the renal tubules from fresh, non-macerated fetuses submitted to post mortem examination.

Finding	Score (%)			
	0	1	2	3
Vacuolation	0	7 (23.3)	4 (13.3)	19 (36.3)
Dilatation	2 (6.66)	4 (13.3)	6 (20)	18 (60)
Necrosis	14 (43.6)	6 (20)	6 (20)	4 (13.3)
C9 positivity	6 (20)	19 (63.3)	5 (16.6)	0

Scores: 0= absent; 1= occasional (finding noted in a few cells and in focal areas); 2= moderate (intermediate between occasional and diffuse); 3= diffuse (finding in multiple cells and in multiple areas)

attached Nikon DS-Fi1 digital camera. The presence or absence of the following changes in the proximal tubules was recorded: deeply eosinophilic epithelial cells (interpreted as necrotic cells), vacuolation of epithelial cells and dilatation of tubular lumen.

Immunostaining for C9 was performed on formalin-fixed, paraffin-embedded sections of representative sections of the kidney. Sections were cut at a thickness of 4 μm, mounted onto coated Dako IHC Flex slides (Dako Cambridge House, St Thomas Place, Ely, Cambridgeshire, U.K., CB7 4EX) and stored overnight at 37°C. Slides were deparaffinized, rehydrated via xylene and graded alcohols, followed by heat-induced epitope retrieval. Pre-treatment was performed using Dako PT-Link at pH 6.0 for 20 min at 97°C. Endogenous peroxide was blocked with Envision tm Peroxide-blocking reagent (SM801) for 5 min. Primary anti-C9 antibody was applied to the slides for 60 min at room temperature, then incubated with Envision™ Flex⁺ Mouse for 15 min. Subsequent steps were carried out on a Dako Autostainer Link 48 immunostainer. Slides were incubated with anti-C9 antibody (Abcam plc, Cambridge, U.K.) at a dilution of 1:50 for 60 min at room temperature using Dako Envision™ Flex⁺ Plus. The tissues were then treated with Envision™ Flex⁺ mouse linker for 15 min and subsequently with Envision™ Flex/HRP for 30 min. DAB was applied for 10 min and the slides were counterstained with Hematoxylin for 5 min. Acute myocardial infarction was used as a positive control. The same tissue was used as negative control by omitting the primary antibody.

C9 was considered positive when crispy, brown DAB pigment was identified on tubular epithelial cells in dilated tubules.

The scores for all findings, whether histological or immunostaining, were defined as 0= absent; 1= occasional (finding noted in a few cells and in focal areas); 2= moderate (intermediate between occasional and diffuse); 3= diffuse (finding in multiple cells and in multiple areas).

The study was approved by South Yorkshire Ethics Committee REC reference number 09/H1308/132 and funded by Sheffield Children's Hospital Charity CA090018.

Results

The mean gestational age of the 30 cases studied was 18.6 weeks, ranging from 13 to 22 weeks. The cause of death was acute chorioamnionitis in 13 cases (43.3%), termination of pregnancy for fetal anomalies in 13 (43.3%) and miscarriage due to different causes in 4 (13.3%) (2 cases of small placenta, one twin fetus with associated hydatidiform mole in the other placenta and one case of unknown cause). None of the cases had any macroscopic abnormality in the kidneys, ureters or the lower urinary tract.

Histological evaluation revealed adequate maturation for gestational age and a range of changes summarised in **Table 1**. The most constant finding (present in all cases) was vacuolation of epithelial cells from proximal tubules (**Figure 1B**), which was frequently diffuse. Widespread dilatation of proximal tubules was another common finding, seen in 93.4% of cases (**Figure 1B**). Intense cytoplasmic eosinophilia, indicative of cell necrosis (**Figure 1C**), was present in 16 (53.4%) cases.

Immunostaining for C9 was positive in 24 cases (80%), of moderate intensity in 5 cases (16.6%) and occasional in 19 cases (63.3%), with no case showing diffuse and intense positivity. Positive C9 immunostain was most of the times
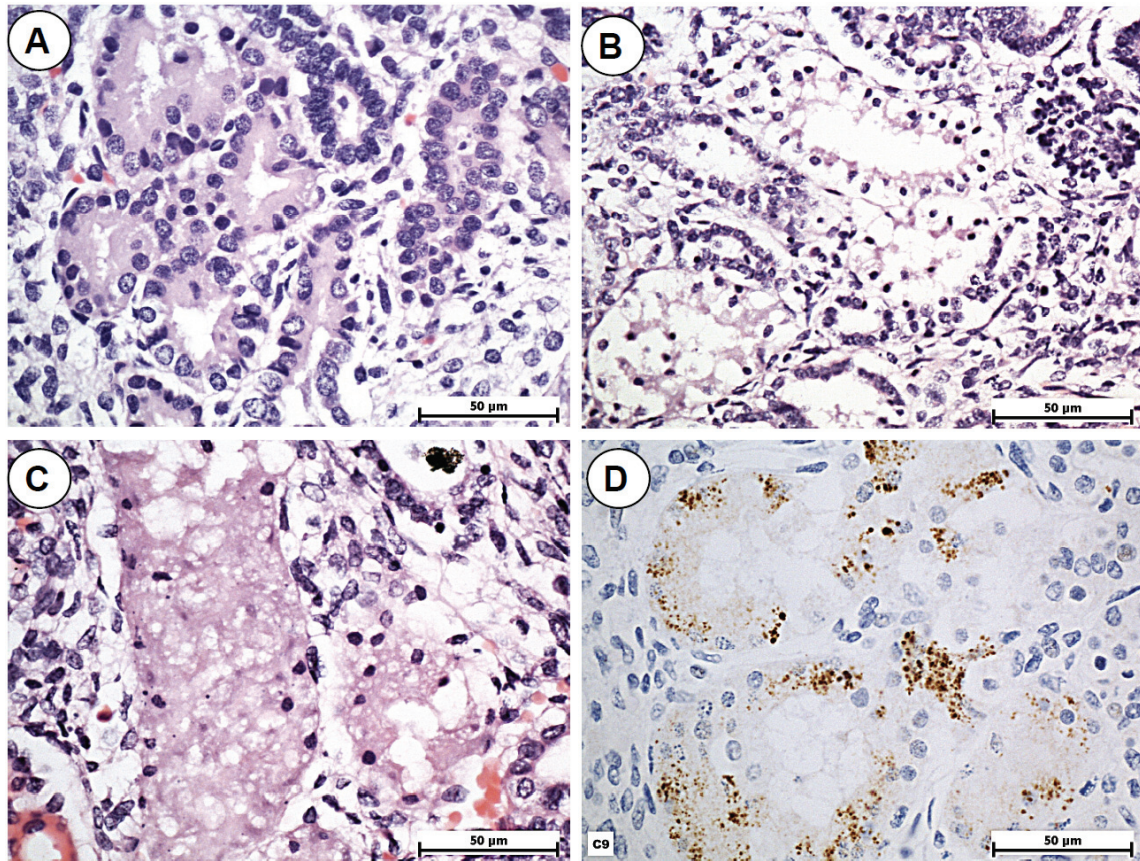
Figure 1. All pictures correspond to kidneys of 13-week fetuses. A. fetus with no acute tubular necrosis for comparison. Note the small size of the tubules, narrow lumen and preserved tubular cells (H&E, x60). B to D is from a case with acute tubular necrosis. B. Histology of kidney depicting dilated tubules and vacuolated tubular cells (H&E, x60). C. In another area the dilated proximal tubules contain eosinophilic tubular cells indicative of necrosis (H&E, x60). D. Immunostain with C9 of this kidney reveals strongly positive cells in the dilated tubules, confirming the necrosis (C9 immunostain, x60).

noted on the cells which were eosinophilic on histological examination, confirming their necrotic nature, but it was also noted on vacuolated cells, and therefore it was more sensitive in identifying necrotic cells than histology. Positive C9 immunostain was noted in all gestational ages, the youngest being 13 weeks (**Figure 1D**). There was no difference regarding positivity for C9 and the cause of death among the three groups.

Discussion

Tubular dilatation, tubular epithelial cell vacuolation, representing tubular cell oedema, and necrosis of epithelial cells are histological features observed in ATN [1]. These features were present in a significant proportion of our cases

and are not the result of autolysis. Although ATN is frequently seen in post mortem examinations of neonates, children and adults, mainly in the context of hypoxic-ischemic conditions [7, 8], we have been unable to identify any previous observation of the occurrence of tubular cell necrosis in the non-viable human fetus. This is mainly due to the rapid autolysis of tubular epithelial cells after death [9]. In our study, all cases were non-macerated in order to avoid false positive or false negative results.

C9 immunostaining proved to be more sensitive in the identification of necrotic tubular cells than using H&E-stained sections alone. This is in keeping with previous studies which demonstrated that C9 identifies necrotic cells in acute myocardial infarction [4], hypoxic-ischemic brain

lesions [6], and laminar necrosis of the placental membranes [10].

Tubular dilatation and tubular cell vacuolation represent cell oedema and therefore are reversible changes, whereas tubular cell necrosis, whether identified with histology or C9 immunostain, is not and from the morphological point of view are necessary for the diagnosis of ATN. According to this, 80% of the cases presented ATN, which is explained by the acute mode of death.

One interesting finding was C9 immunostaining positivity at all gestational ages studied. C9 positivity has been previously demonstrated in the serum of fetuses with gestational age over 18 weeks [11] and it has been assumed that it is not seen in very low gestational age. Our findings contradict this view, as we found C9 expression even at 13 weeks (**Figure 1D**), demonstrating that even in such a very low gestational age both the mechanisms responsible for tubular cell necrosis and C9 deposition are operative.

Although we did not restrict gestational age, all our cases were at or below 22 weeks and this can be explained by the main inclusion criteria of non-macerated fetuses.

It is well recognised that acute chorioamnionitis is a trigger of spontaneous abortion in this age group and fetuses are often non-macerated [12]. Acute chorioamnionitis can cause ATN through secondary fetal sepsis, which induces the release of inflammatory cytokines, increased blood vessels permeability and acidosis.

The processes of miscarriage and termination of pregnancy involve the separation of the placenta with resultant reduction and loss of blood supply to the fetus, inducing hypoxic-ischemic lesion.

Due to the widespread use of ultrasound scan in early pregnancy, most of the major congenital abnormalities can be detected and termination of pregnancy is offered to the parents. Termination of pregnancy in our cases was done by induction of labour and not feticide as this is the usual procedure in this gestational age period [13]. This explains why these fetuses are born non-macerated and may undergo a hypoxic event prior to death.

Hypoxia initially causes increased cardiac output and heart rate to maintain effective circulation. Then it leads to redistribution of the blood flow to fetal organs. The brain, lungs and adrenals are the preferentially perfused organs, whereas blood flow to the gut, spleen, kidneys and limbs is reduced [7]. Previous studies have indicated that irreversibility of "fetal shock" is caused by the development of coagulation disorders and fibrin deposits in fetal organs, fall in blood pressure, fetal acidosis and increased permeability of the blood vessels [14].

Intravascular volume contraction or decreased effective blood volume that reduces blood flow to the kidneys is regarded as a "pre-renal" cause of renal failure, which is reversible when the circulatory changes are restored to normal. When sufficiently prolonged, pre-renal injury can result in AKI due to hypoxic-ischemic ATN [15]. The evolution of pre-renal injury to ATN is not sudden, and several compensatory mechanisms maintain renal perfusion when renal hemodynamics are not optimal. With increasing intensity of renal insults, and importantly, as they are multiplied, tubular injury develops, as has been documented by urinary detection of biomarkers such as cytokines (IL-6, IL-8, IL-18), NGAL, NAG, and KIM-1 [2].

The mechanism of hypoxic/ischemic AKI may involve disturbed vascular tone associated with endothelin or Nitric Oxide release, depletion of ATP, interference with the cytoskeleton and heat shock protein [15].

In summary, tubular epithelial cell necrosis is not an uncommon finding in post mortem examination of second trimester fetuses and its identification and nature can be demonstrated by C9 immunostain. This finding may indicate the occurrence of a terminal hypoxic/ischemic event. Further research is needed to explore this initial observation.

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