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Technical note

Histological changes in the umbilical artery following severe chorioamnionitis and funisitis may be indicative of early atherosclerosis

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ABSTRACT

We investigated whether histological evidence of early atherosclerosis was present in the umbilical artery of 21 pregnancies complicated by severe perinatal inflammation, and 21 controls matched for gestational age, sex and birth weight. Severe chorioamnionitis with funisitis was associated with increased numbers of CD68 and CD45 positive cells (both $P < 0.01$), indicating accumulation of monocyte-derived macrophages in lesion-susceptible regions. A down-regulation of SMA expression ($P = 0.01$) was also observed. These preliminary findings suggest that chorioamnionitis with funisitis may promote changes in the intima and media of the umbilical artery similar to that seen in early atherosclerosis.

1. Introduction

Atherosclerosis is a chronic inflammatory process characterised by immune cell recruitment, lipid accumulation, smooth muscle proliferation and arterial wall thickening [1]. Although the clinical manifestations of atherosclerosis are apparent in adulthood, histological changes indicative of early atherosclerosis, including fatty streaks [2] and intimal thickening [3] may occur in the aorta prior to birth. These features are associated with perinatal cardiovascular risk factors in the mother, including hypercholesterolaemia, diabetes and smoking [4]. Increased umbilical artery intima-media thickness is reported following pregnancies complicated by diabetes [5]. Inflammation is a common mechanism mediating pathogenesis of these maternal risk factors, but there are no data linking perinatal inflammation with early histological changes of atherosclerosis in the umbilical artery. We therefore investigated whether the umbilical arteries from pregnancies complicated by severe chorioamnionitis with funisitis showed histopathologic evidence similar to that seen in early atherosclerosis.

2. Methods

Paraffin-embedded umbilical cord tissue from pregnancies complicated by severe histological chorioamnionitis with funisitis ($n = 21$) and controls matched for gestational age (completed weeks), sex and birth weight (±500 g) ($n = 21$) were identified from recent births at the Royal Women's Hospital (Melbourne, Australia). Severe chorioamnionitis with funisitis was defined by histological evidence of both maternal and fetal inflammatory response ≥ stage 2 by widely used semi-quantitative criteria [6]. All umbilical cords were from pregnancies uncomplicated by maternal and fetal abnormalities, and had two arteries and one vein. Histological examination was undertaken blinded to the case/control status.

3 μm sections were cut from each paraffin embedded tissue block for H&E staining and immunohistochemistry. Primary

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antibodies used included anti-CD68 (macrophages), anti-CD45 (leucocyte common antigen), anti-SMA (alpha-smooth muscle actin), and monoclonal isotype controls mouse anti-IgG1, mouse anti-IgG2a and rabbit anti-1gG (all Dako, Glostrup, Denmark); anti-PCNA (proliferating cell nuclear antigen) (NovoCastra, Newcastle, UK); and anti-LOX1 and anti-ICAM-1 (intracellular adhesion molecule 1) (Abcam, Cambridge, UK). A post-mortem specimen of adult aorta with advanced atherosclerosis served as a positive control. Proteins were visualised using a Dako REAL™ EnVision™ Detection System, Peroxidase/DAB, Rabbit/Mouse, Kit (Dako).

Slides were imaged using high resolution Aperio software (Leica Microsystems Pty Ltd, NSW, Australia) at x40 magnification and processed using the Fiji distribution of ImageJ [7]. Before counting cell populations, the vessel of interest was outlined to select the vessel intima and media, and to exclude the lumen and surrounding tissue. The mean percentage of positively stained cells was compared between groups using Student's t-tests with Stata/IC version 13.0 (StataCorp LP, Texas, USA).

3. Results

Mean birth weight (SD) in cases (3012.91 (675.77) g) was similar to controls (3045.91 (704.19) g; $P = 0.88$), gestational age (mean 37.24 (3.21) weeks) and sex (43% males) were identical between groups. H&E stained sections from inflamed umbilical arteries showed increased numbers of acute inflammatory cells, with smaller numbers of non-foamy histiocytes and occasional eosinophils, associated with apoptotic debris, and oedema (Fig. 1). Where visible in the plane of section, inflamed umbilical arteries showed fragmentation and disruption of the internal elastic lamina, which was not observed in control arteries. Cholesterol clefts, intimal and/or medial hyperplasia were not identified. Positive immunostaining for macrophage markers CD68 and CD45, and for SMA-positive smooth muscle cells was observed (Fig. 2). ICAM-1, LOX1 and PCNA immunostaining was not detected. The mean (SD) percentage of positively stained CD68 (11.79 (7.25) and 2.62 (1.23); $P < 0.01$) and CD45 (21.83 (16.49) and 5.10 (5.19); $P < 0.01$) cells were significantly higher in umbilical arteries from pregnancies exposed to severe chorioamnionitis compared to controls, respectively, whereas the number of SMA-positive cells was significantly lower in those exposed to funisitis (86.86 (7.97) and 94.50 (5.79); $P = 0.01$).

4. Discussion

Severe chorioamnionitis with funisitis is associated with significantly increased numbers of non-foamy CD68 and CD45 positive macrophages in the intima and media of the umbilical artery, indicating accumulation of monocyte-derived macrophages in atherosclerosis-prone regions of the arterial wall. These findings are consistent with a vasculitis, however, they are also analogous with the initial cellular changes observed in early (type I) atherosclerotic lesions that precede the development of type II (fatty streak) lesions [8]. Upregulation of the macrophage scavenger receptor CD68 is a critical step in lesion formation, preceding oxLDL uptake [9].

Pre-atherosclerotic lesions with macrophage abundance have previously been described in premature fetuses of hypercholesterolaemic mothers and closely resemble the cellular distribution in advanced adult atherosclerosis [2]. The increased

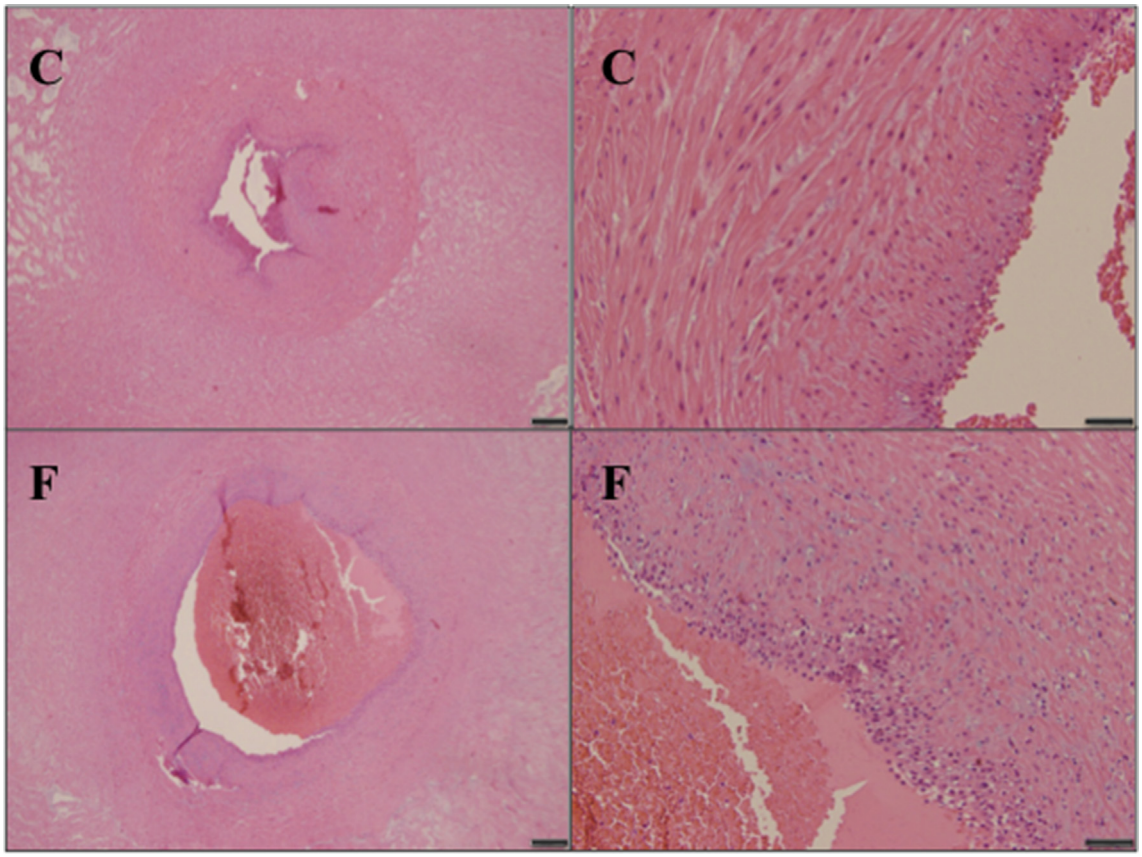

Fig. 1. H&E stained sections of umbilical cord showing umbilical artery of newborns with funisitis (F) and an acute inflammatory infiltrate, and controls (C) showing no inflammation. Magnification x40 with scale bar 200 μm (left), and magnification x200 with scale bar 50 μm (right).