Oesophageal mucosal changes in patients with varices

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SUMMARY Histological examination of oesophageal rings removed at transection for varices reveals dilated intraepithelial blood filled channels. These are present in all oesophageal rings removed at transection for varices. A comparison has been made between rings removed from variceal patients with oesophageal rings removed during resections for oesophageal and gastric tumours. Although a small number of non-varices patients had intraepithelial channels they were significantly larger and more numerous in the varices patients ($p < 0.01$). Similarly, the area and number of dilated subepithelial channels (just beneath the epithelium) and the area of lamina propria channels were significantly greater in the varices patients ($p < 0.05$). Depth of papillae and thickness of the squamous epithelium were also significantly greater in the varices group ($p < 0.05$, $p < 0.01$ respectively). Under electron microscopy the channels were lined with flattened cells which were not typical of endothelial cells but stained positively for Factor VIII related antigen using indirect immunofluorescence. These channels may correspond to the cherry-red spots seen on endoscopy and may have a role in the pathogenesis of variceal haemorrhage.

Oesophageal transection in the management of varices is becoming increasingly popular with encouraging results.¹ The procedure involves excision of a ring of oesophagus from the area of bleeding containing the full thickness of the wall followed by immediate anastomosis with a circular stapling gun. This provides the opportunity to examine tissue from the site of bleeding.

In a previous investigation to assess the incidence of oesophagitis in such tissue dilated blood-filled channels were consistently observed within the oesophageal epithelium in patients with varices.² This study investigates the nature of these channels and assesses their significance.

Methods

PATIENTS

Oesophageal rings were obtained from 27 patients undergoing transection for varices. A control group of 17 rings was obtained, in similar fashion, from patients undergoing oesophageal anastomoses after resection of gastric or oesophageal tumours.

The varices group consisted of 17 men and 10 women with mean age of 53.9 years (SD 19.6 years).

The control group consisted of 10 men and seven women with mean age 60.7 years (SD 13.4 years). The aetiology of portal hypertension is shown in Table 1. In the control group four had squamous cell carcinoma of the oesophagus, 12 had adenocarcinoma of the stomach, and one patient had a carcinoid tumour of the stomach.

The rings, measuring 1-1.5 cm in length, were placed in mercuric formol, opened and the mucosal surface examined. Blocks of this tissue were taken in the longitudinal axis of the oesophagus and the entire circumference of the ring was blocked. Ten to 15 tissue blocks were obtained from each ring. The tissue was then routinely processed for histological examination. The sections were coded and four sections were selected randomly from each case for detailed histological examination.

The sections were projected on to 0.1 inch squared graph paper at a constant magnification ($\times 40$). The outline of the various components of the section were traced on to the paper. By counting the squares, the following information was obtained on each section - the relative area of the squamous epithelium, relative area of lamina propria, relative area and number of dilated intraepithelial channels exceeding 0.05 mm in diameter (normal papillae were not counted). The relative area and number of subepithelial channels, defined as large blood filled channels lying immediately beneath the squamous epithelium, were also counted. Similarly the relative

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Table 1 Aetiology of portal hypertension

	Patients
Micronodular cirrhosis	16
Macronodular cirrhosis	6
Portal vein thrombosis	3
Cystic fibrosis	1
Unknown*	1

* Normal liver biopsy.

area and number of dilated vessels within the lamina propria exceeding 0.05 mm diameter were counted.

In addition a microscopic eyepiece graticule was used to measure the thickness of the squamous epithelium in three randomly selected well orientated sections from each case. The thickness of the basal zone of the epithelium and the depth of penetration of papillae into the epithelium were also measured and expressed as percentages of the thickness of the squamous epithelium. The latter measurements were made on rings from four additional patients with varices in addition to the original 27. In two control patients the rings were unsuitable for assessment owing to poor orientation. Therefore, epithelial morphology was assessed in a total of 31 variceal rings and 15 control rings.

For examination by electron microscopy, tissue was fixed in Karnovsky's fixative, washed in phosphate and S-Collidine buffer, prestained with uranyl acetate, embedded in Epon and stained with uranyl acetate and lead nitrate. Sections were examined using an Hitachi H600 electron microscope.

In two specimens indirect immunofluorescence has been used to assess the presence of Factor VIII related antigen on cells lining the intraepithelial

channels in frozen sections cut from unfixed material and post-fixed in acetone.

Factor VIII related antigen has been shown to be localised only on endothelial cells, platelets, and megakaryocytes.³ Indirect immunofluorescence has been shown to give reproducible results in determining the cellular origin of certain endothelial tumours⁴ and in investigating coagulation disorders.⁵

Oesophageal transection was carried out on four female normotensive greyhounds in order to see if the mucosal changes might be artefactual from the use of the gun. Mean weight was 23.4 kg. In two dogs the procedure was carried out using the Russian gun and in two using the American gun. The oesophageal rings were processed in the standard fashion, stained with haematoxylin and eosin and examined with light microscopy.

The means of measured values within the two groups were compared using the Student's *t* test.

Results

Dilated blood filled channels lying within (Fig. 1) and immediately beneath (Fig. 2) the squamous epithelium were found in all specimens from patients with portal hypertension. They were also found in 23% of specimens from non-varices patients although they were smaller and less numerous in this group. The channels were clearly visible on inspection of the mucosal surface of the oesophagus and were seen as haemorrhagic petechiae with occasional larger lesions resembling blood filled blisters.

These lesions were present in both deep and superficial parts of the squamous epithelium and

Fig. 1 Dilated blood-filled channels within squamous epithelium of oesophagus in patient with varices. Connection between one of channels and papillary capillary is seen. H & E $\times 80$ (original magnification).

Fig. 2 Large subepithelial channel. Blood is separated from oesophageal lumen only by thin layer of squamous cells. H & E $\times 40$ (original magnification).

occasionally were seen to have ruptured through the epithelial surface. The intraepithelial channels measured up to 0.3 mm in diameter. Subepithelial channels were larger and measured up to 2.5 mm in

diameter. All channels were congested with red blood cells but no evidence of clotting of blood was seen within the lumen.

Intraepithelial channels were usually symmetrical. In some cases direct communication was seen between papillary capillaries and the dilated channels (Fig. 1). Serial sectioning was carried out on some blocks and confirmed that the dilated channels were derived from capillary vessels.

Channels within the squamous epithelium were lined by flattened cells. Methenamine silver staining showed they were not enclosed by basement membrane (Fig. 3) and were thus not conventional blood vessels. In some cases small fragments of basement membrane were present. Electron microscopy confirmed the absence of basement membrane and showed that the channels were not lined by endothelial cells but that the red blood cells were in direct communication with flattened squamous cells showing evidence of degeneration but identified by tonofilaments and desmosomes (Fig. 4).

Immunofluorescent staining for Factor VIII related antigen, however, showed the presence of the antigen lining the dilated intraepithelial channels despite the absence of endothelial cells (Fig. 5).

Table 2 shows the results of the comparison of single variables between the two groups. Highly significant differences were found in the number and size of epithelial channels, in the length of papillae within the squamous epithelium. These variables were all greater in the varices group ($p < 0.01$). In addition, the ratio of epithelial channel area to squamous epithelium area was significantly higher in the varices group ($p < 0.01$).

The thickness of the squamous epithelium, the

Table 2 Single variables test

Variable	Mean (SE)		t value	Significance level
	Control (n=17)	Varices (n=27)		
Area of squamous epithelium	360.00 (38.20)	480.00 (40.80)	2.13	
Number of epithelial channels	0.28 (0.085)	2.81 (0.41)	5.96	$p < 0.01$
Area of epithelial channels	0.37 (0.11)	18.43 (4.10)	4.40	$p < 0.01$
Area of lamina propria	472.00 (175.00)	527.00 (77.10)	0.29	
Number of lamina propria channels	4.62 (0.65)	5.61 (0.57)	1.15	
Area of lamina propria channels	10.85 (2.33)	30.25 (4.71)	3.70	$p < 0.05$
Number of subepithelial channels	0.08 (0.04)	0.33 (0.08)	2.81	$p < 0.05$
Area of subepithelial channels	0.95 (0.54)	11.20 (3.93)	2.58	$p < 0.05$
Thickness of squamous epithelium*	0.31 (0.038)	0.36 (0.029)	2.86	$p < 0.05$
	(n=15)	(n=31)		
Basal zone thickness†	15.40 (3.70)	15.97 (2.45)	0.24	
	(n=15)	(n=31)		
Depth of papillae†	48.50 (4.20)	60.20 (4.10)	4.81	$p < 0.01$
	(n=15)	(n=31)		

* mm. † Percentage of total thickness of squamous epithelium. Except where stated the units refer to the number of graph paper squares.

Fig. 3 Methenamine silver staining shows basement membrane (B) lining intact intraepithelial capillaries. No basement membrane is seen lining intraepithelial channel (C). Methenamine silver $\times 150$ (original magnification).

number and area of the subepithelial channels and the area of dilated channels in the lamina propria were also significantly greater in the varices group ($p < 0.05$). No significant difference was observed in the other variables assessed. The variables were also analysed with respect to age, the aetiology of portal hypertension and type of tumour, where applicable. No significant difference was found in any of the variables with respect to these parameters.

Numerous sections were obtained from the dog transected rings and none showed any formation of intraepithelial channels.

Discussion

We have observed intraepithelial channels in all oesophageal transection rings from patients with

oesophageal varices. These channels have been described as vascular ducts by Cheli *et al.*,⁶ as vascularised epithelium by others,⁷ and have been interpreted as evidence of oesophagitis.

Geboes and others⁸ have termed these channels congestive venules and have suggested that they represent the early changes in oesophagitis. They have described these intraepithelial 'venules' separated from the oesophageal lumen by only a few epithelial cells and they feel that this corresponds to the erythema seen on endoscopy in some patients with symptoms of reflux oesophagitis.⁹ Kobayashi and Kasugai have expressed similar views on their significance.¹⁰ Others have expressed doubt that the venular dilatation correlates with early oesophagitis.¹¹ Evidence of oesophagitis as assessed

Fig. 4 Electron photomicrograph of the edge of large intraepithelial channel. No basement membrane or endothelial cells are seen lining channel. Red blood cells (R) appear to lie in contact with flattened squamous cells. Desmosomes joining the squamous cells and tonofilaments within cells are prominent. Original magnification $\times 1500$, inset $\times 8500$.

Fig. 5 Distribution of factor VIII related antigen around periphery of intraepithelial channel. Antigen is also seen within endothelial cells of nearby intact intraepithelial capillary. Indirect immunofluorescence $\times 100$ (original magnification).

using the criteria of Ismail-Beigi¹² and Goldman and Antonioli,¹¹ however, was seen in only 45% of our cases² despite the fact that the intraepithelial channels were present in all cases of oesophageal varices which were studied.

In the present study histological evidence suggests that the channels are not lined by endothelial cells and basement membrane and are thus not true blood vessels. These findings are similar to those of Geboes *et al* who found the channels were lined with flattened cells not resembling the basal epithelial cells.⁹ Indirect immunofluorescence for Factor VIII related antigen is a reliable technique for showing endothelial cells.¹³ Megakaryocytes and platelets also fluoresce positively with this technique but there was no evidence of platelets lining the channels under electron microscopy. It is therefore possible that the flattened cells lining the channels are functioning as endothelial cells.

Burnand *et al*¹⁴ have shown an increase in the size of the skin capillary bed in response to sustained local venous hypertension in the dog and similar findings have been reported in the human skin of the lower leg in the presence of venous hypertension.¹⁵ It is possible that prolonged portal venous hypertension may similarly induce an increase in the size of the capillary bed of the lower oesophagus and that the increased length of papillary capillaries, which we have shown, is a manifestation of this rather than of oesophagitis.

The possibility of these blood filled channels being artefact caused by the operative procedure must be considered as they have been found in a small proportion of the non-varices patients. A tumour in

the region of the cardia, however, may readily cause an element of local venous obstruction and hence give rise to the occasional dilated intraepithelial channels. No evidence of clotting was seen in any of the channels in either group.

In our normotensive dogs there was no evidence of the formation of any intraepithelial channels. If the circular stapling device was causing artefacts, some sign of extravasation of red blood cells among the epithelial cells would be expected.

Endoscopic examination of oesophageal varices frequently reveals red spots and streaks lying on the surface of the varices. These have been described as 'cherry red' spots and 'red wale markings'.^{16 17} Other workers have interpreted the endoscopic appearances as 'varices overlying varices'¹⁸ implying dilated small channels lying superficial to the much larger variceal channels which are found in the submucosa. A retrospective study has recently suggested that the endoscopic findings accurately correlate with the risk of bleeding.¹⁹

This endoscopic interpretation corresponds to our histological findings. While we are unable to rule out that some of these channels may be artefact, nonetheless we have shown that they are larger and more numerous in transection specimens removed from patients with varices in comparison with non-variceal patients. This indicates that the small superficial mucosal vessels are more fragile as a consequence of long-standing hypertension and are thus more liable to rupture.

It is established that there is a sudden increase in pressure within oesophageal varices during coughing, sneezing, and yawning.²⁰ It seems likely that fragile, poorly supported, congested channels extending close to the mucosal surface may rupture during such circumstances. Although such channels are of small calibre the rupture of such lesions especially in patients with poor haemostasis could give rise to substantial haemorrhage.

We propose that these intraepithelial channels may represent the cherry red spots (channels viewed end-on) and the red wale markings (longitudinal epithelial channels). Thus these small superficial channels may play an important role in the pathogenesis of bleeding oesophageal varices.

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