

Short reports

Sample dilution to resolve mistaken identification of haemoglobin D as haemoglobin E using the Variant automated system

S Thomas

Department of Pathology, Christian Medical College Hospital, Vellore 632004, India
S Thomas

Correspondence to: Dr Thomas.

Accepted for publication 11 November 1997

Abstract
High performance liquid chromatography (HPLC) is increasingly being used to estimate variant haemoglobins. A case of haemoglobin S/D (HbS/D) is presented, which was misdiagnosed as haemoglobin S/E (HbS/E) by HPLC. The patient was a 22 year old woman with sickle cell anaemia. Subsequent haemodilution by blood transfusion clearly elucidated the haemoglobin D peak on HPLC. Sample dilution experiments, using the pretransfusion sample, were done resulting in correct elution of the peak in the D window. Troubleshooting in similar problematic haemoglobin variant peaks seen on HPLC can be done by sample dilution.

moglobin variant peaks seen on HPLC can be done by sample dilution.

(J Clin Pathol 1998;51:253-254)

Keywords: high performance liquid chromatography; haemoglobin variants; haemoglobinopathies

High performance liquid chromatography (HPLC) is a precise and accurate method for the detection of abnormal haemoglobins.¹ Automated systems like the Variant (Bio-Rad, Hercules, California, USA) are easy to use and provide results rapidly. They are being increasingly used to separate and measure various haemoglobin fractions. However, minor variations in the mean elution time can lead to mistaken identity of the eluate. A case initially misdiagnosed as double heterozygous for haemoglobins S/E (HbS/E) and later confirmed to be a case of haemoglobin S/D (HbS/D) is described. A simple technique of sample dilution, which can be used to solve this problem on the HPLC system, is detailed.

Case report

A 22 year old woman with sickle cell anaemia was referred to this hospital. Sickle haemoglobin was confirmed by the sickle preparation. HPLC for quantification of the variant haemoglobin was done using the Variant haemoglobin testing system from Bio-Rad following the manufacturer's instructions. Briefly, 5 µl of whole blood in Na₂EDTA was added to 1 ml of the haemolysis reagent and subjected to automated HPLC analysis using the β thalassaemia short program. Analysis of the patient's blood sample revealed four peaks (fig 1A) corresponding to HbF 13.9%, HbA 11.6%, HbA₂/E 44.9%, and HbS 34.8%. We received a second blood sample for analysis from the same patient after blood transfusion (haemoglobin before transfusion 93 g/l, after transfusion 162 g/l). This time no peak eluted in the HbA₂ window, instead a peak was seen in the D window (fig 1B). The total peak areas for both chromatograms were within the range recommended by the manufacturer (1 000 000 to 3 000 000 µV/s). The presence of a small amount of HbA in the first sample was found to be due to a blood transfusion the patient had

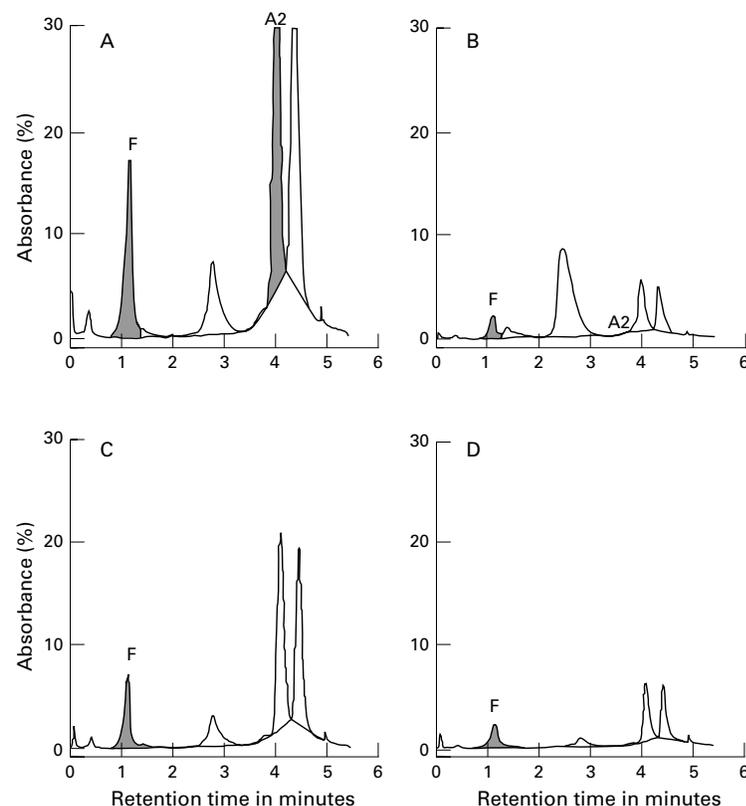


Figure 1 Chromatogram reports; HbF and A₂ appear as shaded peaks. (A) First blood sample showing HbF 13.9%, HbA 11.6%, HbA₂ 44.9%, and HbS 34.8%. (B) Post-transfusion sample showing peaks of HbF, A, A₂, D, and S. (C) Sample dilution using 3 µl of blood showing HbF, A, D, and S. (D) Sample dilution using 1 µl of blood showing same peaks as in (C) but with smaller peak areas.

received nine weeks previously. A final diagnosis of HbS/D was made, which was confirmed on citrate agar electrophoresis.

Surmising that this result was caused by the effect of haemodilution following the recent transfusion, we experimented with the first blood sample that had been preserved at -80°C . Smaller blood samples ($3\ \mu\text{l}$ and $1\ \mu\text{l}$) were added to the same amount of haemolysis reagent, in effect diluting the sample, and analysed using the Variant system. One tube with the usual $5\ \mu\text{l}$ was also added. This time, with the exception of the first tube containing $5\ \mu\text{l}$, which gave the same result as initially, the tubes with the diluted samples no longer showed the peak in the HbA₂ region, instead the peak correctly eluted in the D window (figs 1C and D).

Discussion

HPLC is a method for the rapid detection and measurement of normal and variant haemoglobins. It is capable of resolving HbC, O-Arab, and Agenogi from HbA₂.^{2,3} The Variant system, which uses a cation exchange method, resolves HbA, A₂, F, S, C, and D. In HPLC, presumptive diagnosis of the commonly occurring haemoglobin variants (D, S, C, and E) is made using retention time windows, such as "D window", "S window", and "C window". These windows are time ranges in which common variants have been observed to elute using the Variant β thalassaemia short program. They may change with different batches of the supplied kit. The A₂ window generally ranges from 3.68 to 3.98 minutes followed immediately by the D window (3.98 to 4.12 minutes).⁴ These identification windows are so close they could cause misinterpretation if the variant haemoglobin elutes a fraction of a second earlier, as was seen in this case. It was noted that the HbD peak in the first blood sample eluted at 3.94 minutes, which is within the time range for HbA₂. The post-transfusion sample identified an HbA₂ peak at 3.74 minutes and the HbD peak at 3.96

minutes. Both the diluted samples showed the HbD peak at 4.00 and 4.07 minutes, well within the established elution time range.

Of the haemoglobin variants that co-migrate with HbA₂, it is principally HbE that interferes with the HbA₂ estimation. HbD is not known to cause this problem on electrophoresis but may co-elute with HbA₂ on HPLC.⁴ The presence of a variant haemoglobin (HbE or HbD) is suspected when HbA₂ is more than 15–20%. This needs to be confirmed by other laboratory investigations. We resolved this problem of co-elution by the simple technique of sample dilution.

We have since used this technique for three other cases (one each of HbA/D, HbD/ β thalassaemia, and HbS/D), all of which showed the HbD peak eluting initially in the HbA₂ window, then in the D window after dilution. The presence of the HbD mutation was confirmed at the molecular level in these three cases by the polymerase chain reaction and restriction enzyme digestion.

In conclusion, the simple technique of sample dilution using a smaller volume of blood to prepare the haemolysate for HPLC analysis can be helpful in resolving questionable variant haemoglobins.

The author thanks Drs Mammen Chandy and Alok Srivastava (department of hematology, CMCH, Vellore, India) for critically reviewing the manuscript. For confirmation of HbD the assistance of Dr Uma Khanduri (Sultan Qaboos University Hospital, Muscat, Oman), Ms Patricia Fleming, and Ms Anne Gilbert (Department of Haematology, ICPMR, Westmead Hospital, Australia) is greatly appreciated.

- 1 Tan GB, Aw TC, Dunstan RA, *et al*. Evaluation of high performance liquid chromatography for routine estimation of haemoglobins A₂ and F. *J Clin Pathol* 1993;46:852–6.
- 2 Wilson JB, Headlee ME, Huisman THJ. A new high performance liquid chromatographic procedure for the separation and quantitation of various haemoglobin variants in adults and newborn babies. *J Lab Clin Med* 1983;102:174–85.
- 3 Samperi P, Mancuso GR, Dibenedetto SP, *et al*. High performance liquid chromatography (HPLC): a simple method to quantify HbC, O-Arab, Agenogi and F. *Clin Lab Haematol* 1990;13:169–75.
- 4 *Instruction manual, VARIANT β -thalassaemia short program*. Hercules, California: Bio-Rad, 1994.

Biopsy specimen appearances of ischaemic gastritis in splanchnic arterial insufficiency

J E Trowell, G D Bell

Abstract

A 74 year old man presented with a one month history of epigastric discomfort, anorexia, weight loss, and postprandial vomiting. The diagnosis of ischaemia was made on endoscopic biopsies from the stomach and duodenum. He was too ill for major vascular surgery and died eight days after admission. Postmortem examination confirmed the diagnosis of splanchnic arterial insufficiency caused by atheroma and thrombosis. Ischaemic gastritis is rare but could easily be missed in unrepresentative biopsy specimens. Prompt diagnosis with revascularisation surgery is the only hope for long term survival.

(*J Clin Pathol* 1998;51:255-256)

Keywords: ischaemic gastritis; splanchnic arterial insufficiency

Ischaemic gastritis caused by thrombo-atheromatous disease of the aorta and main splanchnic arteries is extremely uncommon because of the rich collateral blood supply of the stomach. The few cases recorded (all heavy smokers) generally had similar endoscopic appearances¹⁻⁶ with multiple aphthoid ulcers superimposed on a friable congested mucosa. In two cases^{4,5} the endoscopic appearances returned to normal following revascularisation. Biopsies, when taken, have usually not contributed to the diagnosis,^{1,2,5} although some have been suggestive of ischaemia, but not illustrated.⁶ The gross postmortem appearances of the stomach⁷ are similar to the endoscopic appearances with multiple ulcers of varying size in a haemorrhagic mucosa. Atheromatous emboli in arteries in the stomach wall have been described as causing ischaemic gastric necrosis in postmortem histology⁷ and in a surgical resection specimen.⁸

In the case described here the endoscopic biopsy appearances were sufficiently distinctive to make a diagnosis of ischaemia, the cause of which became apparent over the subsequent few days and at postmortem.

Ischaemic ulceration of the stomach can also be caused by polyarteritis nodosa and leucocytoclastic vasculitis,⁹ and recently some ischaemic changes have been described due to secondary arterial changes in portal hypertension.¹⁰

Case report

A 74 year old man was admitted because of frequent vomiting associated with periumbilical pain and some diarrhoea. For the previous month he had epigastric discomfort, no appetite, profound weight loss, and intermittent vomiting after meals. In the past a right inguinal hernia had been repaired twice; he was

a heavy smoker, up to 60 cigarettes daily throughout his adult life.

On examination he was pale, dehydrated, and cachectic with abdominal tenderness and guarding. A bruit was heard over the renal arteries. Laboratory investigations were normal apart from a total white cell count of $20 \times 10^9/l$ with a polymorph leucocytosis, and slightly raised serum amylase of 282 U/l (normal < 220). Plain abdominal radiography and abdominal ultrasound showed some fluid filled, non-dilated small bowel loops, but nothing else of relevance. Intra-abdominal sepsis was suspected and he was treated with antibiotics, intravenous fluids, and nasogastric suction.

Endoscopy of the oesophagus the following day appeared normal; 300 ml of faeculent fluid was aspirated from the stomach revealing severe gastritis and duodenitis, and biopsy specimens were taken.

Two days later the patient developed a cold numb left foot with absent left femoral pulse. He had a left femoral embolectomy under epidural anaesthesia, but because of poor proximal pulses he required a femorofemoral crossover graft. This was successful, but then the endoscopic biopsy reports were received showing ischaemic gastritis and duodenitis. A presumed diagnosis of mesenteric ischaemia was made but he was too ill for major surgery. He developed increasing abdominal tenderness with absent bowel sounds and died eight days after admission.

Pathology

Two biopsy specimens from the greater curve of the body of the stomach, and two from the second part of the duodenum were investigated. Both gastric specimens showed focal full thickness coagulative necrosis of the mucosa with sloughing of the necrotic debris. Although the necrotic areas contained some neutrophil polymorphs, it was possible to identify the ghost outline of glands and pits represented by vertical columns of necrotic epithelial cells containing pyknotic nuclei (fig 1). The adjacent mucosa showed some atrophy of the cells lining the glands, with hyalinisation of the intervening stroma, consistent with ischaemia. Some of the mucosa was normal with no significant inflammation, and the muscularis mucosae was normal.

The duodenal biopsy specimens also showed focal full thickness mucosal necrosis with atrophy of adjacent villi, together with a pseudomembranous exudate of fibrin and polymorphs (fig 2). Elsewhere the mucosa was normal, as was the muscularis mucosae and submucosal blood vessels.

The combination of coagulative-type necrosis, ischaemic atrophy of the adjacent mucosa,

Department of Pathology, The Ipswich Hospital NHS Trust, Heath Road, Ipswich, Suffolk IP4 5PD, UK
J E Trowell

Sunderland Hospital, Kayll Road, Sunderland, Tyne and Wear SR4 7TP, UK
G D Bell

Correspondence to: Dr Trowell.

Accepted for publication 10 December 1997

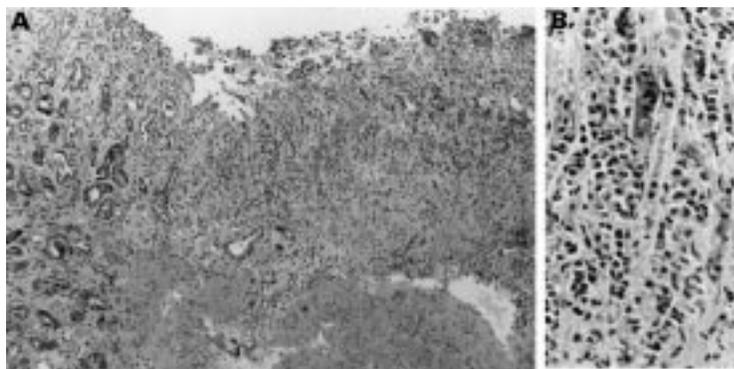


Figure 1 Gastric biopsy (haematoxylin and eosin stain); (A) low power; (B) high power. There is full thickness mucosal necrosis with vertical columns of necrotic epithelial cells admixed with some neutrophil polymorphs (B). The capillaries are congested. The adjacent mucosa shows ischaemic atrophy.

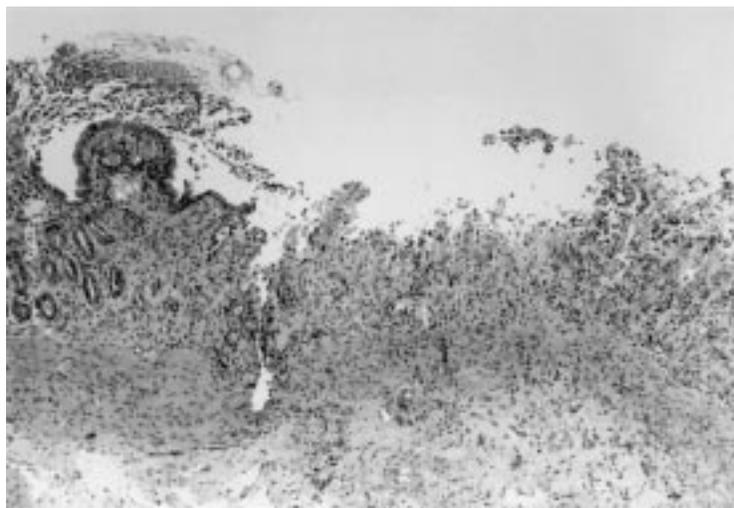


Figure 2 Duodenal biopsy (haematoxylin and eosin stain). There is full thickness mucosal necrosis with a surviving atrophic villus. There is a pseudomembrane of fibrin and polymorphs on the left.

the multiplicity of ulcers, and the chronic history were regarded as diagnostic of ischaemic ulceration.

At postmortem examination there was purulent peritonitis over some loops of small bowel showing haemorrhagic infarction. The distal half of the stomach, the remainder of the small bowel, and the ascending colon were dusky purple and had several ulcers. There were also a few ischaemic ulcers in the transverse colon. The oesophagus, proximal stomach, distal colon, and rectum were normal. The abdominal aorta was severely atheromatous and there was a disc of mural thrombus 3 cm diameter covering the origins of the coeliac and superior mesenteric arteries; this extended into and occluded the proximal 0.5 cm of both arteries, which were narrowed by atheroma. The origin of the inferior mesenteric artery was severely narrowed by atheroma, and the left common iliac artery was occluded by thrombus and atheroma. The heart (400 g) had moderate coronary artery atheroma; the myocardium was normal and there were no endocardial thrombi or vegetations. The liver, gall bladder, and pancreas were normal, and the kidneys (280 g) and renal arteries were unremarkable.

Discussion

Most erosions and ulcers of the stomach, regardless of cause, have a final common pathway of acid and pepsin digestion that results in progressive liquefaction necrosis of the surface. However, the gastric erosion illustrated here shows full thickness coagulative necrosis of the mucosa with sloughing of the necrotic debris, there is also some ischaemic atrophy of the adjacent mucosa. One gastric biopsy included some normal mucosa, and the focal distribution of the ischaemic changes may explain the non-contributory findings in two of the previous cases in which biopsy was done.^{1 2}

Ischaemic gastritis caused by splanchnic arterial insufficiency is extremely rare because of the rich collateral blood supply of the stomach, and is only seen when at least two of the three main splanchnic arteries are occluded or severely stenosed.^{5 6} The main blood supply of the stomach is from branches of the coeliac artery, which anastomose freely with each other, and there is collateral supply from the first branch of the superior mesenteric artery. However, there is also systemic collateral circulation from the oesophageal arteries, and should it arise directly from the aorta, from the left inferior phrenic artery.

This systemic collateral arterial supply probably explains the focal nature of the ischaemic necrosis in this case, and the sparing of the proximal stomach.

Ischaemic ulceration of the stomach has also been reported caused by polyarteritis nodosa and leucocytoclastic vasculitis,⁹ and rarely in portal hypertension,¹⁰ but none of these factors were relevant in our case.

Splanchnic arterial insufficiency causing ischaemic gastritis and duodenitis is a rare disease confined to heavy cigarette smokers, which has a grave prognosis.⁶ Revascularisation is occasionally successful⁴⁻⁶ provided that the patients are not too ill for major vascular surgery.

The various features enabling a biopsy diagnosis of ischaemic gastritis and duodenitis to be made in this case were the chronic history, coagulative-type necrosis in the ulcers, the presence of similar ulcers in the stomach and duodenum, and the ischaemic atrophy of the adjacent mucosa.

- Force T, MacDonald D, Eade OE. Ischaemic gastritis and duodenitis. *Dig Dis Sci* 1980;25:307-10.
- Allende HD, Ona FV. Coeliac artery and superior mesenteric artery insufficiency; unusual cause of erosive gastroduodenitis. *Gastroenterology* 1982;82:763-6.
- Talansky AL, Katz S, Naidich J. Aphthous ulcers in ischaemic gastroenterocolitis; a case report. *Am J Gastroenterol* 1985;20:257-9.
- Hojgaard L, Krag E. Chronic ischaemic gastritis reversed after a revascularisation operation. *Gastroenterology* 1987;92:226-8.
- de Widt-Levert LM, Nelis GF, Jorning PJ. Dyspepsia as initial symptom of splanchnic vascular insufficiency. *Eur J Gastroenterol Hepatol* 1996;8:815-18.
- Casey KM, Quigley TM, Kozarek RA, et al. Lethal nature of ischaemic gastropathy. *Am J Surg* 1993;165:646-9.
- Taylor NS, Gueft B, Lebowich R. Atheromatous embolization: a cause of gastric ulcers and small bowel necrosis. *Gastroenterology* 1964;47:97-103.
- Harvey RL, Dobeneck RC, Black WC. Infarction of the stomach following atheromatous embolization. *Gastroenterology* 1972;62:469-72.
- Lopez LR, Schocket AL, Stanford RE, et al. Gastrointestinal involvement in leucocytoclastic vasculitis and polyarteritis nodosa. *J Rheumatol* 1980;7:677-84.
- Leone O, Zanelli M, Piana S, et al. Late stage congestive gastropathy. *J Clin Pathol* 1997;50:350-2.

Value of assessing parathyroid hormone-like activity in a case of extreme hypercalcaemia

L Ranganath, H Jamal, L Jones, P F Goddard

Abstract

A previously well 70 year old woman was admitted to hospital following a three day history of vomiting and confusion. Her serum calcium was 6.58 mmol/l, phosphate 1.09 mmol/l, and alkaline phosphatase 91 iu/l. The mechanism of this hypercalcaemia was not obvious as there was no evidence of a primary malignancy, lymphadenopathy or hepatosplenomegaly. The calculation of indices of urinary excretion of calcium and phosphate suggested the presence of excessive parathyroid hormone (PTH) activity as the mechanism of hypercalcaemia. Plasma intact PTH, 25-hydroxycholecalciferol, and 1,25-dihydroxycholecalciferol were not raised suggesting the presence of PTH related peptide (rP). This led to a systematic search for a malignancy, which revealed the presence of a high grade B cell non-Hodgkin's lymphoma confined to the bone marrow. Plasma PTH-rP was subsequently shown to be raised confirming the interpretation of the initial urinary and calcium excretion indices. This case highlights the value of standard laboratory measurements such as urinary calcium and phosphate excretion in cases of hypercalcaemia of obscure aetiology, which can complement measurements of PTH and other calcitropic hormones.

(*J Clin Pathol* 1998;51:257–258)

Department of
Chemical Pathology,
Epsom General
Hospital, Epsom, KT18
7EG, UK
L Ranganath
P F Goddard

Department of
Haematology, Epsom
General Hospital
H Jamal
L Jones

Correspondence to:
Dr Ranganath.

Accepted for publication
9 December 1997

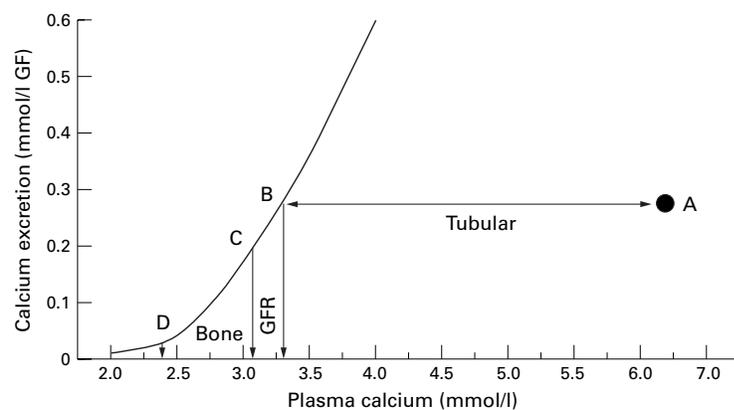


Figure 1 Schema of the steps for resolving the components of hypercalcaemia. Urine indices were obtained on fasting samples (plasma calcium, 6.35 mmol/l; plasma creatinine, 0.143 mmol/l; urine calcium, 7.8 mmol/l GF; urine creatinine, 4.1 mmol/l). Step 1, plot A from essential biochemistry. Step 2, move point A to point B on the normal slope (A minus B is the tubular component; 3.05 mmol/l in our patient). Step 3, move point B down the normal line depending on the ratio of plasma creatinine (0.143 mmol/l) to 0.1 mmol/l (mean value in normals) (B minus C is the GFR component; 0.25 mmol/l in our patient). Step 4, move point C to D (2.4 mmol/l; the mean value in health) (C minus D is the flow of calcium from resorption in the fasting state; 0.7 mmol/l in our patient).

Keywords: hypercalcaemia; non-Hodgkin's lymphoma; PTH-rP; nomogram for calcium excretion; parathyroid hormones

Primary hyperparathyroidism and malignancy are common causes of hypercalcaemia.¹ Parathyroid hormone (PTH) excess is characterised by increased gastrointestinal and tubular calcium absorption as well as increased bone resorption.² Elimination of the gastrointestinal mechanisms by assessment of blood and urine in the fasting state allows the contribution of bone resorption, glomerular filtration rate (GFR), and tubular reabsorption to be identified.³ We applied the scheme proposed by Peacock and colleagues³ for the resolution of the components of hypercalcaemia to a case of extreme hypercalcaemia.

Case report

A previously well 70 year old woman was admitted to hospital following a three day history of vomiting and confusion. Her serum calcium was 6.58 mmol/l, phosphate 1.09 mmol/l, and alkaline phosphatase 91 iu/l (normal range 35–230). The mechanism of hypercalcaemia was not obvious as there was initially no evidence of a primary malignancy, lymphadenopathy or hepatosplenomegaly. She was taking Tenoretic (Stuart, Wilmslow, Cheshire, UK), a combination of chlorthalidone and atenolol, but the severity of the hypercalcaemia made a thiazide effect an unlikely cause. Circulating parathyroid hormone (0.7 pmol/l; normal range 0.5–5.5), 25-hydroxycholecalciferol (25-OHD) (22 nmol/l; normal range 15–75), and 1,25-hydroxycholecalciferol (1,25-OHD) (< 20 pmol/l; normal range 40–150) were low and excluded an excess of these hormones as an aetiological factor in hypercalcaemia. Fasting urine and blood samples were obtained and the components of bone resorption, GFR, and tubular reabsorption assessed (fig 1). These indicated that tubular reabsorption was the predominant mechanism of hypercalcaemia in this patient despite low concentrations of PTH, 25-OHD, and 1,25-OHD. Urine phosphate excretion was high (0.44 mmol/l glomerular filtrate (GF)) with a low tubular maximal reabsorption of phosphate (0.67 mmol/l GF; normal range 0.71–1.36). Parathyroid hormone related peptide (PTH-rP) was 10.3 pmol/l (normal range < 2.5) at a serum calcium of 4.22 mmol/l. Following treatment with rehydration, pamidronate, and chemotherapy, the PTH-rP concentration was 1.9 pmol/l at a serum calcium concentration of 2.54 mmol/l. A diagnosis of PTH-rP producing high grade B cell non-Hodgkin's lymphoma confined to the

bone marrow was made; these tumours have been shown to produce PTH-rP.⁴⁻⁶

Discussion

Raised serum calcium leads to increasing urinary calcium excretion; whether the urinary calcium excretion is appropriate for a given serum calcium can be determined from previously described nomograms (fig 1).³

A two hour timed urine collection and a blood sample during the middle of the urine collection are obtained after a 12 hour overnight fast for the measurements of calcium, phosphate, and creatinine. Calcium excretion is expressed as urine calcium multiplied by plasma creatinine, the product divided by urine creatinine. The calcium excretion and serum calcium in our patient were plotted and resulted in the point marked A on fig 1. The extrapolation of this point to intersect the curvilinear line at B represents the tubular reabsorption. The tubular component was estimated to be 3.05 mmol/l using this scheme in our patient. To correct for calcium retention because of renal failure, calcium excretion was recalculated using a value for plasma creatinine of 0.1 mmol/l (mean value of the reference range). Replotting calcium excretion against plasma calcium and extrapolating to the curvilinear line results in point C, the difference between B and C being the component due to reduced GFR. These investigations also showed an inappropriately high phosphate excretion for the concentration of serum phosphate.

PTH, 25O-HD, and 1,25-OHD can all cause increased tubular reabsorption of calcium, although only PTH can produce relative

phosphaturia. As PTH was low, the relatively increased phosphate and reduced calcium excretion in urine in our patient was suggestive of the presence of another molecule with PTH-like activity. The finding of increased PTH-like activity in the presence of a suppressed plasma immunoreactive PTH indicated the possibility of a malignant tumour producing PTH-rP, and allowed us to intensify the search for a malignancy. This was confirmed by the documentation of raised immunoreactive PTH-rP. The use of this simple diagnostic test proved extremely helpful in the management of this patient with occult lymphoma. The clinical usefulness of the calcium and phosphate nomograms has diminished since the availability of reliable and speedy PTH assays. However, this case proves the value of simple and readily available laboratory measurements demonstrating PTH-like activity, which can aid the appropriate investigation and management of calcium disorders, and deserves to be more widely used in cases of obscure hypercalcaemia.

- 1 Fiskens RA, Heath DA, Somers S. Hypercalcaemia in hospital patients. *Lancet* 1981;i:202-7.
- 2 Marcus R. Laboratory diagnosis of primary hyperparathyroidism. *Endocrinol Metabol Clin North Am* 1989;18:647-58.
- 3 Peacock M. Renal excretion of calcium. In: Nordin BEC, ed. *Calcium in human biology*. Berlin: Springer-Verlag, 1988:125-69.
- 4 Davies SV, Vora J, Wardrop CAJ. Parathyroid hormone related protein (PTHrP) in hypercalcaemia of lymphoproliferative disease [letter]. *J Clin Pathol* 1993;46:188.
- 5 Firkin F, Seymour JF, Watson AM, et al. Parathyroid hormone-related protein in hypercalcaemia associated with haematological malignancy. *Br J Haematol* 1996;94:486-92.
- 6 Kremer R, Shushik C, Tabak T, et al. Parathyroid-hormone-related peptide in haematological malignancies. *Am J Med* 1996;100:406-11.

Neuroendocrine cell hyperplasia in colonic tissue used for long term augmentation cystoplasty

K Oien, M Akyol, G Lindop

University
Department of
Pathology, Glasgow
University, Western
Infirmary, Glasgow
G11 6NT, UK
K Oien
G Lindop

Transplant Unit, Royal
Infirmary of
Edinburgh, 1
Lauriston Place,
Edinburgh, EH3 9YW,
UK
M Akyol

Correspondence to:
Dr Oien.

Accepted for publication
9 December 1997

Abstract

A case is described of neuroendocrine cell hyperplasia in intravesical colonic mucosa, implanted previously during augmentation cystoplasty. The patient was a 28 year old man born with posterior urethral valves, a non-functioning right kidney, and a poorly functioning dilated left kidney. The hyperplasia consisted of pure neuroendocrine acini and tubules within the lamina propria, separate from the normal intestinal glands. Adjacent intraepithelial colonic neuroendocrine cells were increased diffusely. Rectal biopsy and previous biopsies of intravesical colonic tissue contained normal neuroendocrine cell populations. Implantation of gut segments into the urinary tract pre-

disposes to late neoplasia, but there is only one report of carcinoid tumour in uroenteric tissue. Intestinal neuroendocrine cell hyperplasia usually occurs diffusely rather than as aggregates, except when associated with adjacent carcinoid tumour. Both diffuse and nodular hyperplasia were present in this case, with an unusual and striking morphology. This is the first report of neuroendocrine cell hyperplasia in gastrointestinal tissue implanted into the urinary tract; this raises the possibility of a risk of late carcinoid tumour in uroenteric segments.

(*J Clin Pathol* 1998;51:258-261)

Keywords: bladder augmentation; neuroendocrine cell hyperplasia

In urinary tract reconstruction, gastrointestinal segments are used to create urinary conduits and for partial or complete bladder replacement, including augmentation cystoplasty. These procedures may give rise to complications such as metabolic imbalance, infection, urolithiasis, and neoplasia.^{1,2} The oldest of these procedures is ureterosigmoidostomy. Its association with late neoplasia is well established, with a high risk of colonic adenocarcinoma at the anastomosis. When intestinal mucosa is exposed to both faecal stream and urine, faecal bacteria may produce carcinogens, particularly nitrosamines, from urinary contents. Mechanical trauma, suture material, chronic inflammation, and the abnormal interaction of different mucosae at the anastomosis may also be important.^{1,2} It has been recognised recently that bowel implants, including bladder augmentations, that are exposed to urine without faeces also have an increased risk of malignancy; this has been estimated at 2% after more than 10 years.^{2,3} The tumour types (predominantly adenocarcinoma and transitional cell carcinoma), site, and latent periods are similar in both circumstances.

Gut neuroendocrine cell proliferations comprise neoplasms, including carcinoid tumours and neuroendocrine carcinomas, and hyperplasias.^{4,5} Hyperplasias of antral gastrin producing cells (G cells) or of enterochromaffin-like (ECL) cells in oxyntic (acid producing) mucosa, have long been recognised in the stomach. G cell hyperplasia may be primary or secondary, particularly in low acid states, whereas ECL cell hyperplasia is caused by hypergastrinaemia. In both, increased numbers of neuroendocrine cells are present either in their normal intraepithelial position in gastric glands or as aggregates in the lamina propria.⁴

Diffuse hyperplasia of mucosal intraepithelial enterochromaffin cells occurs in the small intestine in untreated coeliac disease, and in the colon in chronic inflammatory bowel disease.⁵ Neuroendocrine cell hyperplasia in the form of nodules in the lamina propria may occur beside large and small intestinal carcinoid tumours,⁵ but to our knowledge, this has only once been reported without co-existing malignancy, in a case of megacolon.⁶

We present a case of combined nodular and diffuse neuroendocrine cell hyperplasia, without concurrent carcinoid tumour, in colonic mucosa used for augmentation cystoplasty. We know of no previous reports of neuroendocrine hyperplasia in uroenteric implants.

Case report

This 28 year old man was born with posterior urethral valves, a non-functioning right kidney, and a poorly functioning dilated left kidney. In infancy he had a right nephrectomy and left cutaneous ureterostomy. The left ureter was later implanted into his bladder but, because of upper urinary tract deterioration, ileal loop urinary diversion was performed in childhood. He then suffered intermittent stomal obstruction with worsening renal function.

At 18 years old the urinary diversion was reversed; at operation the ileal conduit was grossly dilated with significant renal distension. To avoid a second failed ureteric implantation, the bladder was augmented using a sigmoid colon segment and the ureter re-implanted into the latter. Four years later, reduction cystoplasty, removing part of the colonic tissue, was performed because of poor bladder emptying. In addition, there were multiple ureteric re-implantations because of stenoses.

Eventual dialysis was followed after four years by a renal transplant. His renal function was then normal and stable with good bladder function. Two months after the transplant, several episodes of urinary tract infection necessitated a left native nephroureterectomy, which is the subject of this report.

Pathological findings

The specimen comprised a severely hydronephrotic kidney, measuring 130 × 60 × 30 mm, with a 90 mm length of dilated ureter. Microscopy of the kidney showed end stage disease. The unexpected finding was in a fragment of large intestinal tissue, which had previously been used for augmentation cystoplasty, attached to the distal ureter.

The colonic mucosa showed an unusual form of neuroendocrine hyperplasia, composed of individual clusters, acini, and tubular glands within the lamina propria (fig 1). These aggregates contained a pure neuroendocrine cell population, without enterocytes or goblet cells. This was clearly demonstrated by Masson Fontana/periodic acid Schiff (PAS) (fig 2A) and diazo staining (that is, both argyrophil and argentaffin), and by immunohistochemistry with an antibody against chrom-

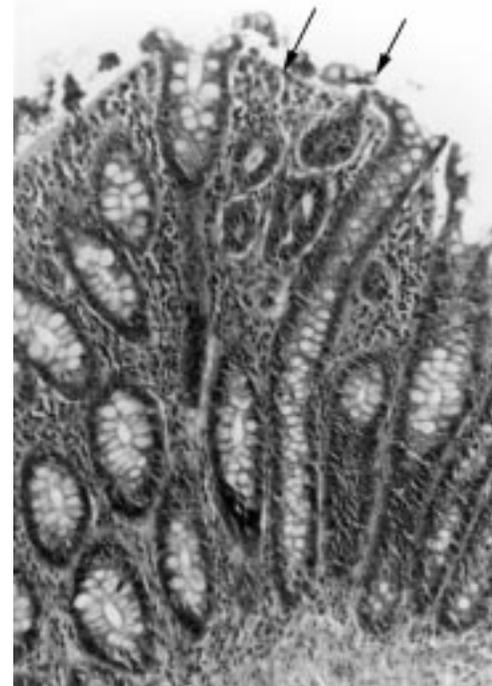


Figure 1 Colonic mucosa with neuroendocrine cell hyperplasia in the form of individual acini and glands in lamina propria (arrows) (haematoxylin and eosin).

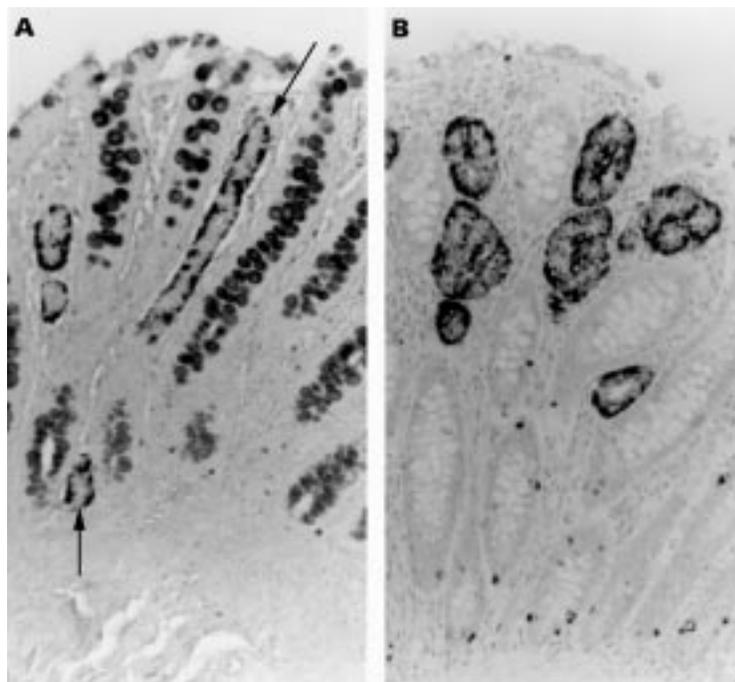


Figure 2 (A) Colonic mucosa with neuroendocrine cell hyperplasia (arrows) (Masson/PAS). Neuroendocrine tubule extends to luminal surface: serial sections do not show communication with colonic epithelial glands. (B) Colonic mucosa with neuroendocrine cell hyperplasia (chromogranin). Intraepithelial neuroendocrine cells are also diffusely increased.

ogranin A (Dako Ltd, High Wycombe, Bucks, UK) (fig 2B). Additional immunohistochemistry showed strong positivity for neurone specific enolase, somatostatin, and serotonin, and weak positivity for gastrin, pancreatic polypeptide, and prostatic acid phosphatase. The neuroendocrine aggregates were distributed mainly superficially in the lamina propria, contrasting with the usual predominantly basal location of intraepithelial neuroendocrine cells. Serial sections showed that the neuroendocrine clusters did not communicate with colonic epithelial glands but that some of the neuroendocrine tubules opened onto the luminal surface and a few extended into the lower mucosa (fig 2A).

The colonic intraepithelial neuroendocrine cells were also diffusely increased in number, with occasional linear aggregates, but remained mostly basal. They did not extrude into lamina propria or communicate with the lamina propria neuroendocrine aggregates. The neuroendocrine hyperplasia was confined to the mucosa, without involvement of submucosa or of ureteric or renal tissue.

Rectal biopsy was normal and showed no increase in neuroendocrine cells. Intravesical colonic mucosal biopsies six years earlier showed only moderate chronic inflammation.

Discussion

We are not aware of previous reports of neuroendocrine hyperplasia in uroenteric implants. Previous histological studies of the mucosa of colonic conduits, exposed to urine only, showed progressively increasing chronic inflammation, without dysplasia, during long term follow up.⁷ Augmentation cystoplasty in

animals produced transitional metaplasia and hyperplasia at the anastomotic junction, together with occasional papillary tumours.⁸ These studies did not specifically discuss the neuroendocrine cell population. There has been a single case report of a goblet cell adenocarcinoid tumour in a ureteroileal conduit⁹; the tumour was predominantly submucosal, and the mucosal neuroendocrine cells were not described.

In our case, the normal rectal neuroendocrine population suggests that the hyperplasia is unlikely to have been a generalised large bowel abnormality. The previous normal biopsies of intravesical colonic tissue indicate that the hyperplasia was either focal or had recently developed. Gut neuroendocrine cell density appears to be related to the requirement for coordination of mechanical and secretory activities.⁴ The previous case of colonic nodular neuroendocrine hyperplasia with formation of acini occurred in a megacolon.⁶ Our patient suffered poor bladder emptying, requiring bladder reduction, and ureteric stenoses. Obstruction and dilatation are features of both cases; neuroendocrine hyperplasia may therefore represent a response to mechanical dysfunction. It has also been postulated that chronic inflammation may cause gut neuroendocrine hyperplasia,⁵ but as most chronic inflammatory gastrointestinal diseases are also associated with altered motility, the precise pathogenesis of neuroendocrine hyperplasia remains unclear.

The diffuse and nodular pattern of intravesical colonic neuroendocrine cell hyperplasia seen here is similar to that occurring adjacent to intestinal carcinoids.⁵ The latter may be associated with diffuse intraepithelial neuroendocrine cell hyperplasia, with intraepithelial aggregates and neuroendocrine buds into the lamina propria³; such budding was not identified in our case. Diffuse and nodular neuroendocrine hyperplasia also occurs in gastric G cell and ECL cell hyperplasias.⁴ It is known that patients with chronic renal failure often develop hypergastrinaemia.¹⁰ Most gastric ECL cell carcinoids are gastrin dependent,^{4,5} while intestinal carcinoid tumours are considered not to be gastrin dependent. Nevertheless, it is possible that increased gastrin could have contributed to the colonic neuroendocrine hyperplasia, perhaps superimposed on altered motility and chronic inflammation.

While our case resembles these other forms of gut neuroendocrine hyperplasia, its morphology, with well defined intramucosal neuroendocrine acini and tubules connected to the luminal surface, is striking and highly unusual. Together with the previous report of a goblet cell adenocarcinoid tumour in a ureteroileal conduit,⁹ this case raises the possibility of a risk of late development of carcinoid tumour in uroenteric implants.

We are grateful to Peter Kerrigan for help with photography.

¹ Husmann DA, Spence HM. Current status of tumor of the bowel following ureterosigmoidostomy: a review. *J Urol* 1990;144:607-10.

- 2 Filmer RD, Spencer JR. Malignancies in bladder augmentations and intestinal conduits. *J Urol* 1990;**143**:671–8.
- 3 Shokeir AA, Shamaa M, El-Mekresh MM, *et al.* Late malignancy in bowel segments exposed to urine without fecal stream. *Urology* 1995;**46**:657–61.
- 4 Dayal Y, DeLellis RA, Wolfe HJ. Hyperplastic lesions of the gastrointestinal endocrine cells. *Am J Surg Pathol* 1987;**11**(suppl 1):87–101.
- 5 Moyana TN. Gastrointestinal endocrine cells and carcinoids: histogenetic and pathogenetic considerations. *Pathol Annu* 1995;**30**:227–46.
- 6 Lindop G. Enterochromaffin cell hyperplasia and megacolon: report of a case. *Gut* 1983;**24**:575–8.
- 7 Moorcraft J, DuBoulay CEH, Isaacson P, *et al.* Changes in the mucosa of colon conduits with particular reference to the risk of malignant change. *Br J Urol* 1983;**55**:185–8.
- 8 Little JS, Klee LW, Hoover DM, *et al.* Long-term histopathological changes observed in rats subjected to augmentation cystoplasty. *J Urol* 1994;**152**:720–4.
- 9 Kochevar J. Adenocarcinoid tumour, goblet cell type, arising in a ureteroileal conduit: a case report. *J Urol* 1984;**131**:957–9.
- 10 Mitchell CJ, Jewell DP, Lewin MR, *et al.* Gastric function and histology in chronic renal failure. *J Clin Pathol* 1979;**32**:208–13.