(98·1%) was calculated, suggesting that the Cytoclone A + B kit would make a useful screening test.

To our knowledge the CD-TOX kit has not been evaluated before. Although the figures for specificity and PPV were not as high as those for the other two kits, the high NPV (96·0%) would also make the CD-TOX a good screening test.

Procedures for using the Premier and the CD-TOX kits were similar. With the Premier kit, results were available in about two hours 15 minutes, while with the CD-TOX, results take only about one hour 15 minutes. The Cytoclone kit was slightly more time consuming involving a centrifugation step and also more wash steps than the other kits, and results were not available for about three hours.

Both the Premier and the Cytoclone kits cost about $9·40 (roughly £4·60) per specimen while the CD-TOX kit cost is about $6·25 (roughly £3·00) per specimen. These costs do not allow for controls and it should be noted that each batch of specimens must have at least one positive and one negative control. The CD-TOX kit actually recommends the use of four controls with each batch, thus increasing the relative cost per specimen. It is difficult to estimate costs of tissue culture techniques as there are many hidden factors requiring consideration. The laboratory must also have appropriate facilities to maintain the cell lines and have staff familiar with these techniques.

In conclusion, small laboratories without facilities for tissue culture should find the EIA kits an excellent alternative, with all three kits performing well. An automatic plate washer and reader would further enhance the efficiency of these techniques. The CD-TOX kit is the least expensive, but if the recommendations of the manufacturer are followed, and four controls per batch are used, the cost per specimen is substantially increased. The Cytoclone kit gave the most impressive performance indices, but the test takes slightly longer to complete. All kits can be recommended and it must be left to individual laboratories to decide whether tissue culture methods or EIA kits meet the logistic needs or financial requirements of their laboratory.

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Imerslund-Grasbeck syndrome in a Chinese family with distinct skin lesions refractory to vitamin B_{12}

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Abstract

Two brothers in a Chinese family with selective malabsorption of vitamin B_{12} associated with proteinuria (Imerslund-Grasbeck syndrome) presented with widespread mottled skin pigmentation, termed poikiloderma. In contrast to anaemia, this pigmentary disturbance remained unresponsive to vitamin B_{12} replacement. This is different from the reported hyperpigmentation sometimes seen in vitamin B_{12} deficiency which is reversible following treatment. As far as is known, an irreversible and persistent skin disorder has not been reported in this syndrome before.

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Familial selective malabsorption of vitamin B_{12} associated with proteinuria was first described by Imerslund (1960)[1] and Grasbeck *et al* (1960)[2] (Imerslund-Grasbeck syndrome). It is inherited as an autosomal recessive trait and is prevalent among small inbred communities such as the Lapps in Scandinavia and North African Jews.[3] Only a few cases had been reported in Orientals.[4] We have seen two young men in a Chinese family with this syndrome associated with a skin disorder, known as poikiloderma. The anaemia in both patients responded to vitamin B_{12} treatment but the skin lesions did

not. The refractoriness of the skin lesions to vitamin B_{12} treatment has not been reported before.

Case reports

A 21 year old Chinese man was admitted because of proneness to fatigue and dyspnoea on exertion, and for evaluation of a skin disorder. The latter was initially noted at the age of 4 years and was characterised as a generalised symptomless pigmentation. At the age of 5, he sought medical attention because of dizziness and fainting spells and was found to have megaloblastic anaemia. Routine blood picture showed haemoglobin of 51 g/l, mean corpuscular volume (MCV) of 121 fl, leucocyte count of $2{\cdot}8 \times 10^9$/l, and a platelet count of 122×10^9/l. He improved with cyanocobalamin 1000 μg monthly given intramuscularly and has continued this treatment since then. However, the skin disorder remained. His dietary history was normal. Both parents, who are not consanguineous, and his two sisters appear normal and have no anaemia. His younger brother (case 2) had megaloblastic anaemia as a result of vitamin B_{12} deficiency and similar skin lesions.

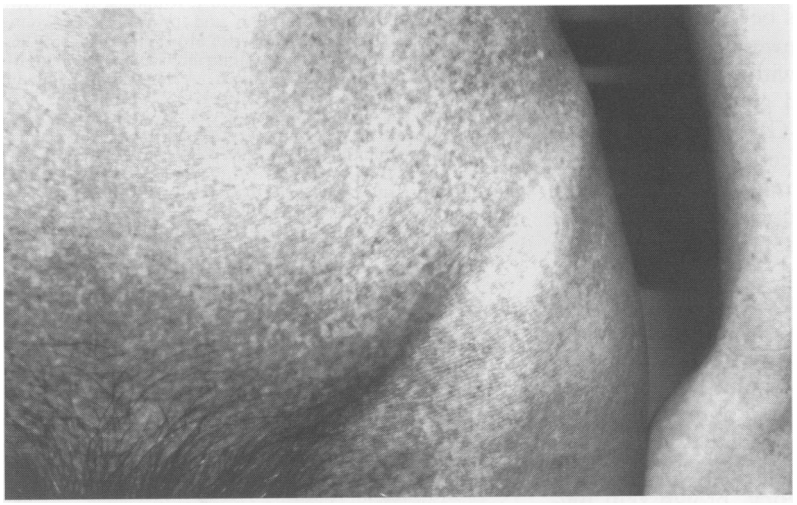

Figure 1 Poikiloderma with widespread mottled pigmentation, particularly in the region of abdomen and both groins, cannot be reversed as a result of vitamin B_{12} replacement.

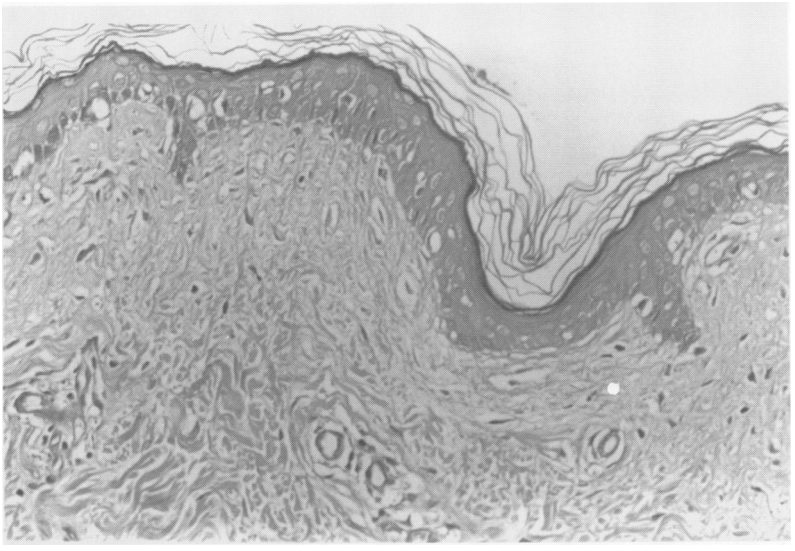

Figure 2 Hyperpigmentation after treatment, showing flattened epidermis with moderate thinning of the stratum malpighii and hydropic degeneration of basal cells associated with pigmentary incontinence (Haematoxylin and eosin).

Haematological investigations in two brothers after discontinuation of vitamin B_{12} replacement for two months

Variables (normal ranges)	Case 1	Case 2
Haemoglobin (g/l)	135	142
MCV (80–100 fl)	96	94
White cells ($\times 10^9$/l)	4·4	9·9
Reticulocyte count (%)	0·3	0·4
B_{12} (150–560 ng/l)	223	178
Serum folate (7–36 nmol/l)	8·7	7·6
Ferritin (20–210 μg/l)	178	145
Dicopac vitamin B_{12} test		
^{57}Co excretion (14–40%)	0·83	0·14
^{58}Co excretion (14–40%)	6·23	0·40
^{57}Co:^{58}Co ratio (0·7–1·2)	0·13	0·35
Parietal cell antibody	—	—
Serum intrinsic factor antibody	—	—
Gastric juice intrinsic factor antibody (14–147 μg/l)	70	20
B_{12} unsaturated binding capacity (805–1465 ng/l)	2517	1398
Transcobalamin		
I and III (235–465 ng/l)	887	808
II (680–960 ng/l)	1630	590
24 hour urinary protein (0·05–0·15 g/day)	0·65	0·85

On admission, the patient appeared to be a well developed and well nourished man. Clubbing, koilonychia, organomegaly, and neurological deficits were absent. The results of a xylose tolerance test and biopsy specimens of stomach and small intestine were normal. Cytogenetic studies were normal. Cortisol concentrations were within normal ranges. Other studies, including a dual isotope (Dicopac) vitamin B_{12} absorption test, and pentagastrin test taken two months after discontinuing vitamin B_{12} replacement, are shown in the table. His skin lesions presented as widespread mottled pigmentation over the whole body, especially over both groins (fig 1), back, neck, and both flanks. These lesions were exaggerated by exposure to the sun and were identified as poikiloderma. The skin biopsy specimen showed flattened epidermis, hydropic degeneration of basal cells, and pigmentary incontinence (fig 2).

The 19 year old younger brother presented with a similar clinical course. Skin manifestations appeared when he was 3 years old and megaloblastic anaemia was noted a year later. His original blood count showed a haemoglobin of 63 g/l, an MCV of 114 fl, a leucocyte count of $4{\cdot}5 \times 10^9$/l, and a platelet count of 141×10^9/l. Despite regular intramuscular cyanocobalamin treatment, the skin lesions remain unchanged. His haematological investigations (table) were also taken two months after discontinuing vitamin B_{12} replacement.

Discussion

The diagnosis of Imerslund-Grasbeck syndrome in our patients was confirmed by isolated vitamin B_{12} deficiency; malabsorption of vitamin B_{12} as demonstrated by a dual isotope vitamin B_{12} absorption test; intact intrinsic factor and gastric secretion; absent intrinsic factor antibody in serum; normal serum transcobalamin I, II, and III; no morphologically identifiable lesion in the stomach and

small intestine; and persistent proteinuria with normal renal function.

The pathogenesis of vitamin B_{12} malabsorption in this syndrome remains unknown. In one study, the uptake of IF-B_{12} complex in vitro by homogenised ileal biopsy specimens from one patient was found to be normal and it was therefore postulated that the defect lay at a later stage after the attachment of the IF-B_{12} complex to the surface of the ileal cell.[5] In another detailed study of two brothers with the syndrome, IF-B_{12} uptake in the ileum in vivo was examined by subcellular fractionation of ileal biopsy specimens.[6] No uptake of vitamin B_{12} was detected in the ileal tissue.

Reversible cutaneous pigmentation sometimes occurs in vitamin B_{12} deficiency. In 1963 Baker *et al* described hyperpigmentation as a sign of vitamin B_{12} deficiency,[7] and noted its frequency in dark-skinned peoples. Indeed, almost all reports of this association have involved people from Africa or India. The importance of the association between hyperpigmentation and vitamin B_{12} deficiency rests in its reversibility with vitamin B_{12} replacement. The mechanism for this reversible change is unclear. It has been postulated that vitamin B_{12} deficiency lowers the intracellular reduction potential, with a concomitant decrease in the reduced glutathione:oxidised glutathione (GSH:GSSG) ratio.[8] Once the tyrosinase inhibiting effect of GSH has been diminished, the epidermal melanocytes are then stimulated to produce melanin. At the same time, the lowered GSH concentration results in retardation of mitosis, as well as diminished DNA synthesis. This could result in epidermal atrophy and hyperpigmentation. The exaggeration of hyperpigmentation by exposure to sunlight in vitamin B_{12} deficiency, as observed in our patients, may be due to a further lowering of intracellular GSH content by ultraviolet light.[9]

In vitamin B_{12} deficiency, brown skin pigmentation usually occurs over the dorsal aspects of fingers and toes, around the nails, and also on the finger pulp. These dermatological signs usually disappear after treatment. In our patients the skin manifestation was widespread mottled hyperpigmentation, especially over both groins, lateral abdomen and neck, termed poikiloderma. This peculiar pigmentary disturbance is similar to that of the patients with this syndrome described by Gillian *et al*[8] and Watson-Williams *et al*.[10] Of interest, was the persistence of the skin lesions in our patients, similar to the proteinuria which also persists despite vitamin B_{12} treatment. The reason for this refractoriness to vitamin B_{12} in our patients is unclear but may have been related to racial or genetic differences and may in part explain the heterogeneous nature of this syndrome, which may be polygenic in origin.

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