

Postheparin plasma diamine oxidase values in the follow up of patients with small bowel Crohn's disease

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Abstract

Measurement of postheparin plasma diamine oxidase (PHD) activity has been proposed to assess mucosal integrity in several diseases of the small intestine. In Crohn's disease, PHD values identify a group of patients with predominantly small bowel mucosal damage. To determine the role of mucosal involvement in the progression of small bowel Crohn's disease and whether different PHD values can predict different outcomes the changes in PHD values in 41 patients with small bowel Crohn's disease admitted consecutively to our department were investigated. The test was performed during periods of active disease and after either medical or surgical treatment had resulted in improvement. PHD values were significantly lower than in normal subjects (normal range 3.7-7.7 U/ml). In 35 patients with active disease (Crohn's disease activity index (CDAI) >150) two groups were identified by choosing a cut off value of 2 U/ml: 93% of the 15 patients with PHD values lower than 2 U/ml (mean (SD) 1.36 (0.46) U/ml) relapsed at least once in the following year, while only the 20% of the 20 whose values were higher than 2 U/ml (mean (SD) 3.69 (1.50)) relapsed in the same period. The data were statistically significant (Yates's corrected $\chi^2=15.63$; $p<0.0001$). The positive and negative predictive values of the test were 93% and 80%, respectively. During relapses, PHD values were consistently lower than previous values, and increased significantly after effective medical or surgical treatment. In the six patients in whom there were no changes in disease activity (CDAI persistently <150), there was no change in PHD values. This test may be useful for identifying Crohn's disease patients who are likely to relapse. Furthermore, the data indicate that mucosal damage is common in active small bowel Crohn's disease and improves at least in part after treatment.

The course of Crohn's disease is extremely unpredictable. Although some patients have frequent relapses, up to 20% have no further episodes for as long as 20 years after the first, or even the second.¹ Very little is known about the factors that determinate the different outcomes of the disease, nor has the role of mucosal damage in the progression of the disease ever been investigated.

Numerical indices are generally used to assess the disease activity and the severity of the

relapses.² None of the indices, however, correlates with the subsequent outcome.³

In the past few years there have been several reports on the usefulness of the postheparin plasma diamine oxidase (PHD) test in evaluating small intestinal mucosa integrity.⁴⁻¹⁰ The test is based on the ability of heparin to release the enzyme diamine oxidase (DAO) from the small intestinal mucosa into the circulation.¹¹⁻¹³ Since the enzyme is synthesised by the differentiated and non-proliferating enterocytes,¹¹ its plasma activity after an intravenous injection of heparin correlates with the morphological integrity of the small intestinal epithelium.^{4,5,10,14} The test proved to be helpful in quantitating the remaining mature enterocyte mass in patients with coeliac disease before^{4,6} and after⁵ gluten free diet, in small bowel lymphoma,^{4,10} and in Crohn's disease.^{7,8}

In the last group, PHD values were significantly lower than in normal subjects and correlated inversely with the Crohn's disease activity index (CDAI),⁷⁻⁹ while no correlation was found with the radiological extent of the disease.⁷ The test was able to identify a cluster of patients with predominant involvement of the mucosal layer.⁷ This finding prompted us to investigate whether different PHD values can predict different disease outcomes. In addition, since we had previously observed in a small number of patients with active disease that PHD values increased after anti-inflammatory treatment,⁷ we also wished to investigate the changes in PHD values during clinical relapse and after medical or surgical treatment in a larger series of patients to clarify the importance of mucosal involvement in the progression of small bowel Crohn's disease.

Methods

PATIENT SAMPLE AND EXPERIMENTAL DESIGN

We studied 41 patients (21 men, 20 women; mean (SD) age 36 (12) years) with small bowel Crohn's disease (12 ileal, five diffuse small bowel disease, and 24 ileocolonic disease) consecutively admitted to our department in the last five years. Diagnosis of Crohn's disease was made by us in 20 patients but in the other 21 we confirmed a diagnosis made elsewhere. Activity of the disease was assessed by the CDAI.¹⁵

In all patients, the PHD test was performed for the first time at admission to our department and then when the disease relapsed (CDAI >150). In the six patients in whom there were no changes in

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disease activity (CDAI persistently lower than 150), the test was repeated every six months.

PREDICTION OF RELAPSE OCCURRENCE

The PHD test was carried out in 35 patients with CDAI >150. Fifteen patients in this group presented with active disease at hospital admission and 20 had clinical relapses during follow up. These patients were divided in two groups according to the PHD value recorded in the active phase (group A: PHD <2 U/ml; group B: PHD >2 U/ml) in order to investigate whether different PHD values can predict different disease outcomes. To this end we evaluated the occurrence of clinical relapses over one year in each group. Follow up started after clinical improvement (CDAI <150) induced by anti-inflammatory drugs and supportive therapy administered according to the National Cooperative Crohn's Disease Study criteria.¹⁶

CHANGES IN PHD VALUES AFTER TREATMENT

To verify the effectiveness of treatment in the recovery of small bowel mucosal integrity, the test was repeated in 23 patients with active disease two to three months after clinical improvement (CDAI <150) resulting from medical treatment. In six patients who subsequently underwent intestinal resection, the PHD value was assessed before and six months after surgery.

PHD TEST AND DAO ASSAY

Heparin (15 000 IU) was administered by intravenous bolus. Blood (7 ml) was drawn 60 minutes after the injection, collected in heparinised tubes, and centrifuged at 3000 g. The plasma was stored at -20°C and assayed for DAO within a week. To simplify the test, plasma DAO activity was assessed only in the blood sample taken 60 minutes after heparin injection instead of in the several samples necessary for the calculation of the area under a 0-120 minute curve, as described in our previous study.⁷ In fact, the 60 minute sample correlates very well with the corresponding area under the curve and is the best discriminator among patients with different intestinal diseases.¹⁷ DAO was assayed in triplicate by a ¹⁴C-putrescine method as described in a previous study.¹⁷

DAO activity was expressed as U/ml (1 U=1 nmol of putrescine dihydrochloride oxidised for 1 hour at 37°C, pH 7.2). The normal range (44 subjects) is 3.7-7.7 U/ml; mean (2SD)=5.3 (2) U/ml.

In each patient, liver function (prothrombin time, alanine aminotransferase, bilirubin, alkaline phosphatase) was tested to avoid the possibility of impaired liver uptake of DAO causing high plasma DAO values that were not related to mucosal integrity.¹⁸ No abnormalities were found.

Written informed consent was obtained from all patients. No complication resulted from the administration of heparin.

In all subjects partial thromboplastin time (PTT) was monitored. Three hours after the

beginning of the test, PTT was still 2-2.5 times the maximum value of the normal range (22-44 seconds). It returned to normal within five hours of heparin injection.

Whenever the test was performed as an out-patient procedure, patients were allowed to leave the hospital four hours afterwards.

STATISTICAL ANALYSIS

Differences in PHD values before and after treatment were evaluated by Student's *t* test for paired data. Differences in outcomes of patients and the relative risk of relapse in the two groups of patients were assessed by the χ^2 test using the EPIINFO version 5 (Public Domain Software for Epidemiology and Disease Surveillance). Results are expressed as mean (SD) and differences were considered statistically significant when $p < 0.05$.

Results

PHD values recorded in the active phase of the disease in patients who relapsed during the one year follow up and in those who did not were 2.1 (1.8) and 3.3 (1.2) U/ml respectively. Choosing a PHD cut off value of 2 U/ml we identified two groups of patients; 14 out of 15 patients (group A) whose PHD values were lower than 2.0 (1.36 (0.46) U/ml) during the active phase of the disease relapsed at least once in the following year, while only four of the 20 patients (group B) with PHD values above 2.0 (3.69 (1.5) U/ml) relapsed during follow up (Table). The mean PHD values of the A and B groups of patients were significantly different ($p < 0.001$).

The average treatment time required for clinical recovery ranged from one to three months and was not different in the two groups studied.

Using the 2 U/ml cut off value, the test sensitivity and specificity in identifying the patients who are likely to relapse were 77% and 94% respectively; the positive and negative predictive values of the test were 93% and 80% respectively.

The Yates's corrected χ^2 value was 15.63, with $p < 0.0001$. The relative risk (RR) of relapse in patients with a PHD value <2 U/ml was =4.67 (1.92 < RR < 11.33).

Biochemical markers of inflammation such as C reactive protein, erythrocyte sedimentation rate, and blood white cell counts, performed during the active phase, were not significantly different in patients who relapsed within 12 months and in those who did not. Thus, these indices were not useful in predicting patient outcomes.

During follow up we performed the PHD test

Clinical relapses in 35 patients with active small bowel Crohn's disease according to plasma diamine oxidase values after iv heparin (PHD)

	Group A	Group B
PHD values (mean (SD))	1.36 (0.46)	3.69 (1.5)
No of patients	15	20
No of relapsed patients/year	14	4

Group A: PHD <2 U/ml; group B: PHD >2 U/ml.
(Yates's corrected $\chi^2 = 15.63$; $p < 0.0001$.)

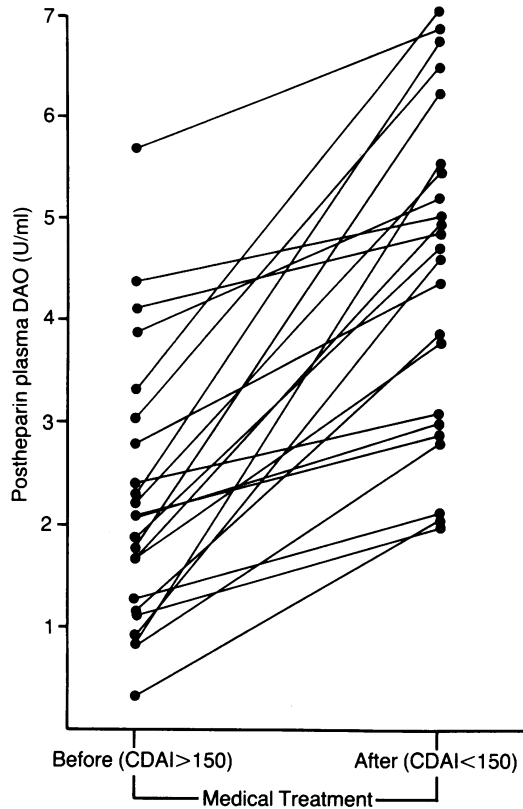


Figure 1: Changes in plasma diamine oxidase (DAO) values after *iv* heparin induced by clinically effective anti-inflammatory treatment in 23 patients with small bowel Crohn's disease. Differences in mean values are statistically significant ($p < 0.001$). CDAI = Crohn's disease activity index.

in 22 patients who relapsed. In all but two PHD values fell consistently: the drop ranged between 23–80% in respect of basal values (data not shown).

A significant increase in PHD values ($p < 0.001$) was recorded in the 23 patients in

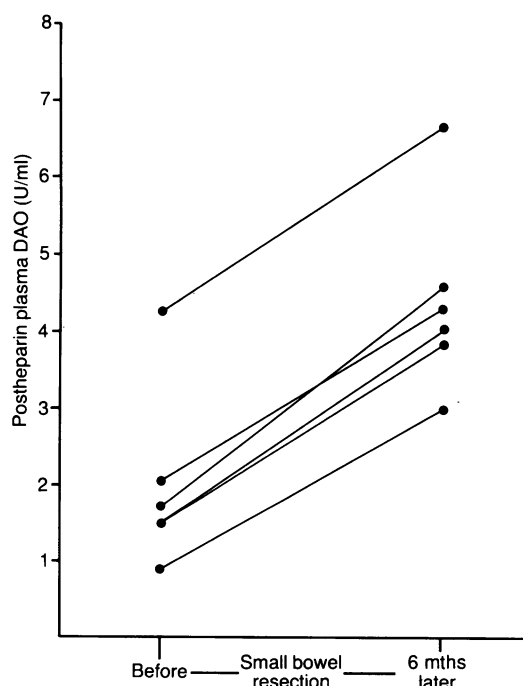


Figure 2: Changes in plasma diamine oxidase (DAO) values after *iv* heparin induced by surgery in six patients with small bowel Crohn's disease. Differences in mean values are statistically significant ($p < 0.001$).

whom the test was repeated after remission induced by anti-inflammatory treatment. Values before and after treatment were 2.30 (1.33) and 4.44 (1.62) U/ml, respectively (Fig 1).

Five patients underwent resection because of complications (three had intestinal obstruction, one abdominal abscess, and one ileocolonic fistula). One additional patient whose disease remitted after medical treatment, needed to be operated on for intestinal obstruction 12 months later. Figure 2 shows the PHD values in these six patients before and six months after surgery. A significant ($p < 0.001$) increase in PHD was observed in all patients.

Six patients whose disease was not active when admitted to our department did not relapse over a mean (SD) follow up period of 38.6 (17.6) months; their PHD values (4.5 (1.1) U/ml) did not change during the follow up.

The specificity of the test for small bowel mucosal damage in Crohn's disease was confirmed by the low PHD values in two patients with Crohn's colitis in whom unsuspected ileal involvement was subsequently found. Normal values were observed in a patient with Crohn's colitis only and in a group of six patients with idiopathic proctocolitis (unpublished data).

Discussion

The results of this study show that PHD, a marker of mucosal damage in patients with small bowel Crohn's disease,⁷ can be used for identifying patients who are likely to relapse. Furthermore, this test may be useful in assessing the response to treatment and in following the recovery of mucosal integrity in Crohn's disease patients.

PHD has been proposed as a new test to assess the morphological and functional integrity of the small bowel mucosa.¹⁹ The blood measurement of an enzyme activity that is almost exclusively of small intestinal origin^{20,21} and that correlates with the differentiated enterocytic mass^{4,5} represents a new approach to the evaluation of diseases affecting the small intestinal mucosa, the assessment of which is otherwise based on clinical tests of digestion and absorption.²² In Crohn's disease results of absorption tests may be affected by bacterial overgrowth rather than mucosal damage, thus making the assessment of mucosal lesions difficult. Thompson *et al*²³ found that in resected specimens of small bowel from patients with Crohn's disease, mucosal DAO activity correlated inversely with histological grading of the severity of inflammatory changes.

In the present study, PHD values decreased when Crohn's disease patients relapsed, indicating that mucosal damage is very common during the active phase of the disease and suggesting that mucosal involvement may be an important factor in determining clinical relapse. The ability to predict, with some degree of confidence, whether or not Crohn's disease will recur in an individual patient would be valuable because it would provide a better basis for decisions about treatment. To this end, there have been studies of prophylactic steroid treatment^{24,25} in subsets of patients with a high risk of relapse.²⁶

This study showed that patients with very low

PHD values during active disease had a poorer prognosis and suggests that the PHD test may have a role in the prediction of short term relapses in patients with small bowel Crohn's disease. If our results are confirmed in a larger series of patients and over a longer follow up, it will be interesting to study subsets of patients with Crohn's disease and very low PHD values to see if they benefit from prophylactic treatment.

Furthermore, our data indicate that effective drug treatment restored, albeit partly in some patients, intestinal DAO values.

Surgery, performed in six patients for complications, also caused an increase in the PHD values six months after the operation. The higher PHD values after surgery may be a result of restoring mucosal integrity after the removal of inflamed gut and the resumption of the luminal nutrition, which has a trophic effect.²⁷ Moreover, the resection of part of the bowel may have induced an adaptive response that quickly increases functional capability.²⁸ In fact, resection is a strong stimulus to compensatory growth of the small bowel.^{29,30} After partial resection the villi become hyperplastic and this persists for at least three to six months, and probably indefinitely.³⁰ Furthermore, increased DAO values were detected in the small bowel mucosa of rats which underwent intestinal resection.³¹

In conclusion, we have confirmed low PHD values in patients with active Crohn's disease. Treatment, either medical or surgical, increased PHD values, indicating that mucosal damage is common during active disease but can be repaired by therapy. Patients with very low PHD values had a poorer prognosis in terms of number of relapses, suggesting that mucosal lesions are important in the progression of the disease.

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