FECAL LACTOFERRIN AS A DIFFERENTIATION MARKER BETWEEN

ULCERATIVE COLITIS AND IRRITABLE BOWEL SYNDROME

By

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ABSTRACT

This study was designed to estimate

fecal lactoferrin (LF) concentration

and to evaluate its clinical applicability

as non-invasive modality for differentiation

between cases with ulcerative

colitis (UC) and irritable bowel

syndrome (IBS) and its relation to disease

activity. The study included 15

patients with UC and15 patients with

IBS and 15 healthy volunteers as controls.

All patients were evaluated clinically

for disease activity and underwent

colonoscopy for diagnosis assurance.

The study participants supplied

fresh fecal samples for qualitative and

quantitative assay for LF. There were

11 patients with active UC and 6 patients

with active IBS. There was a

significant (P<0.05) increase of fecal

LF in patients with UC (1118.21277.8

μg/gm feces) compared to controls

(1.35 10.48 μg/ gm feces) and IBS patients

(1.33 10.36 μg/ gm feces). Moreover,

there was a significant (P<0.05)

increase of fecal LF in patients with

active UC compared to those with inactive

UC, whereas non-significantly

(P>0.05) different in patients with active

IBS compared to those inactive

IBS. Furthermore, there was a significant

correlation between fecal LF level

and score of severity of inflammation

in patients with UC (r=0.623, P=0.013),

whereas the correlation was nonsignificant

in patients with IBS,

(r=0.225, P>0.05). Qualitative determination

of LF could identify patients

with UC with sensitivity of 93.3%,

specificity and positive predictive value

of 100% and accuracy of diagnosis by

97.8% irrespective of the severity of

the disease. It could be concluded that

qualitative determination of fecal LF

could differentiate between patients

with UC and IBS with specificity 100%

and accuracy 97.8% and quantitative

estimation of its level could define

cases with active UC.

INTRODUCTION

Inflammatory bowel diseases and

irritable bowel syndrome are intestinal

diseases perceived differently by patients

and doctors: inflammatory bowel disease

is considered essentially as an 'organic'

disease, i.e. an illness in which the role of

stress or psychological factors is at best

secondary to the disease itself, whereas

IBS is acknowledged as a 'functional'

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disorder, i.e. illness thought to be more in

the 'mind' than in the body of the patient

(Pace et al., 2003).

Both inflammatory bowel disease

and IBS are two chronic conditions that

can present with similar symptoms such

as diarrhea and abdominal pain, but have

very different underlying pathophysiology.

Inflammatory bowel disease is a

chronic, idiopathic inflammatory condition

affecting varying layers of the GI

tract. The anatomic location and degree

of the inflammation determines the predominant

symptoms that may include

rectal bleeding, diarrhea, and recurrent

abdominal pain (Thjodleifsson et al.,

2003). In contrast, IBS is a functional

non-inflammatory disorder, which can

present with abdominal pain and diarrhea.

This syndrome is thought to be

caused by abnormal GI motility or altered

pain perception (Adeniji et at.,

2004).

Clinically, it can be difficult to differentiate

between the two conditions in

the absence of rectal bleeding and systemic

illness. The diagnosis of inflammatory

bowel disease requires invasive testing

to determine the anatomic location of

inflammation and for obtaining tissue

samples. A noninvasive diagnostic test to

screen patients for GI inflammation as a

cause for diarrhea would have clinical

utility. Ultrasonography has been previously

used as a noninvasive test but it

requires special expertise and equipment

and so not widely used in clinical practice

(Minderhoud et al., 2004).

Lactoferrin, an iron-binding glycoprotein,

is secreted by most mucosal

membranes and is a major component of

the secondary granules of polymorphonuclear

neutrophils, a primary component

of the acute inflammatory response

(Baveye et al., 1999). Other hematopoietic

cells such as monocytes and lymphocytes

do not contain lactoferrin (Naidu et

al., 1997).

Fecal lactoferrin levels increase

quickly with the influx of leukocytes into

the intestinal lumen during inflammation.

The protein is resistant to proteolysis and

unaffected by multiple freeze thaws, providing

a useful marker in feces as an indicator

of intestinal inflammation

(Buderus et al., 2004).

This double-blinded comparative

study was designed to determine fecal

lactoferrin concentration and to evaluate

its clinical applicability as non-invasive

modality for differentiation between

cases with inflammatory bowel disease

and irritable bowel syndrome and its relation

to disease activity.

SUBJECTS AND METHODS

This double-blinded, selective,

comparative study was conducted at Departments

of Hepatology, Gastroenterology

& Infectious Diseases and Internal

Medicine at Benha University and Benha

Insurance Hospitals in conjunction with

Medical Biochemistry and Pathology

Departments, Benha Faculty of Medicine

since Oct 2002 till Jun 2004.

The study comprised selection of

15 patients with prolonged history of

recurrent episodes of bloody diarrhea (4-

8 stools/day) and abdominal pain and

diagnosed previously as IJC. Patients on

chronic use of nonsteroidal antiinflammatory

drugs, aspirin, and anticoagulants

or had concomitant other nongastroenterologic

diseases, in particular,

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rheumatoid arthritis or other connective

tissue inflammatory diseases, or respiratory

or urinary tract inflammation/

infection or hepatic diseases were excluded

from the study.

Patients were evaluated using Harvey—

Bradshaw Activity Index for UC

(Harvey & Bradshaw, 1980), including

number of liquid or very soft stools per

day (0=-4, 1=-6, 2=-8, 3=>8 times/day),

abdominal pain: (0= infrequent, 1=mild,

2=moderate, 3=severe), general wellbeing:

(0—well, 1=s1ightly well, 2= poor,

3= very poor, 4= terrible), complications:

(score: Ono & 1=present) Arthritis or

arthralgia, Skin or mouth lesions, kids or

uveitis; Anal fissure, fistula, or perianal

abscess and bleeding per rectum:

(0—none, 1=slight, 2=moderate,

3=severe). A total score of >4 indicates

disease activity. Patients underwent sigmoidoscopy

if under 40 years of age or

colonoscopy if over 40 years so as to

exclude cancer colon Endoscopic examination

revealed extensive superficial

ulcerations of the colon. Endoscopic biopsies

obtained for assurance of the diagnosis

of UC revealed multiple crypt abscesses,

no granuloma, nor cancer colon

or patches of low-grade dysplasia.

Another 15 patients with chronic

alternating diarrhea and constipation with

abdominal pain of varying intensity; diarrhea

is not accompanied by bleeding nor

associated by weight loss or fever. A full

medical history was obtained and a

physical examination was performed;

each patient was evaluated by documentation

of the Rome criteria, which are

considered as a guide for IBS diagnosis.

In addition, all patients underwent laboratory

investigations performed in hospital

laboratory and included complete blood

count, fasting blood sugar and blood

urea, serum creatinine; liver and thyroid

functions tests, ESR and stool examination

for occult blood, pus, and parasites.

Then, patients underwent sigmoidoscopy

or colonoscopy with biopsy. Patients

with negative results for all of the examinations

described above, with normal

average laboratory findings and with a

clinical history indicative of IBS were

considered to be suffering from IBS,

(Drossman et al., 1997). IBS activity

index was evaluated using IBS symptom

questionnaire included nausea, bloating,

abdominal pain, diarrhea, constipation

and anorexia; each symptom was rated

on a scale of 0-3, with 0= no, 1=mild,

2—moderate and 3=severe symptoms. An

activity index of >10 defined IBS activity,

(Mathias et al., 1994).

The study also comprised 15

healthy volunteers with no history of

infectious diarrhea within the previous

month and had stool free of pus, bilharziasis

or active amebiasis.

Lactoferrin Assay

The study participants supplied

fresh fecal samples containing a minimum

of 30 ml. The sample was divided

into 2 parts for qualitative and quantitative

assay and stored at —20°C until assay.

For the qualitative assay, a single

specimen dilution of 1:400 was analyzed

by a polyclonal antibody-based ELISA

(LEUKO-ELISA; TechLab, Blacksburg,

VA) using wells containing immobilized

polyclonal antibodies to human lactoferrin

(Steiner et al., 1997). In this assay,

lactoferrin, if present, binds to the antibodies

during a 30-mM incubation at 37°

C. After the incubation, polyclonal antibodies

coupled to horseradish peroxidase

enzyme (conjugate) were added and

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allowed to bind to captured lactoferrin

during a second 30-min incubation. Unbound

conjugate was then washed from

the well, and one component substrate

(tetra-methyl-benzidene and hydrogen

peroxide) was added for color development.

After the 15-min substrate incubation,

a 0.6-N sulfuric acid solution was

added to stop the reaction, and the absorbance

was measured spectrophotometrically

at 450 nm (A450) and A450

cutoff of 0.200, previously optimized for

the detection of fecal leukocytes, were

used. This corresponds to a concentration

of 12.8 μgig feces. These results

were reported as positive (A450 >0.200)

or negative (A450 <0.200) for a "yes/no"

indicating the presence of intestinal inflammation.

A positive control consisting

of diluted human lactoferrin and a

negative control consisting of test diluent

were evaluated with each ELISA analysis

to facilitate comparisons of results.

For the quantitative assay, fecal specimens

were serially diluted 10-fold and

analyzed as in qualitative assay, and the

absorbance was measured spectrophotometrically

at 450 nm (A450). Lactoferrin

concentration (p.g/g wet weight of

feces) was obtained by multiplying the

concentration by the dilution factor.

Statistical analysis

Data were analyzed using t-test and

Chi-square test. Possible relationships

were investigated using Pearson linear

regression. Statistical analysis was conducted

using the SPSS (Version 10,

2002) for Windows statistical package. P

value <0.05 was considered statistically

significant.

RESULTS

The study comprised 30 patients;

16 males and 14 females with a mean

age of 43.4±5.7; range. 30-52 years and

15 control volunteers; 8 males and 7

females with mean age of 40±9, range:

29-51 years. There was a non-significant

(P>0.05) difference between patients and

controls as regards age and sex distribution.

All UC patients had bloody diarrhea;

9 patients had mild, 5 patients had

moderate and only one patient had severe

bleeding. As regards frequency of

stool times/day, 2 patients had about 4

times, 3 had about 6 times, 8 patients had

about 8 times and only 2 patients had >8

times/day. Two patients had infrequent

abdominal pain, 6 patients had mild, 5

had moderate and 2 patients had severe

abdominal pain, 7 patients feel slightly

well, 6 patients feel poor general wellbeing

and 2 patients had very poor general

well-being. Five patients had arthralgia

and 2 patients had recurrent anal fissures

after surgical excision. There were

11 patients with active UC with a mean

activity score of 8.112.1; range 5-1 land

4 had inactive disease with an activity

score <4, (Table 1).

Clinical evaluation of patients with

IBS, revealed that 10 patients had anorexia;

7 mild and 3 moderate, 9 patients

had nausea; 7 mild and 2 had moderate.

All patients had abdominal pain and

bloating varying from mild to severe, 8

patients had diarrhea, 3 had constipation

and 4 had alternating diarrhea and constipation.

There were 6 patients with

active IBS with a mean activity score of

10.7±1.2; range 10-13 and 9 had inactive

disease with an activity score <10,

(Table 2).