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Assessment of high-sensitivity CRP as a marker of micro-inflammation in irritable bowel syndrome

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Abstract

Background *The diagnosis of irritable bowel syndrome (IBS) is symptom-based. Although considered a functional disease, accumulating evidence supports a low-grade gut inflammation as an element of its pathophysiology. Thus, high-sensitivity C-reactive protein (hs-CRP), a marker of micro inflammation, may be elevated in IBS. Our aim was to assess whether hs-CRP is higher in IBS patients compared to healthy controls (HC) and does it differ among the IBS clinical subgroups and correlate with disease severity.* ***Methods*** *A diagnostic case control study was conducted in two gastroenterology departments. Eighty-eight IBS patients who were recruited prospectively answered the Rome III diagnostic questionnaire. They all completed the Functional Bowel Disorder Severity Index (FBDSI), dietary, and general health questionnaires. All patients underwent blood sampling for hs-CRP levels. Each IBS patient was matched to four HC by age, gender, and BMI. Blood samples were obtained from the HC at a periodic health survey.* ***Key Results*** *The mean hs-CRP level in the IBS group was significantly higher than in HC (1.17 ± 1.26 mg* L^{-1} *vs 0.72 ± 0.91 mg* L^{-1} *respectively, P = 0.001). Hs-CRP levels were highest in patients with diarrhea-predominant IBS and in patients with greater disease severity. A cut-off value of 1.08 mg* L^{-1} *had a sensitivity of 60.2% and a specificity of 68% for differentiating IBS from HC.* ***Conclusions & Inferences*** *Hs-CRP levels are higher in IBS patients than HC, but still in the normal laboratory range. This may reflect the low-grade gut inflammation believed to occur in IBS and support its existence.*

Keywords *high-sensitivity C-reactive protein, irritable bowel syndrome, low grade inflammation.*

Abbreviations: ESR, erythrocyte sedimentation rate; FBDSI, functional bowel disorder severity index; GI, gastrointestinal; HC, healthy controls; Hs-CRP, high-sensitivity C-reactive protein; IBS, irritable bowel syndrome; IBS-C, constipation predominant IBS; IBS-D, diarrhea predominant IBS; IBS-M, mixed type IBS; TAM-CIS, Tel Aviv Medical Center Inflammation Survey; WBC, white blood cells; Wr-CRP, wide range C-reactive protein.

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal (GI) disorders. Its diagnosis is based on the Rome III criteria that include chronic complaints of abdominal pain or discomfort associated with a change in bowel habits. Patients are categorized by their predominant symptom into diarrhea predominant (IBS-D), constipation predominant (IBS-C) or mixed type IBS (IBS-M). Severity of symptoms may be assessed by validated questionnaires such as the Functional Bowel Disorder Severity Index (FBDSI) that are used mainly for research purposes.[1,2]

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Hypotheses regarding the pathophysiology of IBS include genetic predisposition,[3–6] environmental triggers such as infection,[7,8] change in bowel microflora,[3,9,10] immune system activation, and brain-gut dysfunction.[11]

Despite intense research, the pathophysiology of IBS is still not fully understood. Low grade gut inflammation is one of the postulated mechanisms responsible for the increased visceral hypersensitivity and altered bowel motility seen in IBS patients. The role of increased gut inflammation in IBS patients is supported by the elevated number of mast cells[3] and T cells[11] found in the gut wall and by evidence of immune activation.[12] In addition, IBS patients are genetically more prone to develop inflammation compared to healthy controls (HC) as they are more likely to express high TNF-α and low IL-10 secretion genotypes.[6,13] Furthermore, the ratio of IL10 to IL12 secreted from peripheral blood monocytes is lower in IBS patients compared to healthy controls[14] indicating a skew towards a Th1 cytokine profile.

Hence, low grade inflammation may play a role in IBS, but to date no serum inflammatory marker has been found that reflects this putative inflammation.

High-sensitivity C-reactive protein (hs-CRP) is a useful marker for overt inflammatory processes such as in inflammatory bowel diseases, but is sensitive enough to detect low grade inflammatory processes as well, such as in the case of Crohn's disease in remission or atherosclerosis.[15–17]

The aim of this study was to evaluate whether hs-CRP is elevated in IBS patients compared to HC, and whether its levels differ among the three IBS subtypes.

MATERIALS AND METHODS

Patients

One hundred IBS patients were recruited prospectively in this case control study between January 2008 and August 2008 from the IBS units in two Gastroenterology departments in the tertiary hospitals Tel Aviv Sourasky Medical Center and Rabin Medical Center, both located in central Israel.

All patients were over 18 years old and had an established IBS diagnosis according to the Rome III criteria made by experienced gastroenterologists specializing in IBS (RD, RD, AS, YR). The patients were classified into the three IBS subgroups (IBS-D, IBS-C, or IBS-M). The study was approved by the local Helsinki committee and all patients signed informed consent.

Patients with conditions that could mimic IBS, or are known to affect CRP levels, were excluded. These included a medical history of malignancy, asthma, thyroid disease, celiac disease, inflammatory bowel disease, use of steroids, immune-suppressants or antibiotics during the previous month, pregnancy, any inflammatory state or infection in the month prior to recruitment, CRP≥10 mg L^{-1},[18] or abnormal blood test results for TSH or anti-TTG.

Healthy control (HC) group

The medical history and the results of laboratory tests for the HC group were retrieved retrospectively from the Tel Aviv Medical Center Inflammation Survey (TAMCIS), which is a registered data bank of the Israeli Ministry of Justice.[19] From November 2002, more than 15 000 apparently healthy subjects attending a center for periodic health examinations participated in this survey. They answered the same questionnaires as the IBS patients including items on medical background, demographic, socioeconomic, dietary, and lifestyle habits. Each IBS patient was carefully matched to four different HC by gender, age (±4 years), and BMI (±2 kg m^{-2}). Healthy controls were excluded if they suffered from diseases known to affect CRP levels (as detailed in the Patients section above) or if hs-CRP levels were above 10 mg L^{-1}.

All patients were interviewed for demographic data and medical history and their height and weight were measured. Their medical files were reviewed and they completed the following questionnaires:

1 General health, background diseases, treatments and recent infections or inflammatory conditions and other diseases, conditions and habits that are known to affect CRP levels, other than age, BMI, and gender[20] such as physical activity,[20] smoking,[20,21] nutrition,[21–24] alcohol consumption, and the use of contraceptive pills.[20]
2 Functional Bowel Disorder Severity Index (FBDSI) – a validated questionnaire used to assess IBS disease severity.[2] Patients were classified into mild with a score <37, moderate with a score of 37–110, and severe with a score >110.

Hs-CRP level determination

Blood was drawn from patients and HC after a 12-h fast and was analyzed for hs-CRP, wide range CRP (wr-CRP), fibrinogen, erythrocyte sedimentation rate (ESR), complete blood count, anti-TTG antibodies, IgA, TSH and FT4, glucose, and HbA1C.

The quantitative concentration of hs-CRP was determined by the Boering BN II Nephelometer (DADE Boering, Marburg, Germany) according to the Rifai method.[25] Normal hs-CRP levels are in the range of 0–5 mg L^{-1}.

Statistics

All data were summarized and displayed as the mean ± SD for continuous variables, as geometric mean ± SD for non-normal distribution continuous variables and as the number of patients plus the percentage in each group for categorical variables.

Sample size calculations were based on a pilot study of 37 IBS patients and 147 HC, assuming a difference of 0.4–0.5 mg L^{-1} in hs-CRP levels between patients and HC, to achieve statistical significance ($\beta = 0.2$, $\alpha = 0.05$). Therefore, at least 61–95 patients and 244–380 controls were needed for the study.

As hs-CRP, wr-CRP, and WBC (white blood cells) have a non-normal distribution; we used a logarithmic transformation to convert them to a normal distribution for all statistical procedures. The one-sample Kolmogorov–Smirnov test was used to verify that the logarithmic transformation was normally distributed.

All hs-CRP results in the tables and in the graphs are the geometric mean and SD. Univariate conditional logistic regression was used for comparisons between cases and HC, adjusting for the matching ratio of 1 : 4, in terms of socioeconomic variables, health status, and markers of inflammation.

A multiple conditional logistic regression was used, again adjusting for the matching ratio, to investigate the relationship

between IBS (as a dichotomous variable) and a set of prognostic factors, such as inflammatory markers, socioeconomic status, smoking and dietary habits, and health factors.

The Spearman correlation was used to assess correlations between hs-CRP and IBS severity. Two-way ANOVA was used to examine the effects of two IBS subgroups, severity levels and the primary symptom, together.

The level of significance used for all analyses, was two-tailed and set at $P < 0.05$. The SPSS statistical package (Version 15, SSPS Inc., Chicago, IL, USA), was used for all statistical analyses.

Receiver operator curve (ROC) analyses were performed for all inflammation markers to determine sensitivity and specificity rates that distinguish between IBS patients and HC, and to calculate the positive predictive value (PPV) and negative predictive value (NPV) for each variable.

RESULTS

One hundred IBS patients were recruited into the study. Twelve patients were excluded: five suffered from asthma or hypothyroidism, one had rheumatoid arthritis, two had acute inflammatory diseases and no suitable HC could be found for four patients who were in the age range of 68–74 years old. The remaining 88 patients were matched to 352 HC (four HC to each patient) according to age, gender, and BMI (Table 1).

There was no difference between patients and HC regarding cardiovascular risk factors, although IBS patients performed significantly less physical activity, which is probably due to the decreased functional and physical capabilities of these patients. Interestingly, IBS patients drank less coffee and alcohol and consumed a diet lower in fiber compared to HC (cardiovascular risk factors and dietary habits are detailed in Table 1). A higher percentage of IBS patients were treated by SSRIs (selective serotonin reuptake inhibitor), PPIs (proton pump inhibitor), and laxatives (Table 1).

Hs-CRP levels were significantly higher in the IBS group compared with the HC (1.17 ± 1.26 *vs* 0.72 ± 0.91 mg L^{-1}, $P < 0.001$). Moreover, wr-CRP and fibrinogen were also significantly higher in IBS patients (Table 2). Through a ROC curve analysis (Figure S1 Supporting information) we found that a hs-CRP value of 1.08 mg L^{-1} reflects the highest degree of specificity (68%) and sensitivity (60.2%) for the diagnosis of IBS, with a PPV of 31.5% and a NPV of 87.1%.

Hs-CRP was higher in all IBS severity subgroups compared to HC but the difference reached statistical significance only in the high severity subgroup. Hs-CRP levels were lower in the mild severity subgroup than in the high severity subgroup (Table 3).

Among men, IBS severity correlated with hs-CRP levels ($r = 0.501$ $P = 0.004$). However, a similar correlation was not found among women. Hs-CRP also varied in the three clinical IBS subtypes, lowest in IBS-C and highest in IBS-D (Table 3). Fig. 1 shows that

Table 1 Comparison of demographic characteristics, cardiovascular risk factors, dietary and lifestyle habits and medical treatment between IBS patients and controls

	IBS	Healthy controls	*P*-value*
Demographic characteristics			
Gender (% Women)	64.8	64.8	ns
Age (mean ± SD) (years)	37.11 ± 13.78	37.28 ± 13.43	ns
BMI (mean ± SD) (kg m^{-2})	23.40 ± 3.90	23.51 ± 3.8	ns
Cardiovascular risk factors (% of participants)			
Hyperlipidemia (%)	13.6	9.1	ns
Hypertension (%)	5.7	6.8	ns
Currently smoking (%)	20.5	16.5	ns
Diabetes mellitus (%)	1.1	1.4	ns
Coronary heart disease (%)	2.3	2.0	ns
Dietary and lifestyle habits (mean ± SD)			
Physical activity (%)	62.5	61.1	ns
Physical activity – hours per week	1.84 ± 2.31	3.69 ± 2.71	<0.001
Alcohol consumption at least once a week (%)	30.2	40.6	ns
Alcohol consumption – cups per week	0.76 ± 1.94	2.22 ± 2.16	<0.001
Fruit consumption – portions per day	1.38 ± 1.69	2.47 ± 2.13	<0.001
Vegetable consumption-portions per day	2.20 ± 1.88	3.68 ± 2.53	<0.001
Fry food consumption – portions per day	0.44 ± 0.61	1.01 ± 1.14	<0.001
Coffee consumption – cups per day	1.56 ± 1.85	2.48 ± 2.00	<0.001
Medical treatment (% of treated participants)			
Proton pump inhibitor	15.9	0.6	<0.001
Selective serotonin reuptake inhibitor	14.8	0.3	<0.001
Laxative	10.2	0.0	<0.001
Contraception	17.2	22.7	ns
Statin	3.4	4.5	ns

*Conditional Logistic Regression; ns, Non-significant.

Table 2 Comparison of inflammatory markers levels between IBS patients and controls

	IBS	Healthy controls	*P*-value*
Hs-CRP (mg L^{-1}) (mean ± SD)	1.17 ± 1.26	0.72 ± 0.91	<0.001
Wr-CRP (mg L^{-1}) (mean ± SD)	0.86 ± 1.78	0.47 ± 1.22	<0.001
Fibrinogen (mg dL^{-1}) (mean ± SD)	302.70 ± 59.05	277.53 ± 53.99	<0.001
WBC ($10e^3$ μL^{-1}) (mean ± SD)	6.17 ± 0.28	6.45 ± 0.21	ns
ESR>20 (mm h^{-1}) (%)	26.7	18.1	ns

*Conditional Logistic Regression. ns, Non-significant. Data of hs-CRP, wr-CRP and WBC are displayed as geometric mean.

Table 3 Geometric mean of hs-CRP levels in IBS patients by severity and by disease subtype in comparison to matched healthy controls

	IBS mean ± SD (*n*)	Healthy controls mean ± SD (*n*)	*P*-value*
IBS by severity			
Mild	0.89 ± 1.27 (8)	0.54 ± 0.94 (32)	ns
Moderate	1.04 ± 1.19 (23)	0.72 ± 0.80 (92)	ns
Severe	1.28 ± 1.30 (57)	0.75 ± 0.94 (228)	<0.001*
IBS by subtype			
IBS-C	0.82 ± 0.93 (10)	0.71 ± 0.69 (40)	ns
IBS-M	1.07 ± 1.32 (55)	0.75 ± 0.89 (220)	ns
IBS-D	1.67 ± 1.20 (23)	0.65 ± 1.02 (92)	0.001*

*Significant according to Holm multiple comparison technique. ns, Non-significant. Data of hs-CRP are displayed as geometric mean. IBS-C, constipation-predominant IBS; IBS-M, mixed-type IBS; IBS-D, diarrhea-predominant IBS.

hs-CRP correlates with disease severity and its levels are higher in IBS-D compared with IBS-C or IBS-M.

Odds ratio (OR) for IBS among IBS patients and matched healthy controls by hs-CRP was 1.72 (95% CI 1.31–2.25) when adjusted to age, gender, and BMI. Multiple adjustments to known factors that affect CRP hardly change this OR. OR for IBS by other common inflammatory markers (wr-CRP, WBC, and fibrinogen) was lower (Table 4), confirming hs-CRP as the most appropriate predictor for IBS.

DISCUSSION

There is a growing body of evidence that inflammation plays a role in the pathophysiology of IBS. To date, IBS patients have been shown to have a genetic predisposition to inflammation and increased gut inflammatory activity.[6,13] *In vitro* studies have demonstrated an inflammatory profile for cytokines secreted from PBMCs isolated from peripheral blood of patients.[3,12] Although CRP levels have been assessed previously in IBS patients, they have always been in the normal range (0–5 mg L^{-1}).[26] ESR, as assessed in a previous study as well as in the present one, is not sensitive enough to detect low grade inflammation, in contrast to fibrinogen that has been shown here and by others to correlate with low grade inflammatory processes.[27]

The results of this study show, for the first time, that IBS patients have higher hs-CRP serum levels than HC. As their hs-CRP levels is higher than HC but still within normal limits, this finding may reflect the state of micro-inflammation known to occur in IBS patients.

There was a propensity for hs-CRP levels to be correlated with disease severity in IBS patients. The highest hs-CRP levels were found in IBS-D and the lowest in IBS-C. This finding was previously reported by Poulis *et al.* who compared hs-CRP levels in IBD patients to IBS-D and IBS-C.[28] It is also consistent with the report that there is increased serine protease activity, which is speculated to increase local gut inflammation,[29] in IBS-D compared to IBS-C patients.

IBS can have similar symptoms to other diseases. To minimize possible misdiagnosis we recruited all patients from specialized IBS clinics in tertiary centers. All patients were diagnosed according to the Rome III criteria and all underwent blood tests to exclude celiac and thyroid disease. Another strength of our work is the meticulous matching of four HC to each IBS patient by age, BMI, and gender, all variables that are known to affect CRP levels. In addition, the detailed questionnaires used in the study enabled us to adjust for most other factors known to affect CRP levels such as cardiovascular risk factors, dietary habits, smoking status, and physical activity. Indeed, dietary habits of IBS patients compared to healthy participants included less consumption of fried food, fibers, alcohol, and coffee (Table 1). These probably reflect their attempt to eliminate specific foods to which they feel that they are intolerant. Simren *et al.* show that two-thirds (63%) of IBS patients relate their symptoms to nutrition, especially carbohydrates, fatty foods, coffee, alcohol, and hot spices.[30] In addition, our controls, who participated in the health survey as volunteers, represent individuals with enhanced health awareness that results in regular physical activity and healthy diet (rich in fruits and vegetables).

Our study has several limitations: despite our efforts to exclude diseases that may mimic IBS, colonoscopy was not an inclusion criterion for our ≥50 years old patients (although routinely recommended for this population). In addition, several conditions which are commonly associated with IBS and may increase CRP levels, such as depression, chronic fatigue syndrome or sleep disturbances were not excluded unless the

Table 4 Odds ratio for IBS among IBS patients and matched healthy controls by inflammatory markers

	Odds ratio (95% CI)				
Adjusted for	Age, gender, BMI	Age, gender BMI, socioeconomic status*	Age, gender BMI and smoking status†	Age, gender BMI, statins treatment, coronary heart disease, cardiovascular risk factors‡	Age, gender BMI, dietary habits§
Hs-CRP (mg L^{-1})	1.72 (1.31–2.25)	1.65 (1.25–2.17)	1.72 (1.32–2.25)	1.70 (1.29–2.24)	1.69 (1.26–2.26)
Wr-CRP (mg L^{-1})	1.40 (1.15–1.70)	1.33 (1.09–1.62)	1.39 (1.15–1.69)	1.37 (1.12–1.67)	1.34 (1.08–1.66)
Fibrinogen (mg dL^{-1})	1.01 (1.005–1.015)	1.01 (1.005–1.015)	1.01 (1.005–1.015)	1.01 (1.005–1.015)	1.01 (1.006–1.017)

BMI, body mass index; CHD, coronary heard disease. *Socioeconomic Status refers to education (years). †Smoking Status refers to current smoking. ‡Cardiovascular risk factors refer to hyperlipidemia, diabetes, hypertension. §Dietary habits refer to consumption of fruits, vegetables, fry foods, and coffee per day.

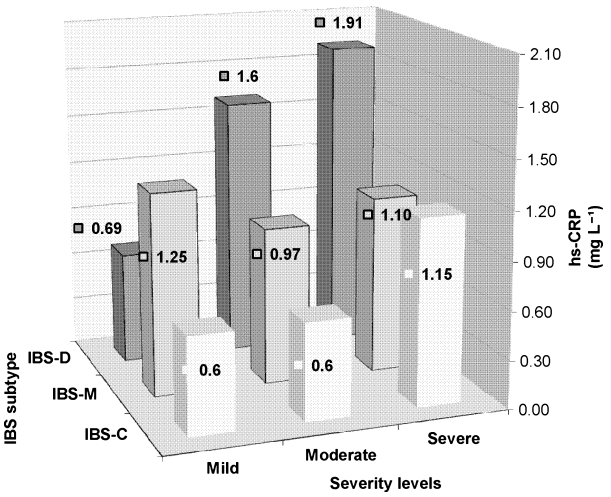

Figure 1 Geometric mean of hs-CRP (mg L^{-1}) levels by irritable bowel syndrome (IBS) subtypes and severity levels. There is a trend towards higher CRP levels in the diarrhea subtype and in more severe IBS.

patients had a formal diagnosis/treatment for these conditions. Furthermore, although participants who were treated with antibiotics, steroids, or immunosuppressants were excluded, we did not record treatment with NSAIDs, which may also affect CRP levels. These may also be considered limitations of this study, although, our control group had the same inclusion/exclusion criteria.

A potential limitation of this study is a selection bias in that our cohort was recruited from tertiary centers and may lack more typical IBS patients. However, the female/male ratio of 1.85 is typical of IBS,[31] and the age range is broad (18–69 years). However, most of the patients are younger than 40 years, which is somewhat lower than the rate reported in a major epidemiological study of IBS in Israel with a mean age of 45.[32] In all, 62.5% of our IBS group were classified as IBS-M, a seemingly atypical proportion.[32,33] This could reflect misclassification or recall bias as the patients were classified according to their complaints at time of the interview. A possible explanation of this finding is that since they were already under treatment in many cases, including laxative use, some patients with constipation as their primary symptom may have reported themselves with mixed-typed IBS as a result of the this treatment. However, further analysis of the data does not support this hypothesis. In a major epidemiological study (mentioned above) of IBS in Israel, the proportion of IBS sufferers with IBS-M was 37.5%.[32] The difference may stem from the small, non-representative number of IBS patients in this study.

Another limitation of this study is the population size, which precluded some IBS sub-group and severity level analyses. Further analyses are needed with larger samples in each subgroup to investigate the differences between these subgroups. An additional potential limitation is the implementation of strict exclusion criteria, especially the exclusion of all patients with conditions and diseases known to affect CRP levels. Although, for the purpose of proof-of-concept, this is a strength of the study, since the study population was carefully chosen our results may not be generalizable to patients with various chronic diseases. However, the majority of IBS patients are young adults who do not suffer from chronic diseases. Finally, as research into micro-inflammation grows, the number of factors and conditions known to affect CRP levels increases. Thus, there may be presently unknown factors that we did not adjust for that could potentially bias the results.

The results of this study do not support the routine clinical use of hs-CRP to differentiate between IBS patients and others. A test using a combination of 10 serum markers, which were chosen from 60 000 biomarkers, yielded a sensitivity of 50% and specificity of 88% for identifying IBS patients.[34] In this study, hs-CRP had a sensitivity of 60.2% and a specificity of 68%. These rates are not high enough to help clinicians

in routine work. In addition, the results were attained comparing IBS patients to asymptomatic HC. In clinical work, a test is needed that would distinguish IBS from symptomatic individuals with other causes.

In conclusion, we believe that our findings support the assertion that IBS, especially D-IBS, may have an inflammatory component. Further studies are needed to determine whether hs-CRP can make other potential contributions to the study of IBS, such as a marker of response to therapy.

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AUTHOR CONTRIBUTIONS

KH designed the research study, acquired the data, analyzed and interpreted the data, drafted the manuscript, and performed statistical analysis; RD and YR acquired the data; AS acquired the data, analyzed and interpreted the data, drafted the manuscript, and study supervision; SM analyzed and interpreted the data, and study supervision; RD acquired the data and drafted the manuscript; ZH acquired the data, contributed administrative, technical, or material support; SB designed the research study, obtained funding, contributed administrative, technical, or material support, and study supervision; NM wrote the manuscript, designed the research study, acquired the data, analyzed and interpreted the data.

COMPETING INTERESTS

The authors have no competing interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Receiver operating characteristic (ROC) analysis for optimal hs-CRP cut-off point to distinguish IBS patients from healthy controls.

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