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Original article

Association between Serum folate with inflammatory markers, disease clinical activity and serum homocysteine in patients with Inflammatory Bowel Disease. Does folate level have an effect on maintaining clinical remission?

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Summary. *Background:* Folate is an important vitamin with protective effect against some human diseases. The aim of this study was to evaluate the relationship between serum folate levels, inflammatory markers and disease clinical activity in patients with inflammatory bowel disease (IBD). *Methods:* The participants were classified into two groups in which 38 IBD patients and 38 healthy controls were studied. Disease clinical activities were evaluated by means of established score systems. Serum folate, homocysteine and C-reactive protein and ESR were measured. Obtained data were analyzed with proper statistical methods and P- value less than 0.05 was considered as statistical significant. *Results:* The level of serum folate was significantly reduced in IBD patients with active disease compared to patients with clinical remission (p=0.043) and also healthy controls (p=0.008). Moreover, there was a significant inverse correlation between serum folate levels and C-reactive protein in IBD patients (r=-0.563 p=0.001). Conclusion: Serum folate levels is associated with inflammatory markers and disease clinical activity in IBD patients, therefore there is a possibility that disease clinical activity is reduced with adequate folate level. (www.actabiomedica.it)

Key words: folate, homocysteine, inflammatory bowel disease, disease clinical activity

Introduction

Inflammatory bowel disease (IBD) is a chronic disease that causes prolonged inflammation of the gastrointestinal tract. Ulcerative colitis and Crohn's disease are two most common types of IBD (1, 2). The role of nutritional factors especially vitamins in developing, progression and even treating various human diseases, including IBD, has always been an interesting topic for researchers. Folate is one of the most important nutritional factors. Folate has a very important role in methyl metabolism in human and is directly involved in some of the vital processes such as DNA methylation. In recent years, folate protective effect against some human diseases such as cardiovascular disease, neurological disease, some cancers, were indicated by several studies) (3-7). This protective effects may be related to folate role in DNA methylation, particularly

in atherosclerosis (8). Furthermore in recent years, some research studies focused on folate utilization as cancer treatment agent (9). Regarding the inflammatory markers, this is should be noted that, although new laboratory markers such as fecal calprotectin and Inducible nitric oxide synthase have been suggested for diagnosis and monitoring of IBD patients, traditional inflammatory markers are still used in this regard (10, 11). Traditional Inflammatory markers such as C-reactive protein (CRP) and Erythrocytes Sedimentation Rate (ESR) increased in IBD patients and widely used for disease monitoring (10). Regarding the association between folate and these inflammatory markers, previous studies reports are controversial, some studies on cardiovascular disease did not report the significant association between folate level and inflammatory markers such as CRP, but other studies on hemodialysis patients demonstrated that treatment with folate reduced the CRP levels.

In the case of IBD, although, some clinical investigations demonstrated significant inverse correlations between homocysteine and folate, hyper-homocysteinemia and folate deficiency in IBD patients (12-15), but the relationship among folate statue, inflammatory markers and disease clinical activity has not been studied properly. Evaluation of disease clinical activity is an important tool for disease monitoring in patients with IBD, clinical activities were assessed based on some established scores systems and give relatively valuable information about disease activity statue, although it may not be correlated with the endoscopic activity of the disease. These score systems mainly consisted of number of variables such as number of liquid stools, abdominal pain, bloody stool, general wellbeing and etc., in fact this score system is highly associated with clinical manifestations of disease and patients complications. Reduction of disease clinical sign and symptoms and maintaining of clinical remission is the main purpose of the treatment in patients with IBD and evaluation of disease clinical activity is useful in this regard. Regarding the relationship between nutritional status and disease clinical activity, a number of studies were done previously and the importance of antioxidant and trace element was demonstrated by some of these studies (16-18). Relationship between folate and disease clinical activity is a neglected issue and further studies are needed in this regard, in the current study, we tried to address this important issue by designing a clinical investigation.

Materials and Methods

Patients

This study was approved by Hormozgan University of Medical Sciences (NO: 93127) and was carried out on Iranian patients admitted to Ayatollah Rouhani Hospital, Endoscopy Department, Babol, Iran, for colonoscopy examination during 2015-2018. All of the subjects included in the project provided signed informed consent of the experimental protocol as recommended by the university ethics committee. A complete clinical history was taken from the patients before colonoscopy. Blood samples were taken after 12 h of fasting, and before colonoscopy serum samples were separated immediately by means of centrifugation for 10 min at room temperature. Colonoscopy was performed up to the cecum. In some patients, colonoscopy was performed up to the terminal ileum. In newly diagnosed IBD cases, biopsy was taken from inflamed mucosa for histopathologic examination and confirmation of IBD existence. At the end of sampling period, according to colonoscopy and histopathologic findings and consultation with a gastroenterologist and according to inclusion and exclusion criteria, among all patients who underwent colonoscopy, diagnosed with IBD and had blood samples, 38 patients (19 men and 19 women) were selected as IBD patient group, in which 22 patients were in the active phase of the disease, and 16 patients were in remission phase.

It should be noted that disease clinical activity was evaluated based on the established score system which is described below. Among IBD patients, 14 patients (7 men and 7 women) were diagnosed with Crohn disease and 24 patients (12 men and 12 women) were diagnosed with ulcerative colitis. Thirty eight healthy subjects were matched in age and sex, with normal colonoscopies and selected as the control group. Clinical characteristics of patient group are shown in Table 1.

Table 1. Clinical characteristics of the patients and control groups

	IBD Patients n= 38	Healthy controls n=38
Disease subgroups	Crohn disease 14 patients(36%) Ulcerative colitis 24 patients (64%)	
Duration of disease (years)	Newly diagnosed 15 patients 1-3 years 10 patients 3-5 years 4 patients 5-10 years: 3 patients >10 years: 6 patients	
Disease clinical activity	Active disease 22 patients (57%) Clinical remission 16 patients (43%)	

Inclusion criteria

Confirmation of IBD was based on clinical, endoscopic, and histopathologic findings. Subjects who were more than 18 years and signed the informed consent were included in the study.

Exclusion criteria

Patients with history of colorectal surgery, treatment with sulfasalazine and methotrexate, pregnancy, diabetes and rheumatoid arthritis, any type of cancer, infectious diseases, renal diseases, liver diseases, genital diseases, metabolic disorders, psychological disorders, and mental retardation were excluded from this study. Furthermore, use of any type of supplements such as vitamins, zinc, selenium, iron, and especially folate were the other exclusion criteria.

Healthy control subjects

Healthy control individuals were selected among individuals who were undergoing colonoscopy because of abdominal pain, positive results of stool occult blood or their regular checkup according to inclusion and exclusion criteria noted above.

Homocysteine measurement

Serum homocysteine level was measured by ELISA method according to the manufacturer's instruction (Axis shield, UK). The absorbance of the samples was read by ELISA reader (RT2100c, Germany).

Folate measurement

Serum folate levels were measured by chemiluminescence method according to manufacturer's instruction, using (Roche, USA) Cobas e 411 analyzer instrument (USA).

Routine lab test

Levels of ESR were measured by routine laboratory method. Serum level of CRP was measured quantitatively according to manufacturer's instruction (Bionic, Iran).

Disease clinical activity

Disease clinical activity in IBD patients was evaluated according to Lichtiger index and CDAI for Ulcerative colitis and Crohn's disease, respectively (19, 20). Clinical remission was defined as a CDAI<150 (21) and Lichtiger index<4 (22).

Statistical analysis

The SPSS software (version 17) was used for data analysis. We used Kolmogorov–Smirnov (KS) test to check variables distribution normality. We used Mann-Whitney U Test for comparison between groups and describe the data with median and interquartile range (IQR). Correlations between variables were also analyzed by Spearman correlation coefficient. P-values less than 0.05 were regarded as statistically significant.

Table 2. Biochemical characteristics of patients and control groups

Parameters	Case group n=38	Control group n=38	p-value
Age(years) (median, interquartile range)	31 (27-41)	33 (29-42)	0.589
Homocysteine (μmol/L) (median, interquartile range)	8.8 (6.62-11.32)	8 (6.57-10)	0.292
Folate (ng/mL) (median, interquartile range)	8.2 (5.05-14.8)	11.2 (9.1-17.42)	0.053

Results

Clinical characteristics of the patients and controls are shown in Table 1. The number of IBD patients was 38 (19 men and 19 women) and the number of healthy controls was 38 (19 men and 19 women). Serum levels of folate and homocysteine did not significantly differ in patients with IBD compared to healthy controls (Table 2). It is obvious that CRP and ESR levels significantly increased in IBD patients compared to healthy subjects. In Figure 1, the correlation among variables in patients with IBD was shown. As presented in this figure, there is an inverse correlation between serum levels of homocysteine and folate (r=-0.491, p=0.002); serum homocysteine levels directly correlated with CRP but this correlation is not statistically significant (r=0.314 p=0.055). There was a significant inverse correlation between serum folate and CRP levels (r=-0.563 p=0.001). It should be noted that the above mentioned correlations were also evaluated separately in IBD patients with active disease (number of cases=22) and the same pattern was observed (Figures 2 and 3). In Table 3, the means of mentioned variables in patients with active disease and patients in clinical remission were shown and compared together and also compared with healthy controls. There was no significant difference in the homocysteine levels among three groups, but serum levels of folate in patients with active disease significantly de-

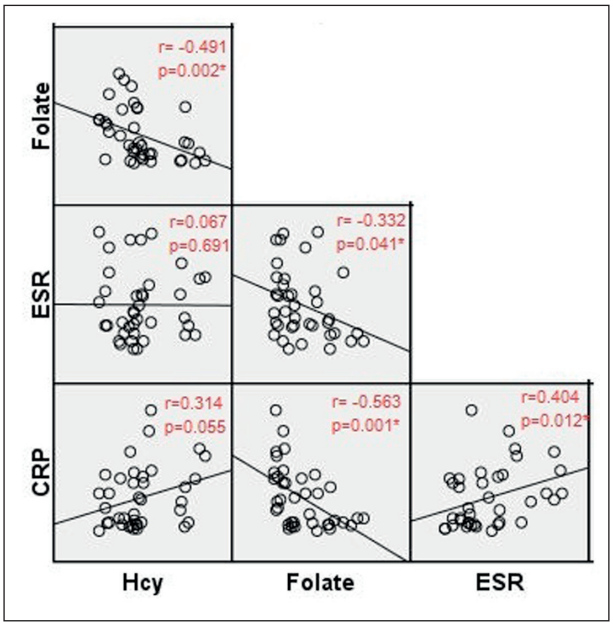

Figure 1. Correlation between variables in IBD patients
Hcy: homocysteine, CRP; C-reactive protein, ESR: erythrocyte sedimentation rate; *statistical significant; r = spearman correlation coefficient

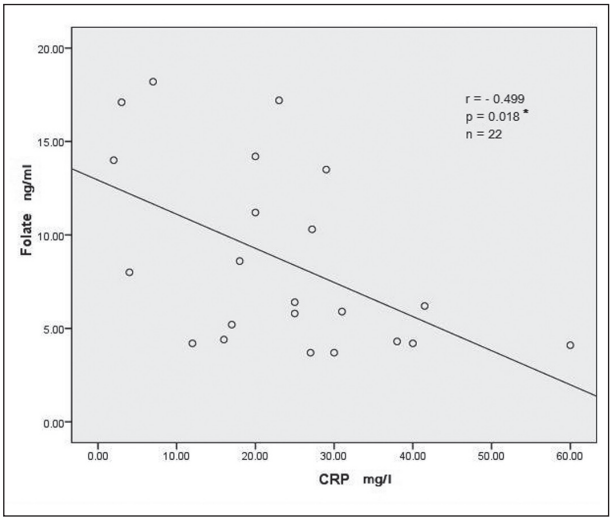

Figure 2. Invers correlation between C-reactive protein and folate serum levels in patients with active IBD
*statistical significant; r = spearman correlation coefficient

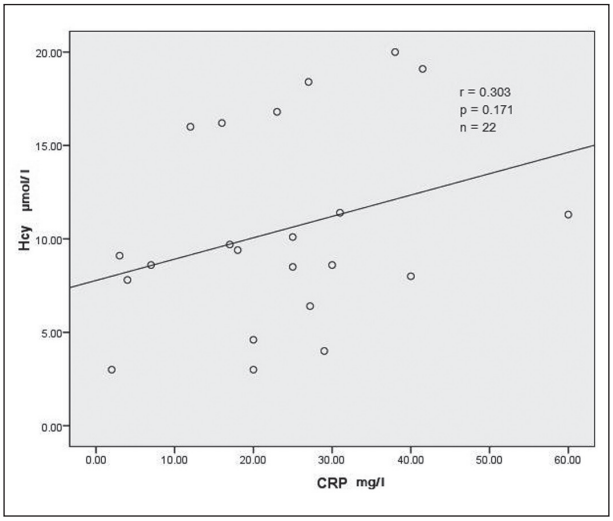

Figure 3. Direct correlation bet ween C-reactive protein and serum homocysteine in patients with active IBD
r = spearman correlation coefficient

creased, in comparison with patients who were in clinical remission and healthy subjects.

Discussion

In the current study, we tried to examine the relationship among folate level, inflammatory markers and disease clinical activity by designing the precise clinical investigation and tried to apply the strict inclusion and exclusion criteria for the patients and controls selection to minimize the effects of other factors on homocysteine and folate levels. According to obtained results, there was no significant difference in serum homocysteine and folate levels between IBD patients and healthy subjects. Serum level of homocysteine was elevated in patients with active form of IBD compared to patients with inactive form of disease, but this elevation is not statistically significant (p=0.344, Table 3). Although some studies demonstrated that the elevation of serum homocysteine levels and reduction of serum folate levels occurred in IBD patients in comparison to healthy controls (14,15, 23), the findings of the current study indicated that folate level reduction occurred only in patients with active disease (Tables 2 and 3). This discrepancy may be due to population difference and disease activity status. Furthermore, a meta-analysis done by Pan et al demonstrated that, factors such as race, geographic region and nutrition pattern may affect the folate levels, this meta-analysis showed, that folate levels in Asian and European IBD patients were significantly lower compared to healthy subjects, but in American and African IBD patients, folate levels did not significantly differ compared to healthy subjects (24).

Table 3. The comparison of means between patients with active IBD, patients in clinical remission and healthy controls

Variables	Active disease n=22	Clinical remission n=16	Healthy subjects n=38	p-value comparison between Active disease and clinical remission	p-value comparison between Active disease and healthy subjects	p-value comparison between clinical remission and healthy subjects
Homocysteine (µmol/L) (median, interquartile range)	9.25 (7.45-16.05)	8.55 (6.32-9.95)	8 (6.57-10)	0.344	0.141	0.917
Folate (ng/ml) (median, interquartile range)	6.3 (4.27-13.62)	10.45 (7.12-19.45)	11.2 (9.1-17.42)	0.043	0.008	0.820
CRP (mg/L) (median, interquartile range)	24 (15-30.25)	6 (4.92-9.5)	4.15 (3-5)	0.005	0.001	0.001
ESR (mm/h) (median, interquartile range)	34 (14.75-62.5)	21.5 (16.25-39)	5 (2-8.25)	0.164	0.001	0.001

The inverse correlation between homocysteine and folate levels in IBD patients was demonstrated in our study (r=-0.491, p=0.002) (Figure 1), these findings are in agreement with the findings of Erzin et al. and Akbulut et al. (12, 14). Therefore, according to the present study results, hyper-homocysteinemia is not a common phenomenon in IBD patients and folate level has a significant influence on homocysteine level and previous reports of hyper-homocysteinemia in IBD patients may be due to only folate deficiency which likely occurred due to the nutritional or medication effects. Furthermore, according to current study results, disease activity should be considered in this regard, because it is highly possible that the activity of disease and therefore the state of inflammation affects folate absorption. Our results demonstrate that the serum folate level in IBD patients with active disease is significantly lower than patients who are in clinical remission (p=0.043) and also healthy subjects (p=0.008), these findings are similar to Erzin et al. findings (14), but there was no significant difference in serum folate levels between patients who are in clinical remission and healthy subjects. According to this observation, folate is likely involved in clinical remission maintenance in IBD patients, further clinical investigations are needed in this regard. The mentioned association between serum folate levels and disease clinical activity and also inverse correlation between serum folate and CRP levels in patients with active IBD (Figure 2) showed that, folate plays an important role in inflammatory processes and IBD pathogenesis. This may be due to the fact that folate has an important role as methyl carrier in the body. Methyl deficient diet aggravates dextran sodium sulfate (DSS) induced colitis in rats (25).

Furthermore, it is shown that folate supplementation reduced murine colitis severity by promoting methylation (26). These findings along with our results demonstrated that insufficient folate level can lead to exacerbation of inflammation; therefore, folate status should not be neglected in IBD patients and needs more attention.

Conclusion

Serum folate levels decreased in patients with active IBD compared to patients with clinical remission and also healthy subjects and this is highly possible because, activity of disease and the state of inflammation affect the folate absorption and therefore folate levels reduction in active IBD. Besides, serum folate levels are inversely correlated with inflammatory markers; therefore, it is possible that adequate folate level may be useful in remission maintenance and reduction of the severity of inflammation.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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