Efficacy of Vitamin B6 Supplementation on Inflammatory Markers, Serum Homocysteine level, Fecal Calprotectin and Clinical Outcomes among Patients with Ulcerative Colitis: A Randomized Double Blind Clinical Trial

Pezhman Alavinejad[1], Ali Mard[1], Fatemeh Panahandeh[2], Saeed Hesam[3], Seyed Jalal Hashemi[1], Mehrnaz Morvaridi[4], Sanaz Taherpour[2]

[1] *Alimentary tract Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[2] *Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[3] *Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical, Sciences, Tehran, Iran*

[4] *Nutrition Sciences, Alimentary tract Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Corresponding Author
Dr Pezhman Alavinejad

Mobile:00989161115880

E mail:
pezhmanalavinejad@gmail.com

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Background and study aim: Inflammatory bowel disease (IBD) include a spectrum of immune-mediated chronic disorders. The aim of this study is to evaluate the efficacy of vitamin B6 supplementation on laboratory markers and clinical outcomes in patients with ulcerative colitis.

Patients and methods: In this double-blind placebo-controlled randomized clinical trial ulcerative colitis patients were randomly divided into two groups, intervention (usual treatment plus vitamin B6 (40 mg / day)) and placebo group (usual treatment plus placebo). The serum levels of inflammatory markers measured and compared at the beginning and the end of intervention.

Results: Overall forty patients were randomly selected to participate in this trial. Age range of participants was between 25-65 years and 3.43% of patients (13 cases) were males. Baseline characteristics of two groups were equal. The mean serum level of homocysteine after intervention in placebo and vitamin B6 groups were 9.05±3.45 and 16.31±20.52 respectively (P= 0.205). There were no significant differences between serum levels of homocysteine, CRP (P=0.328), ESR (P=0.329), calprotectin (P=0.683) and stool frequency after 6 months intervention in univariate analysis. In multivariate analysis stool frequency was significantly greater in vitamin B6 group in comparison with placebo group (P = 0.01).

Conclusion: We couldn't find any significant effect of vitamin B6 supplementation on duration and severity of ulcerative colitis and even stool frequency in vitamin B6 group increased.

INTRODUCTION

Inflammatory bowel disease (IBD) include a spectrum of immune-mediated chronic disorders with two major types of ulcerative colitis (UC) and Crohn's disease (CD) [1]. Etiology of IBD is not well understood and different studies have led to this hypothesis: in genetically susceptible individuals, dysregulation of the enteric immune response leads to the development of acute and chronic inflammation and the pathologic feature of mucosal damage [2, 3, 4]. Principle components of treatment include induction of remission, preventing disease flare up, reducing complications and side effects of medications, and improving quality of life by participation of physician, nurse, surgeon and dietitian in a multi-disciplinary approach [5, 6]. Vitamin deficiency is common in IBD patients and among these deficiencies, one of the most prevalent is vitamin B6 deficiency which is reported in involve about 30% of patients [7, 8, 9, 10]. Pyridoxal phosphate, biologically active form of vitamin B6, is the cofactor of many

biochemical interactions including homocysteine metabolism (conversion of homocysteine to cysteine) and its deficiency could result in hyper homocysteinemia (HHcy) which can lead to life-threatening complications such as thrombosis [11]. Hydrogen sulfide (H2S) is a mediator in biochemical reactions that contribute to tissue repair and attenuation of inflammation. Synthesis of H2S from cysteine naturally occurs within mammalian tissues by pyridoxal-5-phosphate-dependent enzymes, cystathionine gamma-lyase and cystathionine beta synthase [12]. Inhibition of H2S synthesis from cysteine by HHcy can result in mucosal inflammation, increased susceptibility to tissue injury and defective tissue regeneration while H2S administration improve wound healing and has significant anti-inflammatory effects on gastrointestinal tract [13]. Previous studies in experimental colitis have shown that HHcy exacerbate mucosal inflammation and administration of H2S can reduce severity of ulcers in mice [14]. Vitamin B6 supplementation in mice with interleukin-10 induced colitis model can decrease molecular and histological markers of colon inflammation [15]. Since vitamin B6 deficiency is prevalent among patients with ulcerative colitis and synthesis of H2S is dependent on enzymes that require vitamin B6 as a cofactor, and also based on its role in tissue repair and suppression of inflammation in experimental studies, this study designed to evaluate efficacy of vitamin B6 supplementation on reduction of inflammatory markers, serum homocysteine level and clinical outcomes in ulcerative colitis patients.

PATIENTS AND METHODS

This double-blind placebo-controlled randomized clinical trial was conducted as a pilot study on mild to moderate UC patients with active disease referred to IBD clinic of Ahvaz Imam Hospital. Patients were randomly divided into two groups, intervention group (usual treatment plus vitamin B6 (40 mg/day)) and placebo group (usual treatment plus placebo). Their routine medical regimen include 5ASA family (oral ± suppository) with or without Azathioprine tablet. Vitamin B6 pills and placebo were exactly same in appearance, taste and packaging. The statistician who was blinded to patients' information, used block randomization to allocate patients to each group. Intervention and placebo groups encoded, so

participants and researchers were not aware of any assigned code. At first visit, pills were given to patients and explained about their consumption and preservation. Researchers followed up patients via text messages and weekly phone calls. Patients were also visited monthly by researchers and gastroenterologists to monitor clinical symptoms and use of pills. After 6 months of intervention, researchers counted the number of consumed pills. No side effect reported with consumption of vitamin B6 during study period .

Inclusion criteria include age range 18 to 65 years old, diagnosis of ulcerative colitis by gastroenterologist at least 6 months before beginning of study and colonoscopy report plus histology compatible with UC. Exclusion criteria include recent diagnosis of disease (less than 6 months) or current flare, change in medications during the study period, consumption of vitamin B6 over last month before inclusion, Parkinson's disease and co-administration of Levodopa, presence of infectious diseases and or cancer. After confirmation of inclusion criteria, patients filled consent form for enrolling in trial.

Blood samples were taken at the beginning and at the end of intervention, then were frozen at -70 ° Celsius immediately. Stool samples were collected for measuring fecal calprotectin. Serum homocysteine was measured by enzyme-linked immunosorbent assay (ELISA) method using DiaMetra kit (DiaMetra Co, Milan, Italy) and calprotectin was determined by CALPRO ELISA kit (CALPRO AS, Norway, Europe). Serum CRP level have been measured by using ELISA kit (Parsazmoon, Karaj, Iran). Patients were asked to note stool frequency daily and give their records to researchers.

After collecting data, statistician performed statistical analyses using the SPSS package version 22 (SPSS Inc, Chicago, Illinois, USA). Quantitative variables were showed as mean and standard deviations and qualitative variables were reported as frequency and percentage. Normal distribution was tested in quantitative variables by using quantile-quantile (QQ-plot) and Kolmogorov-Smirnov test. For data analysis, Chi-square test, Fischer exact test, student's t-test, Mann-Whitney test, Pearson and Spearman correlation coefficient, and multiple linear regression were used. Probability value less than 0.05 was considered significant. Both univariate

and multivariate analysis was performed for data analyzing.

RESULTS

At first, 40 patients were randomly selected to participate in this trial. 10 patients were excluded from trial due to lack of proper follow-up and finally, thirty patients, 15 in vitamin B6 group and 15 in placebo group, underwent final analysis. Age range of participants was between 25-65 years, 43.3% of patients (13 cases) were males and 56.6% (17 patients) were females. Baseline characteristics of two groups were not different significantly (Table 1). After 6 months intervention, clinical and laboratory data in two groups were evaluated and compared. As shown in Table 2, the mean ± standard deviation of homocysteine after intervention in placebo and Vitamin B6 group was 9.05±3.45 and 20.52±16.31 respectively (P= 0.205) and difference between groups was not significant statistically. The mean ± standard deviation of ESR after intervention in placebo and vitamin B6 group was 21.47±20.32 and 23.80±23.42 respectively. This difference for CRP, calprotectin, and stool frequency was also not statistically significant (P = 0.329, 0.328, 0.683 respectively). In multivariate analysis, there was not any significant differences for homocysteine, ESR, CRP, and calprotectin variables. For stool frequency, difference between placebo and vitamin B6 groups after 6 months intervention was significant (P= 0.01) and stool frequency in vitamin B6 group was greater than placebo group.

Table (1): Baseline characteristics of 2 groups.

Variable	Placebo group	Vitamin B6 group	P value
Age, years	43.93±12.87	36.20±10.58	0.08
Male/ Female	6/9	7/8	0.71
Disease duration, years	7.22±6.33	4.57±4.40	0.43
Type of ulcerative colitis			
Left-sided	3(20%)	5(33.3%)	0.28
Right-sided	1(6.6%)	0	
Proctitis	3(20%)	6(40%)	
Extensive	8(53.3%)	4(26.6)	

Table (2): Laboratory values of 2 groups before and after study period.

Variables	Placebo		Vitamin B6		P-value*		P-value•
	Baseline	After 6 months	Baseline	After 6 months	Baseline/ baseline	After 6 months	After 6 months
Homocysteine, μmol/L	11.79±3.89	9.05±3.45	14.62±8.50	20.52±16.31	0.56	0.20	0.32
ESR, mm/h	25.87±20.34	21.47±20.32	33.40±26.16	23.80±23.42	0.34	0.32	0.93
CRP, mg/L	5.92±5.17	5.69±4.27	3.28±2.63	2.66±2.10	0.34	0.32	0.90
Fecal calprotectin, mg/kg	402.94±264.59	272.79±153.27	285.40±182.24	172.83±103.03	0.71	0.41	0.94
Stool frequency, daily	2.8±1.86	2.20±1.26	2.73±2.37	3.61±3.2	0.65	0.68	0.01

Data are expressed as mean ± standard deviation

*P-values indicate univariate analysis

•P-values indicate multivariate analysis

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein

DISCUSSION

Synthesis of hydrogen sulfide (H2S), a mediator that contributes in tissue repair and inflammation attenuation, is carried out by enzymes which need vitamin B6 as a cofactor. Vitamin B6 deficiency is common among patients with ulcerative colitis and could result in hyperhomocysteinemia (HHcy) and diminished H2S synthesis [16, 17]. According to present evidences, this is the first double-blind placebo-controlled randomized trial for evaluation of the effect of vitamin B6 on inflammatory markers and clinical manifestations of ulcerative colitis, which was conducted as a pilot study. Data analysis in our study showed that there was no significant difference between intervention and control groups in terms of plasma homocysteine level, mean ESR, CRP, and calprotectin excretion before and after intervention (P> 0.05). Flannigan et al. reported that diet-induced HHcy result in exacerbation of colitis in dextran sulfate sodium-induced colitis in rats (DSS Model) but H2S administration can reduce its severity [14]. The hypothesis of this experimental study assumed that HHcy would diminish conversion of homocysteine to cysteine, H2S synthesis, and gene expression of cysteine-converting enzymes to H2S, especially cystathionine γ-lyase. Consequently, administration of vitamin B6, which is a cofactor for H2S synthesis, can lead to decrease in serum homocysteine level and severity of ulcerative colitis. In our clinical trial, there was no significant difference between homocysteine levels before and after vitamin B6 administration. One of differences between our trial and DSS model in this study was that patients had homocysteine levels below 15 µmol/L. HHcy occurs when homocysteine levels is more than 15 µmol/L [18]. Also, in DSS model, H2S was administered directly to rats, while in our study its cofactor, vitamin B6, prescribed. Selhub et al., investigated the effect of dietary intake of vitamin B6 on colon inflammation of mice (interleukin-10 induced colitis model). The results of this study showed that vitamin B6 supplementation suppresses expression of molecular and histological markers of inflammation in colitis [15]. In our study, inflammatory markers, ESR and CRP, which are commonly used clinically for diagnosis and follow up of ulcerative colitis patients, have no significant difference before and after the intervention. These two factors are not specific to ulcerative colitis, and could be affected by other clinical conditions. It is suggested that future studies assess highly sensitive factors such as hsCRP and TNF-α in order to more accurate follow up of treatment process. Calprotectin is a peptide which bond to calcium and zinc with unique properties and acts as a biomarker of pathophysiologic processes in inflammatory diseases, including inflammatory bowel diseases [19] and in many studies, has been used to diagnose and follow up ulcerative colitis [20, 21]. In our study, the amount of fecal calprotectin was measured before and after the intervention to evaluate the effect of vitamin B6 supplementation, but there was no significant difference. Stool frequency as an important indicator for evaluating clinical symptoms, according to Mayo score [22], was checked at each visit. This factor was not statistically different before and after intervention in univariate analysis but in multivariate analysis was significantly more frequent in vitamin B6 group. Jiang et al. reported that polymorphism is common in some genes associated with homocysteine metabolism, hyperhomocysteinemia, and low levels of folate and vitamin B12 in ulcerative colitis patients. In fact, the hypothesis is that the interaction of genetic and nutritional factors in this population can lead to hyperhomocysteinemia and severity of ulcerative colitis symptoms [23]. It seems that for designing clinical trials in future, genetic polymorphisms involved in homocysteine metabolism should be consider with folate, vitamin B12, and B6 supplementation, in order to improve homocysteine levels. We didn't evaluate other possible deficiencies such as folate and vitamin B12 in patients with ulcerative colitis.

CONCLUSION

Based on findings of current study, we were unable to find any significant effect on duration and severity of ulcerative colitis with vitamin B6 supplementation and in vitamin B6 group, stool frequency was greater than placebo group. Our study is the first clinical trial for evaluation of the effect of vitamin B6 supplementation on disease process and inflammatory markers among patients with ulcerative colitis. Further studies are needed with larger sample size, longer duration of intervention, and considering genetic factors such as different genotypes of calprotectin protein or possible deficient factors such as folate and vitamin B12, in order to

achieve definitive results about using vitamin B6 as an adjunct therapy with less side effects plus usual treatments in these patients.

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