Original Research Article

Serum Concentrations of Trace Elements in Patients with Ulcerative Colitis

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ABSTRACT

Introduction: Nutritional status of patients with inflammatory bowel disease is estimated to be lower than optimal. This study was designed to compare the serum level of some trace elements in patients with ulcerative colitis (UC). **Materials and methods:** This cross-sectional study was performed in 2015-2016, on 60 recently diagnosed UC patients (30 with active UC and 30 with inactive UC) who were recruited from registry of the Golestan Research Center of Gastroenterology and Hepatology (Gorgan, Iran) through simple random sampling. Thirty healthy individuals were also recruited as controls. Demographic data and disease activity index were recorded through a checklist. Serum levels of copper (Cu), zinc (Zn), selenium (Se) and magnesium (Mg) were measured by atomic absorption spectroscopy. Data were analyzed using SPSS 16 at significance level of 0.05. **Results:** Mean serum level of Cu in the patients (0.75±0.25 mg/L) was significantly lower than in the controls (1.11±0.3 mg/L) (P=0001). Similarly, mean serum level of Mg in the patients (15.84±4.3 mg/L) was significantly lower than in the controls (19.48±15.7 mg/L) (P=0.001). Mean serum level of Se was slightly higher among the UC patients, while mean serum level of Zn was slightly lower in the UC patients compared with the controls. **Conclusions:** Our results show that UC patients have significantly lower Cu and Mg levels compared to healthy individuals. However, serum Zn and Se levels do not differ significantly between the UC patients and the control individuals. Further studies are required to confirm the results of this study.

KEYWORDS: Inflammatory bowel diseases, Ulcerative colitis, Trace elements

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INTRODUCTION

Inflammatory bowel diseases (IBD) are caused by inappropriate functioning of the mucosal immune system, and include ulcerative colitis (UC) and Crohn's disease [1-3]. The global incidence of UC ranges from 2.2 to 3.14 per 100,000 people per year, and the global incidence of Crohn's disease is 1.3 to 6.14 per 100,000 people per year [4]. Despite lack of clear statistics, the incidence rate of IBD seems to be on the rise in Iran, with UC reported as the most common type in the country [1-3]. The exact etiology of the disease is not clear yet, but the possible role of several factors including bacteria, nutrition, psychology, immunology, genetics and environmental factors have been implicated in IBD pathogenesis. In recent years, studies have found much stronger correlations between immunological processes, cytokines and the complement system and risk of IBD [5, 6].

Weight loss, malabsorption, diarrhea, systemic inflammation, hypermetabolic state and adverse drug reaction are known causes of nutrient deficiency in IBD patients. Nutritional status is lower than optimal in these patients, and signs and symptoms of this problem may take months or years to appear [2]. Insufficient level of trace elements and minerals can also compromise host defense mechanisms against oxidative damage caused by free radicals. In IBD cases, altered metabolism of minerals occurs mostly during flare-ups. Trace elements particularly copper (Cu) and zinc (Zn), may affect ability of mucosa in dealing with harmful effects of free radicals [7].

Zn is an antioxidant element essential for biological functions, homeostasis, metabolism, absorption and intracellular transport in enterocytes and hepatocytes. Some metaloproteins need Zn ions for structural stability. Zn is also considered as a key factor for maintaining intestinal integrity. Zn plays an anti-inflammatory role in intestinal inflammation through inhibition of nitric oxide [8].

Cu is important for absorption, storage and metabolism of iron. Symptoms of Cu deficiency and anemia are similar. Cu absorption occurs in the stomach and intestinal walls [9]. It is a strong antioxidant that eliminates free radicals and prevents cell damage [9, 10].

Magnesium (Mg) is also an essential trace element required for RNA, DNA and protein synthesis. Regulation of muscle contraction, blood pressure, insulin metabolism, cardiac irritability, nerve conduction and neuromuscular transmission are all among the most important functions of Mg [11].

Selenium (Se) can induce apoptosis and prevent mutations induced by free radicals and carcinogenic metabolites in form of glutathione peroxidase and selenoproteins [7]. Dietary Se affects the intestinal microbial composition and adjustment of various prostaglandins, which reduces reactive oxygen species [2].

Considering the importance of these elements, this study was designed to evaluate their levels in patients with UC and in healthy control individuals without intestinal pathology.

MATERIALS AND METHODS

Study population

The study included 60 healthy individuals and 60 patients with a definite diagnosis of UC (no longer than 6 months ago) who had a medical record in the Golestan Research Center of Gastroenterology and Hepatology, Gorgan, Iran. Demographic data and disease activity index were collected through a checklist. Serum samples were taken from all eligible participants. Exclusion criteria included use of vitamin supplements in the past three months, use of immunosuppressive drugs (such as glucocorticoids) and antibiotics for the last three months, severe intestinal disease, and flare-ups or admission to the hospital in the past 6 months.

Flame atomic absorption spectroscopy

Cu, Zn, Mg and Se values were measured by atomic absorption spectroscopy (Younglin AAS 8020, South Korea) with a hollow cathode lamp provided by the Younglin Company. The lamp was set to warm up for 10-15 min. The flame was lightened up after calibrating the light and ensuring that the air pump was open (required for complete combustion of acetylene). Absorption of the samples was read by detector and concentration was calculated based on standard linear curve for each element. The normal ranges of Zn (0.5 to 1.4 ppm), Cu (0.7 to 1.4 ppm), Mg (17-22 mg/L) and Se (63-160 mg/L or ppm) were also considered in the analysis of results.

Sample preparation

According to the protocol for atomic absorption spectroscopy, the samples were prepared by deproteinizion and dilution of serum samples.

Preparation of Zn standard

Various concentrations of Zn (0.2, 0.4, 0.6, 0.8, 1 and 1.5 mg/L) were prepared from the standard solution of Zn (Chem Lab Company) in deionized water for drawing the linear standard curve. The mean coefficient of variation (CV) and Standard deviation were calculated based on the absorption (10 times repetition) of Zn and Cu (pooled serum) diluted 1/10, separately.

Preparation of Cu standard

Various concentrations (0.2, 0.4, 0.6, 0.8, 1 and 1.5 mg/L) of Cu were prepared from the standard solution (Chem Lab Company) in deionized water for drawing the linear standard curve. Using control serum samples (Zitrol N) for Zn and Cu, absorption of 1/10 and 1/20 dilutions was assessed to find the best dilution factor. Comparing the observed results and expected concentrations, dilution factor of 1/10 was selected for the next experiments.

Preparation of Mg standard

To measure serum concentration of Mg, serum dilutions (1/10, 1/20, 1/40, 1/60 and

1/70 mg/L) were prepared using deionized water. Absorption of each dilution was measured ten times, then CV and SD were calculated separately. The 1/60 dilution was found as the most suitable.

Preparation of Se standard

Standard solution of Se (1000 ppm, Chem Lab Company) was diluted with deionized water to obtain various concentrations of Se (50, 100, 150, 200 and 400 mg/L) and the standard linear curve of the Se was drawn.

Statistical analysis

Data were analyzed in SPSS 16 software using t-test and chi-square test. P-values less than 0.05 were considered as statistically significant.

Ethical considerations

The study protocol was approved by the ethics committee of Golestan University of Medical Sciences, Iran (code: IR.goums.rec.1395.119). Informed consent was taken from all participants before sampling.

RESULTS

There was no significant difference in the mean age and male to female ratio between the UC patients and the controls (Table 1).

Table 1. Mean age and sex ratio in the study groups

Group	Sex		Age (years)
	Male N (%)	Female N (%)	Mean ± SD
Patient (N=60)	31 (51.7%)	29 (48.3%)	40.23 ± 10.60
Control (N=30)	9 (30%)	32 (70%)	39.8 ± 11.8
Total	40	50	0.881
P-value	0.06		

The mean serum level of Cu and Mg was significantly lower in the UC patients compared with the controls (Table 2).

Table 2. Mean serum concentrations of Cu, Zn, Mg and Se in UC patients and controls

Elements	Groups	Mean ± SD	P-value
Zn (mg/L)	Patients	1.63 ± 0.4	0.062
	Control	1.86 ± 0.63	
Cu (mg/L)	Patients	0.75 ± 0.25	0.000
	Control	1.11 ± 0.3	
Mg (mg/L)	Patients	15.84 ± 4.30	0.001
	Control	19.48 ± 15.7	
Se (mg/L)	Patients	93.95 ± 14.96	0.114

level of Zn was lower in the UC patients than in the controls (P>0.05). In addition, Cu and Se serum levels were significantly lower in the patients with inactive UC compared to the healthy controls. The mean serum level of Cu and Mg was significantly lower in the patients with active UC compared to the controls (P<0.005). Serum Se serum levels differed significantly between the patients with active UC and the patients with inactive UC (P<0.05) (Table 3).

Table 3. Mean serum concentrations of Cu, Zn, Mg and Se in patients with active/inactive UC and controls

Mean ±SD	Cu (mg/L)	Zn (mg/L)	Se (mg/L)	Mg (mg/L)
Inactive UC	0.25 ± 0.75	0.31 ± 1.70	3.73 ± 15.66	12.40 ± 98.40
Active UC	0.24 ± 0.82	0.5 ± 1.60	4.87 ±16.02	16.15 ± 89.50
Control	0.3 ± 1.11	0.63 ± 1.86	5.70 ± 19.48	10.41 ± 89.08
P-value	0.000* 0.000**	0.211 0.704	0.003 0.906	0.003 0.014
	0.036***	0.268	0.020	0.753

*between inactive UC and control; **between active UC and control; ***between active and inactive UC

There was no sign of low serum Zn level in the UC patients, and Se deficiency was only found in one patient with active UC. However, serum level of Cu was lower than the normal value in 50% of patients. Low Cu level was detected in 73.3% of the patients with inactive UC and in 26.6 % of the patients with active UC. In addition, low Mg level was seen in 80% of the patients with active UC, 66.7% of the patients with inactive UC and 40% of the healthy controls ($P<0.005$). Cu deficiency was significantly more common among females with UC compared to females in the control group (51.7% vs. 4.8%, P=0.000). Since Cu deficiency was not found in healthy males, the statistical comparison was not possible for the Cu levels.

DISCUSSION

We found a statistically significant difference in the serum levels of Cu and Mg between the controls and UC patients. The mean serum Se level differed significantly between the two groups of UC patients, and was higher in the control group. Patients had lower Zn level compared to the controls, but Zn deficiency was not found in the patients. In the the very first reports of the Zn level in IBD patients, Crohn (1932) reported that Zn level is significantly lower in patients compared to healthy individuals [12]. This finding has been repeatedly reported in future studies [13-15], but we found no significant difference in the serum level of Zn between patients and the controls. This could be due to the small sample size or better nutritional status and supplementation of our subjects.

It has been shown that serum concentrations of Cu can be sufficient to high in IBD patients with a good nutritional status [16]. Serum concentrations of Cu in these patients can be influenced by proper nutrition, but depending on the type of inflammatory bowel disease, either increased (Crohn's disease) or decreased (UC) serum concentrations of Cu has been reported. In addition, in a study by Sturniolo et al., a significant difference in Cu and Zn concentrations was found between control group and UC cases [17] .

In a study by Whineray et al. on 46 IBD patients, serum Mg level of patients was lower than that of the controls [18]. Aghazadeh et al. claimed that the intake of fluids, Mg and vitamin C is lower in IBD patients than in healthy people [19]. In a study conducted in 2014, Song et al. reported that 72% of patients had micronutrients deficiency and about 10% of Crohn's disease patients were Mg deficient [7]. In our study, the mean serum level of Mg was significantly higher in the control group.

In a study by Sikora et al. on 154 children with IBD, mean serum Se level was 1.66 ± 0.32 µmol/L [15]. In the present study, the mean serum Se level in both controls and patients was higher than that of previous studies. This high level of Se in the soil of the study region has been demonstrated in previous studies [20].

Study limitations

In this study, we did not assess the nutritional status of IBD patients and had no data about the nutritional background of the subjects. Moreover, we did not record extension of the disease, which could affect the nutritional status of subjects.

CONCLUSION

Our results show that UC patients have significantly lower Cu and Mg levels compared to healthy individuals. However, serum Zn and Se levels do not differ significantly between the UC patients and the control individuals. Further studies are required to confirm the results of this study.

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