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**THE EFFECTS OF PPIs ON SERUM LEVEL OF VITAMIN B12 AND
METHYLMALONIC ACID IN GASTRO-ESOPHAGEAL REFLUX DISEASE**

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ABSTRACT

Gastro-esophageal reflux disease (GERD) is a common gastrointestinal disorder in the people and Proton pump inhibitors (PPIs) are specified for the treatment. It was stated that PPIs may cause vitamin B12 malabsorption. So, it is important to investigate whether treatment with PPIs is associated with a significant vitamin B12 deficiency in patients with GERD and also, measuring of methylmalonic acid (MMA) level could be a beneficial markers for the low level of vitamin B12 or not.

This cross-sectional study carried out at the Shariati hospital, Isfahan, Iran in 2012. GERD patients that used PPIs were recruited. Vitamin B12 status was determined by serum levels of vitamin B12 and MMA. Categorical data were analyzed using Fisher's exact test and Pearson correlation test. Normally distributed continuous data are expressed as the mean±SD. P-value of

<0.05 was considered statistically significant. All analyses were performed using SPSS version 20.

54 patients with the mean age of 48.3 ± 15.5 years participated in our study. Age and gender had no confounding effects on the level of vitamin B12 and MMA. Between the duration of PPIs usage and the serum vitamin B12 level, an inverse correlation was found (0.33) which was significant according to the Pearson correlation test ($p=0.04$); but, there weren't seen any significant association between the frequency of usage and vitamin B12 level ($p=0.33$) and MMA level ($p=0.36$). Also, 36% of patients with the normal level of vitamin B12 had high level of MMA.

PPIs usage has significant association with the vitamin B12 deficiency and with the measuring of MMA concentration, vitamin B12 deficiency may be detected in the early stages.

Key words: Gastro-esophageal reflux disease, Proton pump inhibitors, Methylmalonic acid

INTRODUCTION

Gastro-esophageal reflux disease (GERD) is a common gastrointestinal disorder in the people, and its prevalence is increasing worldwide [1, 2]. Reflux from gastric has symptoms like heartburn and regurgitation, chest pain, and sleep disturbance. Also, these symptoms of GERD have adverse impact on health-related quality of life [3]. Accordingly, Proton pump inhibitors (PPIs) such as omeprazole, Rabeprazole, Lansoprazole, Esomeprazole, and Pantoprazole are indicated for the treatment and relieving the symptoms of GERD and because of the high level of efficacy with low toxicity and long-lasting inhibitory effect on gastric acid secretion, they are used frequently in the world as in the US, more than 119 million PPI prescriptions were written in 2009 [4]. It was stated that

PPIs reduce the formation of gastric acid in the stomach by inhibiting $H^+,K^+-ATPase$ in the secretory membrane of the parietal cells [5].

As gastric acid is essential for the release of vitamin B12 from food particles, daily PPIs usage may inhibit absorption of vitamin B₁₂ and may have effect on its deficiency. Although; in this regard, there were contradictory results. For example, Rozgony *et al* concluded that older individuals who are long-term PPI users are at increased risk of vitamin B12 deficiency and they declared that treatment of PPIs users with cyanocobalamin nasal spray for 8 weeks could improve vitamin B12 deficiency [6]. On the contrary, another study showed that there were no association between long-term proton pump

inhibitor use and vitamin B12 status in the elderly individuals [5].

Since, vitamin B₁₂ deficiency can cause severe damage to the brain and nervous system; and also, it may result in the anemia [18] and the elevation of homocysteine that have adverse effects on the cardiovascular system and cognition [7-9]; in addition to the limited study in this regard, it is important to investigate whether treatment with PPIs is associated with a significant vitamin B12 deficiency in patients with GERD and moreover, we want to know the measurement of methylmalonic acid (MMA) level could be a beneficial markers for the low level of vitamin B12 or not.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Shariati hospital in Isfahan, Iran at 2012. Participants comprised 62 men and women with the GERD who referred to the hospital and they were the users of PPIs included omeprazole pantoprazole and Rabeprazole. After description of the operation and obtaining informed written consent from participants, they were interviewed by structured questionnaire including demographic information, medical history, dietary intake, type of PPIs, the period and the amount of their usage. It should be noted, we excluded individuals with a vegetarian diet,

vitamin B12 supplement usage, and who had helicobacter pylori infection and anemia. So that, among all of the participants 54 patients completed the questionnaire and didn't have exclusion criteria were enrolled in the study. After that, they were asked to visit the laboratory for the blood sampling. The vitamin B12 status was determined by serum levels of vitamin B12, serum levels of MMA and mean corpuscular volume (MCV). All assays were performed using commercially available kits and methods. Vitamin B12 concentrations were measured with the Electro Chemiluminescence method using the Elecsys apparatus (Roche, USA) with a normal range of 200-946 pg/ml. MMA was measured by liquid mass spectrometer (clinmass optimization matrix, USA) with a normal range of 70-270 nmol/mL. The study protocol was taken under the medical ethics standards and was approved by the Ethics Committee of Islamic Azad University, Najafabad Branch. Categorical data were expressed as counts and analyzed using Fisher's exact test and Pearson correlation test. Normally distributed continuous data are expressed as the mean±SD and analyzed using t-tests. A P-value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 20.

RESULTS

In this study 54 patients were followed. Among these 19 (35.2%) patients were male and 35 (64.8%) were female. Average age of male and female were 52.4 ± 16.3 and 46.1 ± 14.7 years, respectively. According to the independent t-test, no significant differences were seen between their mean ages ($p=0.15$). The average duration of omeprazole usage, pantoprazole and Rabeprazole usage were 3.16 ± 2.9 , 2.24 ± 2.4 and 1.42 ± 0.83 years, respectively. According to the one way ANOVA test, a significant correlation was not found between the duration of drugs usage and the type of drugs ($p=0.38$). Also, according to the Pearson correlation test, a significant associations were not seen between the age and the duration of drugs usage ($p=0.97$) and between the age and the vitamin B12 level ($p=0.51$). Between the duration of drugs usage and the serum vitamin B12 level, an inverse correlation was found (0.33) which was

significant according to the Pearson correlation test ($p=0.04$) (**Table 1**). The mean of serum vitamin B12 level in the patients who taking PPIs once a week was reported 267.6 ± 151.2 and in the patients consuming twice a week was shown 184.7 ± 127.6 . According to the Fisher's exact test, the significant association wasn't seen in the level of serum B12 due to the frequency of drugs usage ($p=0.33$) (**Table1**). The mean age of the patients with the normal and high MMA were 51.6 ± 16.9 and 45 ± 14.7 , respectively which according to the t-test there wasn't observed any significant association between the age and the level of MMA ($p= 0.31$). **Table 2** showed that according to the Fisher's exact test there wasn't any significant association between the MMA level and the frequency of drugs usage ($p=0.36$). Figure showed that in the patients with the normal level of vitamin B12, 64% had normal MMA and 36% had high level of MMA.

Table1: vitamin B12 level according to the period and frequency of usage

Vitamin B ₁₂ level	Low	Normal	p-value
Period of usage of PPI (mean±SD)	3.22 ± 2.52	1.93 ± 2.42	0.04
Frequency of usage n(%)			
Once a day	22(44%)	28(56%)	0.33
Twice a day	3(75%)	1(25%)	

Table2: The MMA level according to the frequency of usage

MMA level	Normal	High	p-value
Frequency of usage n(%)			
Once a day	18(66.7%)	9(33.3%)	0.36
Twice a day	0(0%)	1(100%)	

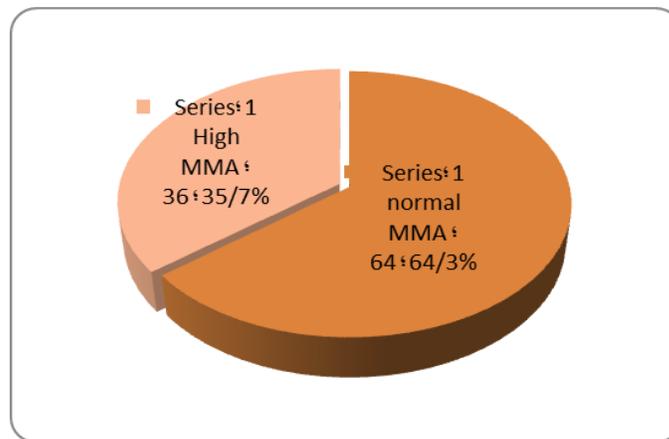


Figure 1: The frequency of the MMA in the patients with the normal vitamin B12

DISCUSSION

The aim of this investigation was to evaluate the serum level of vitamin B12 and MMA in the users of PPIs and the association of the vitamin B12 and MMA concentrations with the frequency of usage, the duration of usage, age and sex of the patients. The results showed that demographic variables such as age and gender had no confounding effects on the level of vitamin B12 and MMA and these variables level was associated with the PPIs usage and the duration of usage. In our study 46% of the patients had low levels of vitamin B12, so that a significant inverse correlation had been seen between the duration of PPIs usage and vitamin B12 level.

According to our results, although there was no significant association between the frequency of PPIs usage and serum vitamin B12 level, however 44% of patients consuming these drugs once a day and 75% of them taking twice a day had low level of

vitamin B12. Data showed that in 36% of patients which had normal vitamin B12 level, MMA concentration was above the normal range. There were many studies to some extent confirmed our results and stated the relationship between serum vitamin B12 levels and the usage of PPIs [10, 11]. Valuck *et al.* findings supported the association between chronic use of PPI or histamine H2 receptor antagonists (H2R) by older adults and the development of vitamin B12 deficiency; while no association was found with past or short-term H2R or PPI usage [12]. Also, Oh in the systematic review confirmed that PPIs have adverse effects such as vitamin B12 deficiency, rebound acid hypersecretion syndrome and others in patients with gastro-oesophageal reflux disease, peptic ulcer disease and functional dyspepsia [13].

Termanini et al revealed that Long-term omeprazole treatment leads to significant

decreases in serum vitamin B12 in patients with severe GERD [14]. In addition, Bellou *et al* concluded that prolonged omeprazole therapy can be responsible for a cobalamin deficiency due to protein-bound cobalamin malabsorption [15]. Another study proposed that the absorption of cyanocobalamin is decreased during treatment with omeprazole. However, no change in serum cobalamin levels was observed in the patients with GERD after treatment with omeprazole for up to 7 years [16]. Moreover, another survey which performed on the dog showed that there was association between the MMA level and cobalamin concentration; since, by increasing the serum MMA concentration, the serum cobalamin concentration decreasing [17]. Also, pervious study showed that at baseline the chronic PPI users had lower serum VB12 levels, higher level MMA [18, 19]. Additional study proposed that the conversion of methylmalonyl coenzyme A to succinyl coenzyme A requires vitamin B12; so, a deficiency of vitamin B12 causes increases in the concentration of MMA [20].

According to our data and the pervious investigation results; it could be mentioned, MMA provides a better index of cobalamin status than the measurement of total vitamin B12 [21], and with the measuring of MMA concentration, vitamin B12 deficiency may be

detected in the early stages [18] and this deficiency could be prevented by the suitable treatment [22].

This study has some limitations, such as the small sample size and the lack of control group to compare with these patients. So, it is recommended that further studies to examine the effect of MMA level and its sensitivity in the diagnosis of vitamin B12 deficiency in the large sample size with the control group.

Conclusion: Finally, we concluded that while our results and many of the studies confirmed an association between the chronic use of PPIs and the development of lower vitamin B12 levels especially in the older adults, at present this is still not firmly established and remained controversial. We can guess, these differences in the results may be existed because of the difference in the cut point of vitamin B12 and MMA levels measuring, the sex, the age and the number of participants. So, vitamin B12 level should be routinely checked in the patients consuming PPIs for a long time, and general practitioners should be aware of these potential adverse effects and ensure that PPIs are used appropriately.

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