Depression, Hematologic Parameters, and Blood Levels of Vitamin B12 in Patients With *Laryngopharyngeal* Reflux Under Use of Proton Pump Inhibitors

(S)SAGE

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ABSTRACT

OBJECTIVE: The objective of this study was to evaluate and correlate hematologic indices, vitamin B12 levels, and depression in patients with suspected *laryngopharyngeal* reflux (LPR) treated with proton pump inhibitor (PPI).

METHODS: This was a prospective study with a population (n = 23) consisted of patients with suspected LPR and treated with 2 daily doses of Omeprazole 40 mg (80 mg or full dose). The study volunteers performed a complete blood count and vitamin B12 dosage before and after 3 and 6 months of treatment, as well as depression screening using the Center of Epidemiologic Studies-Depression (CES-D) questionnaire before and after 6 months of treatment. Scores greater than 16 were considered as suggestive of depression.

RESULTS: The mean score on the CES-D scale of study participants at baseline significantly decreased after 6 months of treatment. A significant decrease was observed in the red blood cells count (RBC) after 6 months in relation to 3 months. Significant increases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were observed from 3 months to 6 months in this study. Vitamin B12 levels increased significantly between baseline and after 6 months of treatment.

CONCLUSION: Decreased scores on the CES-D scale were associated with increased MCV values, with no association with vitamin B12 levels. However, vitamin B12 levels also increased significantly after 6 months of treatment with PPI.

KEYWORDS: *Laryngopharyngeal* reflux, proton pump inhibitor, B12 vitamin, blood count, depression

Introduction

Laryngopharyngeal reflux (LPR) is defined as the retrograde flow of stomach contents to the larynx and pharynx and the contact of this material with the upper aerodigestive tract.[1] Typical symptoms of LPR include dysphonia, pharyngeal globus, mild dysphagia, chronic cough, and throat clearing[2]; it is estimated that up to 15% of visits to the otolaryngologist are related to the LPR.[3]

Considering the low specificity of the laryngoscopic examination and the low sensitivity of pHmetry, the most accepted method used in clinical practice to suggest the diagnosis of LPR is the empirical treatment with a proton pump inhibitor (PPI).[2,4] These medications are the most potent available for decreasing gastric acidity. They are currently among the most prescribed drugs, due to their high efficacy and safety.[5,6] Empirical treatment consists of the use of PPIs twice a day for 2 to 3 months.[7] Although most patients show some improvement after 3 months of treatment, resolution of symptoms and improvement of laryngeal findings usually occurs after 6 months.[1,8]

In addition to preventing the upper airway from exposure to stomach acid, PPIs also reduce the damage resulting from the enzymatic activity of pepsin, which requires an acidic medium for its activation.[9] Although these drugs are considered safe and approved for long-term use, some questions about the safety of continued use, specifically regarding absorption of B-complex vitamins, have been raised.[6,10]

Studies have shown that long-term use (more than 12 months) of PPI is associated to reduction in the blood levels of vitamin B12.[11,12] Even the short-term use of PPI has been shown to decrease the blood levels of vitamin B12.[11,13,14] Long-term use of PPI is believed to be associated with vitamin B12 deficiency in 2 ways: first, by the decrease in gastric acidity, which impairs the release of vitamin B12 from the proteins in the stomach; second, by the increase in gastric pH that would favor the growth of bacteria in the intestine, reducing the absorption of this vitamin.[15,16]

Vitamin B12 deficiency may result in hematologic and neurological problems.[16,17] It is classically associated with megaloblastic anemia and neuropsychiatric disorders, such as paresthesia, psychosis, mental confusion, and depression.[16,18]

The positive relationship between vitamin B12 deficiency and depression would be due to the fact that this vitamin is involved in the metabolic pathway leading to the formation of

S-adenosyl methionine (SAM), a methylating agent that is necessary in the metabolism of neurotransmitters.[19–22]

Depression is a relatively common condition in patients with LPR. It is estimated that more than 50% of these patients present with depressive symptoms during the course of treatment with PPI, ranging from minor to severe symptoms.[23] One of the causes of depression in these patients would be deficiency of B-complex vitamins, especially vitamin B_{12}.[19,21,22]

Depression is a psychopathological construct not directly evaluated by objective measures. Its identification depends on reports of behaviors and symptoms considered as part of a syndrome. Inventories and scales that record the presence and frequency of depressive symptoms were constructed with the objective of tracking, identifying, and evaluating the intensity and presentation of these symptoms.[24]

The aim of this study is to evaluate and correlate hematologic parameters, vitamin B_{12} levels, and depression in patients with LPR-related symptoms treated with PPI.

Materials and Methods

Design

This was a prospective study held between December 2016 and August 2017.

Population sample

The sample consisted of 23 patients with suspected LPR and treated with PPIs. The patients were attended at the otorhinolaryngology outpatient clinic of the Clinical Hospital of Rio Branco (Acre, Brazil), between December 2016 and August 2017.

Inclusion criteria included clinical symptoms of LPR, such as dysphonia, pharyngeal globus, mild dysphagia, chronic cough and throat clearing, and the presence of signs of LPR in the videolaryngoscopy examination. The signs that were considered suggestive of LPR were edema and erythema of the larynx, lymphoid hyperplasia of the posterior wall of the larynx, and the presence of granulomas. Patients who had clinical symptoms and suggestive signs of LPR in the videolaryngoscopy examination were considered eligible to enter the study. Other inclusion criterion was age above 18 years. Only patients younger than 18 years were excluded (Figure 1).

Of the 23 study participants, 20 were women and 3 were men. The age median (Q1-Q3) was 51.5 (38.5-59.0) years. The average ± standard deviation values of body weight in the first, second, and third moments of the study were 67.8 ± 2.39, 66.8 ± 2.47, and 66.52 ± 2.27 kg, respectively. The median height (Q1-Q3) was 1.55 (1.52-1.6) m.

This study was approved by the Research Ethics Committee of the Federal University of Acre under registry number 1,806,320. Eligible individuals signed free and informed consent terms.
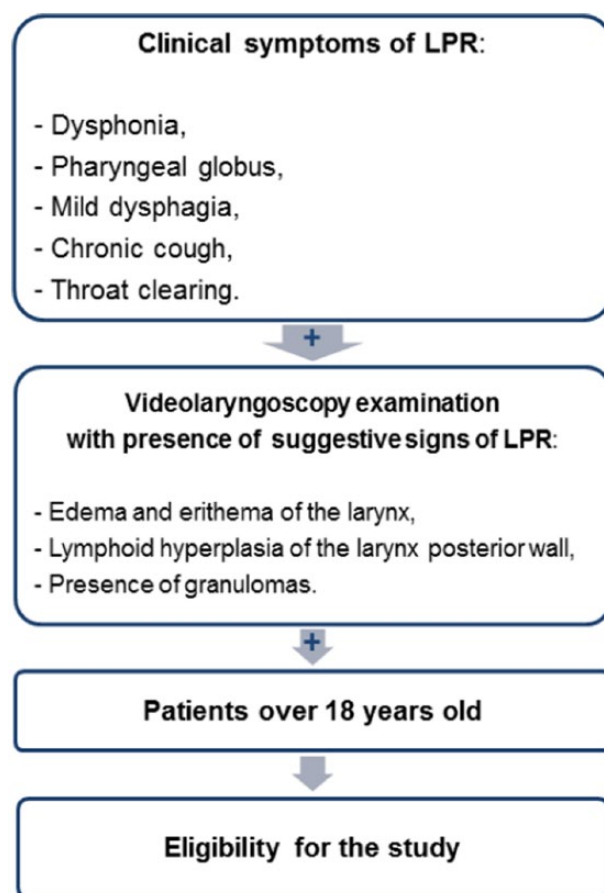

Figure 1. Inclusion criteria used in the study.

Hematologic evaluations and therapy

Volunteers who met the inclusion criteria and agreed to voluntarily participate in the study had an evaluation of their complete blood count and vitamin B_{12} dosage every 3 months in a 6-month period, totaling 3 determinations. Treatment with PPIs consisted of 2 daily doses of Omeprazole 40 mg (80 mg or full dose).

The blood cell count was done using the electrical impedance method in an Abbott-Cell-Dyn 3700 analyzer (Abbott Diagnostics Division, Mountain View, CA, USA). The dosages of vitamin B_{12} were done by electrochemiluminescence in a Roche Cobas 6000 e601 analyzer (Roche Diagnostics, Indianapolis, IN, USA). Vitamin B_{12} values between 211 and 926 pg/mL were considered normal.

Screening for depression

The volunteers were submitted to the depression screening questionnaire titled *Center of Epidemiologic Studies-Depression* (CES-D) at the beginning and end of the treatment, being considered as suggestive of depression scores greater than 16. CES-D is a scale elaborated by the National Institute of Mental Health (USA) that has been translated and validated to be used in parts of the world, including Brazil.[24,25]

Table 1. Values of hematologic variables, vitamin B$_9$ and B$_{12}$ concentrations, and depression scores at baseline and after 3 and 6 months of laryngopharyngeal reflux treatment with proton pump inhibitor.

	REFERENCE RANGE	BASELINE	AFTER 3 MONTHS	AFTER 6 MONTHS
Age (years)	–	51.5 (38.5-59.0)	–	–
Height (m)	–	1.6 (1.5-1.6)	–	–
CES-D (score)	0-60	14.0 (8.5-24.5)*		9 (5-16)*
Weight (kg)	–	67.8 ± 2.4	66.8 ± 2.47	66.5 ± 2.3
RBC (million/mm³)	4.5-6.1 (M) 3.9-5.4 (F)	4.7 ± 0.1	4.7 ± 0.09*	4.6 ± 0.08*
Hemoglobin (g/dL)	12.8-16.3 (M) 11.3-14.5 (F)	13 (12.6-14.4)	13.2 (12.6-14.3)	13.1 (12.7-14.3)
Hematocrit (%)	40-54 (M) 36-48 (F)	39.5 (38.1-42.6)	39.8 (38.6-43.6)	39.5 (38.5-42.6)
MCV (fL)	78-100	87.2 ± 0.9	86.5 ± 0.9*	88.8 ± 0.9*
MCH (pg)	26-32	29.0 ± 0.4	28.7 ± 0.4*	29.5 ± 0.3*
MCHC (g/dL)	31-36	33.3 (32.6-33.8)	32.9 (32.6-33.7)	33.3 (33.0-33.7)
RDW (%)	11.5-15.5	12.2 (12.2-12.2)	15.9 (15.4-16.0)	15.0 (13.9-15.2)
Platelet (10³/mm³)	150-450	226.7 ± 6.56	230.4 ± 7.1	233.1 ± 9.6
Leucocyte (10³/mm³)	4.0-11.0	7.2 ± 0.5	6.4 ± 0.3	6.5 ± 0.3
Vitamin B$_9$ (ng/mL)	3.1-17.5	10.7 ± 0.8	10.9 ± 0.4	13.1 ± 0.9
Vitamin B$_{12}$ (pg/mL)	211-926	341.0 (262.0-410.0)*	313.0 (253.0-503.0)	413.5 (293.1-553.0)*

Abbreviations: ANOVA, analysis of variance; CES-D, Center of Epidemiologic Studies-Depression Scale; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells count; RDW, red-cell distribution width.
*$P < .05$ indicates statistically significant difference between study moments when compared by ANOVA for repeated measures (for data with normal distribution and represented as mean ± SD) and Friedman ANOVA with Wilcoxon post-test and Bonferroni correction (for data with non-normal distribution and represented as median [Q1-Q3]).

Data analysis

The comparison between the different moments of the study was done using analysis of variance (ANOVA) for repeated measures when the data distribution for that variable was normal. When there was no normal distribution in any of the study times or when there was violation of sphericity, the comparisons were made using Friedman ANOVA, followed by the Wilcoxon post-test, adjusted with the Bonferroni correction to avoid that the inflation of the *P* value implied in the occurrence of type 1 error.

For the generation of the correlation matrix, all 3 moments were considered, totaling a value of n of about 69 measurements for each variable. Initially, Pearson and Spearman correlation matrices were generated, and then they were merged into a single matrix. In the final matrix, the values of the Pearson (r) or Spearman (ρ) coefficients were considered when both correlated variables presented normal distribution or when one or both of the correlated variables presented a non-normal distribution, respectively. Data were analyzed using SPSS 22 (IBM Corporation, Chicago, IL, USA).

Results
The values of the variables analyzed in the study population before and after 3 and 6 months of treatment are presented in Table 1.

In this study, the average CES-D score of the participants at the initial time of the study was 14 (8.5-24.5) and the median value after 6 months of treatment was 9 (5-16). As this difference was statistically significant ($P = .01$), there was improvement in the mean score on the depression screening scale (Table 1), possibly associated with clinical improvement of the study volunteers after 6 months of treatment.

A significant decrease was observed in the red blood cell (RBC) count after 6 months (T3) in relation to 3 months (T2) after beginning on treatment (Table 1). However, no difference was observed in relation to hemoglobin concentration and hematocrit values, which are variables that are conceptually associated with RBC counts and that, therefore, showed strong correlations with this variable (Table 2).

Significant increases in the values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)

Table 2. Spearman (*r*) or Pearson (ρ) correlation coefficients for pairs of variables with non-normal (0) or normal (1) distribution, respectively.

NO.	VARIABLE	DISTRIBUTION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			0	0	0	1	1	0	0	0	1	0	1	1	1	0	0
1	Age	0	1.0														
2	Height	0	−0.2*	1.0													
3	CES-D	0	−0.2	0.0	1.0												
4	Weight	1	−0.2	0.8§	0.3	1.0											
5	RBC	1	−0.1	0.2	0.1	0.4§	1.0										
6	Hemoglobin	0	−0.2	0.4§	0.0	0.5§	0.8§	1.0									
7	Hematocrit	0	−0.1	0.4§	0.0	0.5§	0.8§	0.9§	1.0								
8	MCV	0	0.0	0.2	−0.3*	0.1	−0.4§	0.1	0.1	1.0							
9	MCH	1	−0.1	0.2	−0.3	0.1	−0.3*	0.3*	0.2	0.9§	1.0						
10	MCHC	0	−0.3*	0.1	0.1	0.1	0.1	0.5§	0.2	0.2*	0.5§	1.0					
11	RDW	1	−0.2	−0.2	0.2	0.0	−0.1	−0.3	−0.2	−0.4*	−0.4	−0.3	1.0				
12	Platelet	1	0.0	0.0	−0.1	0.0	−0.2	−0.3*	−0.3*	−0.1	−0.1	−0.2	−0.3	1.0			
13	Leucocyte	1	0.0	0.2	−0.2	0.1	0.2	0.2	0.2	0.0	0.0	−0.1	0.0	0.3*	1.0		
14	Vitamin B$_9$	0	0.2	−0.1	−0.2	−0.3*	−0.2	−0.1	−0.1	0.0	0.0	−0.1	0.0	0.0	−0.1	1.0	
15	Vitamin B$_{12}$	0	0.0	−0.1	−0.1	0.0	0.0	−0.1	−0.1	0.0	0.0	0.0	0.2	0.2	0.3*	0.3*	1.0

Abbreviations: CES-D, Center of Epidemiologic Studies-Depression Scale; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; RDW: red-cell distribution width.

*$P < .05$; §$P < .01$.

were observed from T2 to T3 in this study (Table 1). A trivial cause of elevation in MCV values are deficiencies of vitamin B_9 and/or B_{12}.[26] However, there was no decrease in the levels of these vitamins after 6 months of treatment. There was also no significant correlation between MCV and MCH values with blood levels of those vitamins in the study population (Table 2).

Discussion

In patients with LPR, occurrence of depression compared with a control group has been reported in several studies.[27,28] In one of these studies, after treatment with omeprazole for 3 months, the patients showed improvement in quality-of-life scores, without significant difference in the score of the instrument used to estimate the occurrence of depression.[27]

The literature suggests that psychiatric and reflux symptoms may not be mutually exclusive.[23] On one hand, depression and anxiety can aggravate LPR symptoms. On the other hand, the presence of reflux itself may contribute to a worse psychological state.[29] In this way, it is often difficult to know which of the symptoms first began, the neuropsychiatric or the LPR symptom. But, of course, the issue is much more complex, because more than 50% of patients with LPR receiving PPI develop depression,[23] which has been associated with deficiency of B-complex vitamins, especially vitamin B_{12}.[19,21,22]

A retrospective study of PPI users for more than 1 year revealed a decrease in mean hemoglobin, hematocrit, and MCV. The author suggested that the cause would be a lower iron absorption by the PPI users,[30] because the acid produced in the stomach would facilitate the absorption of nonheme iron from the diet by converting it to its absorbable ferrous form.[31] However, there are few studies in the literature that have investigated the association between long-term use of PPI and iron deficiency.

In this study, in addition to a decrease in RBC and an increase in MCV and MCH, there was also a decrease in RBC with both MCV and MCH increase (Table 2). The negative correlation of RBC with MCV suggests a tendency to macrocytic anemia with the increase in the time of treatment with PPI, which could be trivially attributed to the development of deficiency of vitamin B_9 and/or B_{12}.[11] Paradoxically, however, vitamin B_{12} levels increased significantly between baseline and after 6 months of treatment (Table 1).

Meta-analysis on the use of PPIs for 10 or more months showed an increased risk of developing vitamin B_{12} deficiency.[32] This effect was shown to be dose-dependent.[11,33] However, this deficiency may not manifest itself in short-term treatments,[34] as was the case in this study. Indeed, under normal circumstances, the decline in blood levels of vitamin B_{12} may take years to occur.[16,35]

As there are studies that have also found no association of the use of PPIs with vitamin B_{12} deficiency,[35] it is possible that the time of use may not have been sufficient to promote a significant decrease of serum levels of vitamin B_{12}, despite the high dose of PPI used in this study. However, it is not possible to explain why the patients achieved a significant increase in this vitamin after treatment, rather than maintaining pre-therapy serum levels. In any case, it is important to emphasize that the changes reported in this study occurred in the context of a high dose of PPI, which although is within the limits of standardization of this drug, does not correspond to the use of 2 doses of 20 mg employed by most studies.

It is important to note that the significant drop in the CES-D scores at the end of 6 months of PPI treatment (Table 1), as previously mentioned, is consistent with the observed increase in vitamin B_{12} levels after 6 months of study, because depression was associated with deficiency of B vitamins, especially vitamin B_{12}.[19,21,22]

The fact that there was a significant association of the decrease in the scores of the CES-D scale with the increase in the MCV values (Table 2) is consistent with the decrease in the scores of the CES-D scale and the increase in MCV observed at the end of 6 months of treatment with PPI (Table 1). However, these findings do not confirm previous literature findings that the use of PPIs would lead to a decline in vitamin B_{12} absorption, which would result in simultaneous increases in MCV values and CES-D scores.

The association of vitamin B_{12} deficiency with depression is not an absolute finding in the literature. Several studies, like this study, did not find this relationship,[36-38] which suggests that research in this area should be continued to better elucidate this issue. It is important to emphasize that the clinical diagnosis of depression was not made during this study, which used a scale whose elevated scores only suggest the existence of depression, and it is possible that patients with quality-of-life impairment for several reasons, including the LPR itself, may have higher scores on this scale even without depression.

In our study, unlike others who investigated the association of depression with vitamin B_{12}, patients were being treated for LPR, which in itself is already a factor related to depression. As the symptoms of LPR tend to improve with treatment, this may have been a confounding factor.

The limitations of this study include the absence of clinical tools, such as pHmetry, to confirm the diagnosis of LPR. Although many physicians use a therapeutic approach with PPI to confirm the diagnosis,[2] this procedure is not perfect, due to the existence of a significant number of patients with non-acidic or mixed LPR.

Another limitation is the lack of use of other methods to estimate the early deficiency of vitamin B_{12}, such as plasma homocysteine and methylmalonic acid dosage, because the normal reference range of vitamin B_{12} is very broad. Even though the increase in vitamin B_{12} was significant, the results still remained within the reference range. The study could have been developed for a longer time, to better assess changes in hematologic parameters over time.

A major limitation was the lack of evaluation of serum iron and ferritin levels. After all, reduction of stomach acid may decrease the absorption of iron, which could result in anemia.[30]

At least, iron deficiency would affect hematologic variables, as the reduction of iron levels in the body tends to cause hypochromia and microcytosis.[39] In addition, it is important to highlight that the lack of assessment of red-cell distribution width (RDW) in all patients must have interfered in the results related to this variable.

The use of CES-D instead of the clinical diagnosis of depression may have been a limitation in the interpretation of the findings, even though the CES-D has already been used in other studies with this same purpose.[40]

Moreover, the low number of men in the study may have interfered with the results, both because menstrual blood losses in women may influence the variables analyzed and because women are more susceptible to depression.

As all patients with suspected LPR are instructed to modify their diets and lifestyle, it is possible that these changes have affected the study results. However, dietary and lifestyle influences were not analyzed in this study.

Finally, other confounding factors not reported by the patient, such as changes in diet, use of vitamin supplements, and other psychiatric disorders may also have interfered with the results. It also important to note that other disorders, such as long-term rhinosinusitis, allergy, diabetes, and long-term consumption of tobacco and/or alcohol, which have common symptoms with LPR, may have affected the results.

Studies such as this are extremely important in assessing the safety of continued use of medications, such as PPIs, which are widely used in clinical practice today. However, further studies need to be developed to corroborate the findings reported in this study or in studies previously conducted in the scientific literature, not only regarding the use of PPIs and vitamin B_{12} decrease but also in relation to the association between deficiency of this vitamin and depression.

Conclusions

The use of PPI (Omeprazole 80 mg/day) for 6 months in patients with suspected LPR may has led to a decrease in the number of RBCs and an increase in MCV, MCH, and vitamin B_{12}. After 6 months of treatment, there was improvement in the scores of a tool used for depression estimation (CES-D). The decrease in the CES-D scores was associated with an increase in MCV values, with no relation to vitamin B_{12} levels.

Author Contributions

All authors contributed equally in the design and execution of the study and in preparing this paper.

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