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Research Article
Is Long-Term Proton Pump Inhibitor Use a Cause of Leaky Gut Syndrome? A Cross-Sectional Case-Control Study

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Abstract

Background and Objective: Although Proton Pump Inhibitors (PPIs) have a good safety profile, they have been shown to be associated with Small Intestinal Bacterial Overgrowth (SIBO) and *Clostridium difficile* infection if used long-term. Tight junctions in the small intestine epithelium tightly regulate the passage of antigen into the cell. The best-known modulator of tight junctions is the zonulin protein. The gut microbiome is an important factor influencing zonulin release. The aim of current study was to investigate the effect of long-term PPI use on serum zonulin levels, an important indicator of leaky gut syndrome. **Materials and Methods:** A total of 75 patients were included in the study, 45 long-term PPI users and 30 non-PPI users. Use exceeding one tablet a day for 6 months or one tablet three days a week for 2 years was defined as long-term PPI treatment. The control group was selected from individuals who had never used PPIs. Venous blood samples were obtained from all patients to measure serum zonulin levels. **Results:** Eighty percent of the patients were using PPIs daily, while twenty percent were using them every other day. No significant difference was observed in the positive/negative ratios of serum zonulin levels between patients with PPI usage and those without PPI usage ($p>0.05$). **Conclusion:** The lack of change in zonulin levels in patients on long-term PPI use suggested that PPI use has no significant effect on intestinal permeability. In this context, it can be said that PPI use is safe.

Key words: Proton pump inhibitors, long term use, leaky gut, zonulin, chronic disease

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Proton Pump Inhibitors (PPIs) have become one of the most widely used drug groups worldwide. The PPIs are the most effective medications used in the treatment of common diseases such as peptic ulcer disease, Gastroesophageal Reflux Disease (GERD) and functional dyspepsia[1]. Despite often lacking appropriate indications, they are used for prolonged periods, sometimes lifelong, particularly in patients with GERD. These drugs act by irreversibly binding to the H^+-K^+-ATPase pump in parietal cells on the apical surface of the stomach, thereby inhibiting gastric acid secretion[2].

The PPIs have a good safety profile and discontinuation of these drugs by patients due to side effects is rare. The main side effects of short-term PPI use include mild symptoms such as headache, rash, dizziness, nausea, abdominal pain, gas, constipation and diarrhea. Generally, there is little concern about serious side effects of PPIs during a short treatment period of approximately two weeks; however, reports of side effects associated with prolonged use of these drugs are increasing. Complications related to chronic acid suppression due to PPI use can be categorized into three main headings: Malabsorption, infections and gastric effects[3]. Suppression of gastric acid secretion due to PPI use leads to a reduction in the elimination of orally ingested bacteria, creating a predisposition to intestinal dysbiosis. An increased incidence of Small Intestinal Bacterial Overgrowth (SIBO) and *Clostridium difficile* (CD) infections has been observed, particularly with long-term PPI use[4].

In the past, genetic and environmental factors were held responsible for the development of many chronic inflammatory diseases (CIDs), but in recent years, the gut microbiome and its effects on the host genome have been held responsible in the foreground. Leaky gut syndrome is important in the CID epidemic[5]. The intestinal barrier plays a critical role in preventing the entry of unwanted antigens or toxins into the intracellular space[6,7].

Intercellular tight junctions (TJs) and zonulin protein have an important role in regulating intestinal barrier function. The system, normally operating in a reversible equilibrium, can be influenced by genetic or environmental factors[8-10]. Zonulin, a protein that regulates tight junctions in intestinal epithelial cells, is considered a key indicator of leaky gut syndrome[11].

The aim of current study was to explore whether there is a role for the protein zonulin in the development of systemic side effects attributed to long-term PPI use.

MATERIALS AND METHODS

Study design: Patients who applied to the Gastroenterology Department of Abdurrahman Yurtaslan Oncology Hospital in Turkey between October, 2023 and December, 2023 were included in this prospective cross-sectional case-control study.

Ethical approval for the study was obtained from the hospital clinical ethics committee before the commencement of the study (Date: 04 October, 2023, Decision No: 2023-09/369). Clinical Trials registration number: NCT06268834. The research was conducted in accordance with the principles of the Helsinki Declaration. Written and verbal informed consent was obtained from all patients.

Study population: Hertzog[12] suggested that for a randomized controlled trial, a patient or control group of 30 to 40 participants would be needed to estimate the between-group effect size for power analysis. A study group was formed from patients aged 18 and above who applied to the gastroenterology clinics of Ankara Abdurrahman Yurtaslan Oncology Hospital and had been using long-term PPI (omeprazole, lansoprazole, pantoprazole, esomeprazole and rabeprazole) every day for at least 6 months or at least three times a week for at least 2 years. A control group was formed from individuals who had never used PPI. Those with inflammatory bowel disease, celiac disease, type 1 diabetes, active gastroenteritis, acute kidney failure, chronic kidney failure and those who had used antibiotics for any reason in the last 3 months, as well as those receiving probiotic drug support, were not included in the study[13-17]. The duration and dosages of PPI use were examined. Demographic data such as age, Body Mass Index (BMI), educational status, comorbidity status and chronic medication use were recorded.

Zonulin test procedure: Approximately 5 cc of blood was drawn from the antecubital veins of patients to measure serum zonulin levels. After clotting at room temperature for 1 hr and 30 min, the blood was centrifuged at 2000 revolutions per minute for 15 min using a Yuda 800D brand centrifuge. The obtained serum samples were stored at -80°C in a Thermo brand freezer for a period of 3 months. Zonulin levels in the serum samples were measured using commercial BT-LAB Human Zonulin ELISA kits (Cat. No: E1117Hu) (Shanghai Crystal Day Biotech Co. Ltd., Shanghai, China). Enzymatic reactions were measured photometrically. Zonulin values were determined by comparing the optical

density of serum samples with the standard curve. The assay range of the kit was 2-600 ng/mL. The sensitivity of the test was 1.09 ng/mL. The absorbance values of the standards for our test were determined as follows: STD1: 0.13, STD2: 0.25, STD3: 0.48, STD4: 0.96 and STD5: 1.92. An average optical density (OD) was calculated for each standard by plotting the concentration on the horizontal (X) axis against the absorbance on the vertical (Y) axis, creating a standard curve. The most appropriate curve was drawn through the points on the graph. Consequently, serum zonulin levels above 12 ng/mL were considered positive.

Statistical analysis: Descriptive statistics for continuous data included mean, standard deviation, median, minimum, maximum and 25th-75th percentiles (IQR), while for discrete data, count and percentage values were provided. The normality of the data was assessed using the Shapiro-Wilk test. For comparisons between patients with and without PPI usage regarding continuous variables, an independent sample t-test was utilized for normally distributed data and the Mann-Whitney U test was employed for non-normally distributed data. For group comparisons of nominal variables (in cross-tabulations), the Chi-square/Fisher's exact test was applied. The IBM SPSS for Windows 20.0 (SPSS Inc., Chicago, Illinois) software was used for the evaluations and a $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

A total of 75 patients participated in the study, comprising 45 individuals using long-term PPIs and 30 individuals not using PPIs. In Table 1, there were no significant differences in age, gender or BMI values between patients not using PPIs and those using PPIs ($p>0.05$). The rates of coronary artery disease (CAD) and diabetes mellitus (DM) detection were found to be higher in patients using PPIs compared to those not using PPIs ($p<0.01$). In Table 2, 80% of the patients were using PPI daily, while 20% were using it every other day. The mean duration of PPI use in our PPI users was 8.18±7.39 years. Additionally, 51.1% of them were using lansoprazole. In Table 3, there was no significant difference in the positive or negative ratios of zonulin values between patients using PPIs and those not using PPIs ($p>0.05$).

In current study, a significant difference did not find in serum zonulin levels between patients using PPIs for an extended period and those not using PPIs. While there have been numerous studies on the gut side effects of PPIs, there have been very few studies investigating their effects on leaky gut syndrome and its best indicator, zonulin.

The two main triggers of zonulin release identified so far are bacteria and gliadin. Many enteric pathogens capable of producing enterotoxins affecting the intestinal TJ have been described by El Asmar *et al.*[15]. The interaction of leaky gut syndrome, intestinal bacteria and zonulin was shown in Fig. 1[10].

Dial *et al.*[18] and Leonard *et al.*[19] showed an increase in enteric pathogenic bacteria (*Clostridium difficile*, *Salmonella* and *Campylobacter*) with the long-term use of PPIs. Based on these studies, the impact of increased intestinal pathogenic bacteria investigated due to long-term PPI use on serum zonulin levels. However, this study did not find a significant difference between the PPI-using and non-using groups. This may be explained by the fact that the enteric pathogenic bacteria that El Asmar *et al.*[15] found to increase zonulin release are different from the bacterial species that increase in the intestines with long-term PPI use. According to another meta-analysis, PPI use was found to increase SIBO by 8-fold[4].

Table 1: A comparison of the characteristics between patients not using PPIs and those using PPIs

Characteristics	Total	Using PPI	Not using PPI	p-value
Age (years)				
Mean±SD	54.93±12.59	52.90±12.75	56.28±12.44	0.256[a]
BMI (kg/m²)				
Mean±SD	28.43±4.98	27.63±4.89	28.96±5.02	0262[a]
Sex (n (%))				
Female	45 (60.0)	19 (63.3)	26 (57.8)	0630[c]
Male	30 (40.0)	11 (36.7)	19 (42.2)	
Comorbidity (n (%))				
Hypertension	17 (37.8)	7 (23.3)	17 (37.8)	0.189[c]
Hypothyroidism	11 (14.7)	7 (23.3)	4 (8.9)	0.104[c]
CAD	10 (13.3)	0	10 (22.2)	0.005[c]
DM	13 (17.3)	1 (3.3)	12 (26.7)	0.009[c]
Zonulin (ng/mL)				
Median (IQR)	2.19 (1.45-4.49)	2.12 (1.37-8.99)	2.19 (1.55-4.27)	0.581[b]

Values are presented as the Mean±SD, median (25-75% inter-quartiles) and number (%). Statistically significant values are marked in bold. BMI: Body Mass Index, IQR: Interquartile range, SD: Standard deviation, n: Number of patients, CAD: Coronary artery disease, DM: Diabetes mellitus, PPI: Proton Pump Inhibitor, [a]Mean±Standard Deviation and Student's t test, [b]Median (25-75%), Mann-Whitney U test and [c]Data are presented as n (%) and the Chi-square test

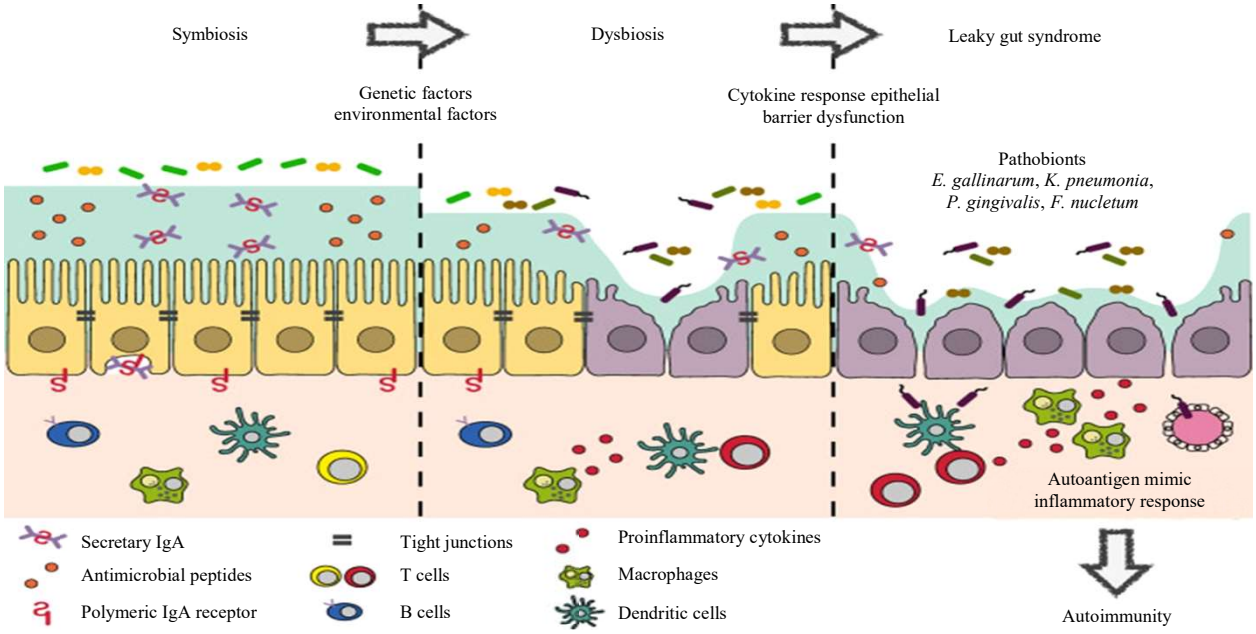

Fig. 1: Interaction of leaky gut syndrome, intestinal bacteria and zonulin[10]

Table 2: Characteristics of the PPI group

Characteristics	Using PPI (n = 45)
PPI duration of use (years)	
Mean±SD	8.18±7.39
PPI dose (n (%))	
Every day	36 (80)
Once in two days	9 (20)
PPI	
Esomeprazol	12 (26.7)
Lansoprazol	23 (51.1)
Pantoprazol	10 (22.2)

PPI: Proton Pump Inhibitor, SD: Standard deviation and n: Number of patients

Table 3: A comparison of zonulin distributions between patients not using PPIs and those using PPIs

Characteristics	Total (n = 75)	Not using PPI (n = 30)	Using PPI (n = 45)	
Zonulin: n (%)				
Positive	13 (17.3)	7 (23.3)	6 (13.3)	0.262[c]
Negative	62 (82.7)	23 (76.7)	39 (86.7)	

Zonulin values positive >12 ng/mL, PPI: Proton Pump Inhibitor, n: Number of patients, [c]Data are presented as n (%) and the Chi-Square test

Cortez *et al.*[20] in their study of 67 overweight and 66 normal-weight adolescents, found no association between SIBO and zonulin levels. Zonulin levels were found to be high in adolescents with a high BMI and zonulin levels were found to be low in adolescents with a low BMI. Although SIBO was not evaluated in this study, the fact that there was no significant difference in zonulin levels between the control and PPI groups was similar to the results of this study. This study did not find a relationship between BMI and zonulin level.

Horvath *et al.*[21] conducted a study investigating the effects of long-term PPI therapy on intestinal inflammation, intestinal barrier function, microbiome composition, routine laboratory parameters and a three-month synbiotic intervention. In the section related to intestinal barrier function, fecal zonulin levels were examined and 44% of patients were found to have slightly elevated levels (i.e., >50 ng/mg). In patients with initial zonulin levels exceeding 50 ng/mg, a significant decrease in zonulin levels was observed after the three-month synbiotic treatment.

However, when patients with high and normal zonulin levels were analyzed together, the decrease was not statistically significant ($p = 0.4$). This study was similar to current study in that it is the only study in the literature that examines the PPI-zonulin relationship. Although our results are similar, the fact that we looked at serum zonulin levels instead of stool may be the reason for some differences.

Long-term use of PPIs has been associated with negative effects on cardiovascular diseases, chronic kidney disease, increased fracture risk, hepatocellular carcinoma and dementia[22-26].

In current study, the frequency of CAD was higher in the group using PPIs compared to the non-PPI-using group. Similarly, the frequency of DM was found to be higher. While the primary aim was not to investigate complications related to PPI use, while an association between long-term PPI use and the presence of CAD and DM in the study groups was observed frequently. This association may also be due to the use of PPIs for protective purposes due to chronic drug use.

A strength of current study was that it is one of the few conducted to explore the impact of chronic PPI use on leaky gut syndrome. While the limitation was that the intestinal bacterial cultures in both patient and control groups were not examined.

CONCLUSION

Current study suggested that long-term PPI use, which is implicated in dysbiosis and intestinal infections, does not have a significant impact on serum zonulin levels, a crucial marker of leaky gut syndrome. In this regard, it can be stated that PPI use is safe. Further studies with larger sample sizes and the exploration of other markers associated with intestinal tight junction function are needed.

SIGNIFICANCE STATEMENT

Despite numerous studies linking Proton Pump Inhibitor (PPI) use to increased risk of dysbiosis and infection, there is a lack of research on the effect of PPI use on intestinal barrier function. The investigators found that long-term PPI use did not have a significant effect on blood zonulin levels. This study is important as it definitively demonstrates that long-term PPI use does not impact zonulin, the most important marker of intestinal tight junction function.

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