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Original Article

Can fecal zonulin be used as a biomarker in the detection of leaky bowel syndrome in patients with intestinal metaplasia? A prospective cross-sectional study

İntestinal metaplazili hastalarda sızdıran bağırsak sendromu tespitinde fekal zonulin biomarker olarak kullanılabilir mi?: prospektif kesitsel çalışma

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ABSTRACT

Aim: Intestinal metaplasia is the most common finding in routine endoscopic studies. The relationship between intestinal metaplasia and intestinal dysbiosis and leaking intestine is now a known fact. In this study, we tried to show the detection of this chain of events, which may cause many diseases, with zonulin in stool, which is a non-invasive method.

Material and Method: Endoscopy was performed on 20 patients with dyspeptic complaints as of January 2022. All patients were tested for zonulin in the stool using the Stool Sample Application System (SAS) and Elisa method. Endoscopic findings of the patients (intestinal metaplasia, atrophic gastritis, helicobacter (Hp) status) and zonulin values in the stool were examined. This study was designed as a prospective cross-sectional study.

Results: The median age of the patients was 48.35 (December: 33-73). In this study, 40% of the patients were female and 60% were male. Of the 20 patients who underwent endoscopy, 14 had intestinal metaplasia (70%), 11 had atrophic gastritis (55%) and 14 had helicobacter (Hp). In our results, while zonulin was high in stool in all patients with these conditions, only statistical significance was observed between intestinal metaplasia and zonulin elevation.

Conclusion: Zonulin excretion has increased in fecal excretion in cases with intestinal metaplasia and may be an important marker in these cases.

Keywords: Zonulin, intestinal permeability, atrophic gastritis, intestinal metaplasia

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ÖZ

Amaç: intestinal metaplazi, rutin endoskopik çalışmalarda en sık görülen bulgudur. İntestinal metaplazi ile bağırsak disbiyozu ve sızdıran bağırsak arasındaki ilişki artık bilinen bir gerçektir. Biz bu çalışmamızda bir çok hastalığa neden olabilen bu olaylar zincirini non invazif yöntem olan gaitada zonulin ile tespitini göstermeye çalıştık.

Gereç ve Yöntemler: Ocak 2022 den itibaren dispeptik şikayetleri olan 20 hastaya endoskopi yapıldı.. Hastaların hepsinden, Stool Sample Application System (SAS) ve Elisa yöntemi ile gaitada zonulin bakıldı. Hastaların endoskopik bulguları (intestinal metaplazi, atrofik gastrit, helicobakter(Hp) durumu) ile gaitada zonulin değerleri incelendi. Prospektif kesitsel çalışma olarak dizayn edildi.

Bulgular: Hastaların median yaşı 48.35(Aralık:33-73) ti. Hastaların %40 ı kadın %60 ı erkekti. Endoskopi yapılan 20 hastadan, 14 hastada intestinal metaplazi (%70), 11 hastada atrofik gastrit (%55) ve 14 hastada helikobakter (Hp) saptadık. Sonuçlarımızda bu durumların var olduğu tüm hastalarda gaitada zonulin yüksek saptanırken, sadece intestinal metaplazi ile zonulin yüksekliği arasında istatistiksel anlamlılık gözlenmiştir.

Sonuç: Zonulin atılımı intestinal metaplazili olgularda dışkıda atılımı artmıştır ve bu olgularda önemli bir belirteç olabilir.

Anahtar Kelimeler: Zonulin, bağırsak geçirgenliği, atrofik gastrit, intestinal metaplazi

Introduction

The human body contains nearly 100 trillion gut microbes. Specifically, the gut microbiota consists of a large number of different bacteria that produce various metabolites and participate in important metabolic functions, such as membrane functions along with energy metabolism (1, 2). The evidence available so far has shown that gut microbiota contributes to the regulation of gastrointestinal function (3). Previous studies have shown that bacterial abundance increases in patients with chronic atrophic gastritis (CAG) with decreased gastric acid secretion, and changes in intestinal microbiota contribute to progression from intestinal metaplasia (IM) to gastric cancer (4,5).

Microbial infections, especially Helicobacterial Pylori (Hp) infection, the imbalance of the gut microbiota and inflammation function together and cause damage to the gastric mucosa and consequently cause CAG (6, 7). CAG is a multi-stage, multifactorial and continuous inflammation process (8), and persistent inflammation in the stomach is one of the important causes of intestinal microbial disorders. An observation has been made that a shift towards opportunistic microorganisms in intestinal microbiota composition balance results in increased secretion of zonulin, a recently discovered protein (9). Such events may lead to various digestive system dysfunctions, systemic infections, food intolerances, and autoimmune diseases. The discovery of zonulin, a protein with strict binding regulatory activity in the epithelium, sheds new light on the role of the intestinal barrier in improving health and understanding the occurrence of diseases.

In this study, we aimed to show that these barriers deteriorated in the gastric mucosa with increased zonulin levels in the stool in patients with intestinal metaplasia in the stomach.

Material and Methods

Ethics committee approval (decision no: 2020/08/04 Non-interventional Clinical Studies Ethics Committee) was obtained from 20 patients who have had dyspeptic complaints since January 2022 and underwent upper gastrointestinal (GIS) endoscopy for this reason. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Endoscopic findings of the patients (intestinal metaplasia, atrophic gastritis, helicobacter (Hp) status) and zonulin values in the stool were recorded. The age and gender status of the patients were recorded.

Zonulin Stool Test Procedure

This ELISA is planning for the quantitative assurance of zonulin family peptides (ZFP) in stool; for in vitro symptomatic utilize as it were. This test is based on the strategy of competitive ELISA. As a to begin with planning step, biotinylated ZFP is included to the tests, benchmarks and controls. A while later, aliquots of the treated tests, measures and controls are exchanged and brooded in microtiter plate wells coated with polyclonal anti-ZFP antibodies. Amid the brooding, the free target antigen within the tests competes with the biotinylated ZFP for the official of the polyclonal anti-ZFP antibodies immobilized on the microtiter plate wells. The unbound components are expelled by a washing step. During a moment hatching step, peroxidase-labelled streptavidin, which binds to the biotinylated ZFP, is included to each microtiter well. After a washing step to remove the unbound components, the peroxidase substrate tetramethylbenzidine is included. At long last, the enzymatic response is ended by an acidic halt arrangement. The color changes from blue to yellow and the absorbance is measured within the photometer at 450 nm.

The concentrated of the yellow color is reverse relative to the ZFP concentration within the test, which suggests that tall ZFP concentration within the sample reduces the concentration of the biotinylated ZFP bound to the immobilized against ZFP antibodies and brings down the photometric flag. A dose-response bend of absorbance unit (optical thickness, OD at 450 nm) concentration is created utilizing the values gotten from the standard.

Statistical Analyses

SPSS 22 (Statistical Package for Social Sciences) statistical program was used to analyze the data. Kolmogorov–Smirnov normality test was performed before the test selection. The Mann-Whitney U test was performed to compare the median values of the group in the data set with a non-parametric distribution, analyzed and considered statistically significant with a $p < 0.05$.

Results

The median age of the patients was 48.35 (December: 33-73) and 40% of the patients were female and 60% were male. Intestinal metaplasia was detected in 14 (70%) of the patients due to the upper GIS endoscopy performed in 20 patients. Zonulin test was high in stools performed in these patients. The median value of zonulin was 1314 (range: 9-3200). The median value of the stool zonulin test results of six (30%) patients without intestinal metaplasia was 27 (range: 8-76). The results are given in Table 1. When the results were evaluated, the zonulin level in the stool was statistically significantly higher in patients with intestinal metaplasia ($p < 0.05$). In patients with atrophic gastritis and Helicobacter Pylori, zonulin values in the stool were high but not statistically significant ($p < 0.05$).

Table 1. General Features, Pearson Chi Square Test

	N (%)	Zonulin (Median-Range), Standard Deviation	P value
Age (Median-Range), Year	4835(33-73)		
Gender			
Female	8 (40 %)	332 (8-3200), 1420	0.215
Male	12 (60 %)	62 (9-3200), 903	
İntestinal metaplasia			
Yes	14 (70%)	173 (9-3200), 1314	0,003
No	6 (30%)	9,5 (8-76) , 27	
Atrophic Gastritis			
Yes	11 (55 %)	133 (9-3200) , 1281	0.137
No	9 (45 %)	10 (8-3200) ,1045	
Helıcobacter Pylorı			
Yes	14 (70 %)	95 (9-3200), 1185	0.679
No	6 (30 %)	148 (8-3200) ,1251	

Discussion

In fact, the intestine can be defined as the pathway that connects the external and internal environments of the body and serves as the determinant of which components enter the bloodstream and in what amount. Therefore, the intestinal barrier plays a vital role in maintaining the immune homeostasis of the intestine and the whole body. Studies have documented that increased zonulin expression and intestinal barrier permeability are associated with celiac disease, type 1 diabetes, and other autoimmune diseases. The dysbiosis of the microbiome may trigger zonulin release, which leads to the penetration of the intestinal lumen content into the epithelial barrier and thus the release of proinflammatory cytokines. The presence of cytokines may cause a large amount of food residues and microbial antigen flow, which leads to the activation of T cells and induces an ever-increasing permeability status (9).

Causes of chronic atrophic gastritis, especially Hp, may lead to intestinal metaplasia after CAG and ultimately to gastric cancer. In CAG, gastric acid decreases and as a result, pathogenic bacteria passage from the stomach to the intestine increases. This causes dysbiosis in the intestine, which leads to zonulin activation from intestinal epithelial cells. As a result of zonulin activation, it leads to the opening of tight compounds in the intestine, causing many pathogenic toxins and microorganisms to pass into the bloodstream. In our study, zonulin level in stool was also high in helicobacter positive and atrophic gastritis patients, but it was not statistically significant. We think that this is due to the lack of patients. Although there were few patients in intestinal metaplasia, the zonulin level in stool was significantly higher.

A groundbreaking period in understanding the role of intestinal permeability in health and disease was marked by the discovery of zonulin, the first human protein to exhibit the regulatory activity of TJ connections. Possibly, zonulin activates the epidermal growth factor receptor (EGFR) using proteinase-activated receptor 2 (PAR 2), which leads to phosphorylation of TJ proteins and rearrangement of actin filaments, followed by suppression of TJ proteins, which consequently relaxes increased intestinal permeability[10]. Research programs have revealed that zonulin affects the relationships between bacteria and the host. Increased secretion of this protein was detected after exposure to non-pathogenic and pathogenic bacterial strains [11]. In a study on small intestinal permeability in different animal species exposed to various types of bacteria, a random increase

in paracellular intestinal permeability was noted with the separation of ZO-1(zonula occludens) protein from TJs [12]. It is assumed that activation of the zonulin pathway may be a defense mechanism that prevents pathogenic bacteria from adhering to and colonizing the small intestine. In this case, modulation of intestinal permeability by activating the zonulin pathway may be in addition to the non-specific response of the body to maintain intestinal homeostasis.

In recent years, much has been discovered about the intercellular structure, its function, function and tight junction (TJ). However, information on how these mechanisms is still lacking. In this regard, progress was made with the discovery of zonula occludens toxin (zot). Zot is an enterotoxin developed by vibrio cholera and a toxin that affects intercellular binding (TJ). It was determined that the COOH terminal part of the toxin (called G) was effective. This G point is located in the specific proteinase activating receptor. In case of binding to the proteinase activating receptor, it leads to the reversible opening of TJ with intracellular signals (13). This has shown that intercellular junction (TJ) is of great importance in regulating the passive solute transition from the epithelial barrier to the paracellular area (14).

Zonulin isolated from human intestinal cadavers shows an increase in permeability in non-human primate intestinal epithelium (15).

When the data were considered collectively, it was found that Zonulin was involved in congenital immunity in the intestine (11) and it was an indicator of TJ dysfunction, which has a regulatory role in the primary damage of autoimmune diseases such as type 1 diabetes, including celiac (16,17).

Since zonulin-related proteins (ZRP) are proteins that increase permeability in the epithelial layer of the small intestine by reversing and modulating tight intercellular connections, they can serve as a new, non-invasive biomarker of disease activity (18).

Zonulin-related proteins are fecal proteins that reflect intestinal permeability, and their increased fecal levels are thought to be a marker of an impaired intestinal barrier, especially in the small intestine (19). The results we obtained in our study also support this.

The small number of our study is a limitation. However, measurement of zonulin in stool and obtaining the kit was very difficult and costly.

In conclusion, in this study, we tried to show that increased zonulin excretion increased in stool with a non-invasive method and that it may be an important marker related to these diseases. Until recently, there have not many studies supporting or showing the use of zonulin other than blood serum values. Thus, although we think that our study is important, there is a need to work in much larger series in this field. Furthermore, little is known about the correlation between serum and fecal zonulin or which may be more useful in diagnosing impaired intestines. Until recently, there have not many studies supporting or demonstrating the use of zonulin other than blood serum values. Therefore, although we believe that our study is important, clinical studies are needed in a much larger series in this field. Therefore, the invention of a new non-invasive biomarker will greatly benefit both scientific (discovery and confirmation of a new disease marker) and clinical/practical perspectives.

Ethicss Approval

Ethics Committee Approval: Approval for the study was given by the ,Non-interventional Clinical Studies Ethics Committee (Date:21/08/2020, decision no: 2020/08/04)

Informed Consent

Consent was obtained from all patients included in the study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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