Original Article

IL-33 Levels in the Esophageal Biopsy Specimen on Patients with Erosive and Non-Erosive Reflux Esophagitis

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ABSTRACT

Background:
IL-33, a tissue-derived cytokine, plays an important role in inflammatory responses. This study aimed to evaluate the interleukin-33 (IL-33) marker in esophageal biopsy samples from patients with erosive reflux disease (ERD) and non-erosive reflux disease (NERD) in comparison with a control group.

Materials and Methods:
In this study, tissue samples were obtained from 54 patients (27 with ERD and 27 with NERD) and 27 controls. Tissue contents of IL-33 were measured using Enzyme-Linked Immunosorbent Assay (ELISA). Additionally, the Bradford method was employed to determine the protein concentration of the samples. Our results were analyzed by SPSS software (Version 22.0). The $P<0.05$ was considered a significant level.

Results:
The mean concentration (mean ±SD) of IL-33 in the NERD, ERD, and control groups was 439.06± 135.47, 511.83±137.75, and 206.09± 76.03 pg/mgp, respectively. A significant difference was observed between patients with NERD, ERD, and healthy subjects in terms of IL-33 levels (P=0.072).

Conclusion:
This study provides new evidence to support a major role of IL-33 in distinguishing patients with ERD and NERD subtypes. IL-33 level may serve as a biomarker for the progression and development of gastroesophageal reflux disease (GERD). These data may support the recommendation of IL-33 measurement in patients with GERD for making informed decisions about further drug treatment strategies and for monitoring this type of disease.

Keywords: Esophageal biopsy; Erosive esophagitis; Gastroesophageal reflux disease; IL-33; Non-erosive reflux disease

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INTRODUCTION

Nonerosive reflux disease (NERD) is a highly prevalent phenotype of the gastroesophageal reflux disease (1). Heartburn and acid regurgitation are the most common symptoms of GERD, although pathologic reflux can result in a wide variety of clinical presentations (2). Gastroesophageal reflux disease (GERD) has become the most commonly seen gastrointestinal disorder in outpatient clinics (3). It involves 18.1% - 27.8% of the population in North America (4). Its prevalence in east asia countries is 2.5%-7.8% (4). GERD is a multifactorial, acid-peptic disorder that results from the reflux of noxious material from the stomach into the oesophagus (5). Long-term exposure to GERD can increase the risk of Barrett metaplasia, resulting in a person susceptible to esophageal adenocarcinoma Reflux esophagitis causes Barrett's metaplasia, an abnormal esophageal mucosa predisposed to adenocarcinoma (5, 6). NERD is the most common manifestation of the disease, in community-based patients (7). Reflux esophagitis is believed to be caused by the caustic effects of refluxed gastric acid on esophageal epithelial cells. For many years, it has been believed that GERD is caused by direct damage to the epithelial cells of the esophagus from gastric acid (8). Studies have shown that in reflux, the effects of direct acid damage quickly manifest, while inflammation occurs several weeks later. It was found that the stimulation of epithelial cells of the esophagus by gastric acid secretes cytokines such as IL-8 and IL-1β, which can cause inflammation (8, 9). Thus, the epithelial cells of the esophagus play a major role in the pathogenesis of GERD, serving as the initiator of the inflammatory process (9). IL-33, a pro-inflammatory cytokine and key mediator, plays a role in regulating the production of other inflammatory factors associated with GERD (9). IL-33 production may be the forerunner and stimulant for the production of other inflammatory factors, such as IL-6 and IL-8 (10). Nuclear IL-33 is upregulated in patients with heartburn. Upregulated IL-33 in heartburn patients is related to the symptoms (10). It is well known that pro-inflammatory cytokines such as IL-33 can stimulate production and secretion of other inflammatory mediators by increasing NF-kB activity (10). It can be used as a factor in determining the disease process and also as a biomarker to assess the effect of treatment on the disease (11). It is also known as a warning factor as its serum levels increase as a result of necrotic processes and induce the production of inflammatory cytokines. Many diseases associated with inflammatory effects appear to be related to this interleukin (12). Interleukin 33 (IL-33) is a member of the IL-1 family of cytokines with a growing number of target cells and a plethora of biological functions(13). In vivo, IL-33 induces the expression of IL-4, IL-5, and IL-13 and leads to severe pathological changes in mucosal organs(14). Therefore, the increase in these cytokines may be related to the features and damage of tissues. It does not work well in differentiating patients with ERD and NERD. This fact prompts us to identify a marker responsive to the differentiation of patients with ERD and NERD. The goal of the current study was to measure the levels of IL-33 in esophageal biopsy specimens from patients with ERD, NERD, and controls to determine the process of reflux esophagitis development.

MATERIALS AND METHODS

Instruments

Vortex mixer labnet, centrifuge (Clement 2000, Australia), water bath (Fanazmagostar Co WM22), Dionizer (HastaranTeb Co) , Microplate reader with 450±10 nm filter (Rayto life and analytical science Model RT -2100 C, Hamburg, Germany).

Patients

Fifty-four patients undergoing upper endoscopy due to reflux symptoms and/or previously reported information were entered in the experiment. The patients were divided into two groups: erosive esophagitis and NERD. 27 patients with ERD, 27 patients with NERD, and 27 controls were studied. The severity and frequency of GERD symptoms were assessed using a standardized questionnaire. Entry criteria: female or male, aged 18–80, with the ability to provide informed consent. Patients with typical symptoms of reflux should experience symptoms at least three times a week. Typical symptoms of reflux were determined as heartburn and regurgitation. Patients with other symptoms of reflux were not included in this investigation. Exclusion criteria were: taking NSAIDs, corticosteroids, anti-allergic drugs or other immunosuppressive drugs, proton pump inhibitors (PPI) and H2 antagonists (at least two months before sampling), having upper digestive system diseases (like cancers, peptic ulcers, polyps, and Barrett's esophagus), mal-absorptive diseases (like celiac and Crohn's disease, vasculitis, ulcerative colitis).

Control subjects

The volunteers in this experiment were from the general population of Mazandaran (including subjects who wanted to have a checkup), as the control group. The WHO definition of health as complete wellbeing is no longer fit for purpose given the rise of chronic disease. Huber and colleagues propose changing the emphasis towards the ability to adapt and self manage in the face of social, physical, and emotional challenges (15). Exclusion criteria were supplementation of micronutrients, smoking, and pregnancy.

Ethical Considerations

Written informed consent was obtained from all patients before endoscopy. This study was approved by the Human Subjects' Ethics Board of Babol University of Medical Sciences and was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. All protocols involving patients and control subjects were confirmed by the Ethics Committee of Babol University of Medical Sciences with the code number of (IR.MUBABOL.HR.REC.1397.089).

Sampling and Assay

During endoscopy, a sample of esophageal biopsy was taken from each patient. Then each biopsy sample was washed in cold phosphate-buffered saline (PBS) and was immediately frozen at -80 °C. After collecting the complete set of samples, all biopsies were weighed, and a predetermined amount of protease inhibitor cocktail and PBS was added for tissue homogenization using an ultrasonic device. After homogenization of each sample, centrifugation was performed to isolate the supernatant for measurement of the factors. The supernatant was stored at −20 °C.

Biochemical Assays

The Bradford test (16) was performed for each sample to determine the protein content of the tissue.

Tissue IL-33 concentrations were measured using the human IL-33 assay kit (product code: E0044Hu) provided by Bioassay Technology Laboratory (China) according to the manufacturer's guides.

Statistical Analysis

For data analysis, we used SPSS software (Version 22.0). The P value<0.05 was considered a significant level.

RESULTS

The esophageal biopsy specimen IL-33 levels were significantly increased in ERD and NERD patients compared with control samples (Tables 1, 2, 3). The results show that the IL-33 level in both patient groups is higher than in control samples (Figure 1). IL-33 levels were higher in patients with ERD compared with the NERD group, which indicates that IL-33 levels are related to the development process of GERD. Thus, the development of acute reflux esophagitis is associated with increased IL-33 in the esophageal squamous epithelium. Our results indicated that IL-33 levels are useful and effective markers for differentiating between ERD and NERD (Table 4, 5).

Table 1. Comparison of mean, standard deviation, standard error, and confidence interval for the mean of IL-33 of patients with GERD and control subjects with one-way analysis.

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	P value
					Lower Bound	
Control	27	206.09	76.03	14.63	176.02	0.000
Erosive esophagitis	27	511.83	137.75	26.51	457.33	0.072
Non-erosive reflux disease	27	439.06	135.47	26.07	385.47	0.072

N= number of sample, Std=standard deviation, Std=standard error

Table 2. Comparison of upper bound minimum, and maximum of IL-33 of patients with GERD and control subjects with one-way analysis

Groups	95% Confidence Interval for Mean	Minimum	Maximum
	Upper Bound		
Control	236.17	98.96	479.60
Erosive esophagitis	566.32	332.83	798.60
Non-erosive reflux disease	492.66	239.07	760.80

Table 3. Comparison of Sum of Squares, df, and Mean Squares of IL-33 in patients with GERD and control subjects with ANOVA analysis

	Sum of Squares	df	Mean Square	F	P value
Between Groups	1377387.133	2	688693.566	47.924	0.000
Within Groups	1120902.564	78	14370.546		0.072

Table 4. Multiple comparison mean difference of IL-33 of patients with GERD and control subjects using the Tukey test

(I) Status	(J) Status	Mean Difference (I-J)	Std. Error	P value
Control	Erosive esophagitis	-305.73407*	32.62644	0.000
	Non-erosive reflux disease	-232.97019*	32.62644	0.000
Erosive esophagitis	Control	305.73407*	32.62644	0.000
	Non-erosive reflux disease	72.76389	32.62644	0.072
Non-erosive reflux disease	Control	232.97019*	32.62644	0.000
	Erosive esophagitis	-72.76389	32.62644	0.072

Table 5. Multiple comparison, lower bound, and upper bound of IL-33 in patients with GERD and control subjects with post hoc tests

(I) Status	(J) Status	95% Confidence Interval	
		Lower Bound	Upper Bound
Control	erosive esophagitis	-383.68	-227.78
	Non-erosive reflux disease	-310.92	-155.01
Erosive esophagitis	control	227.78	383.68
	Non-erosive reflux disease	-5.18	150.71
Non-erosive reflux disease	control	155.01	310.92
	erosive esophagitis	-150.71	5.18

*. The mean difference is significant at the 0.05 level.

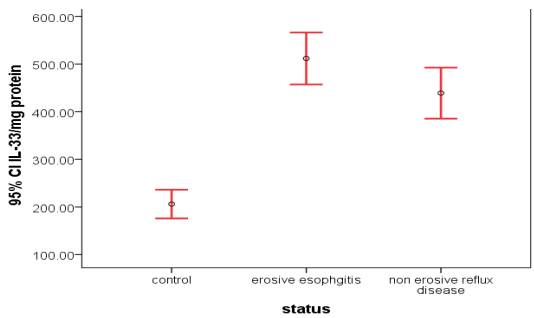

Figure 1. Comparison of IL-33 levels in control subjects, EE, and NERD. As is known, the IL-33 factor in EE patients was higher than in the NERD and control groups.

DISCUSSION

The cause of mucosal damage to the esophagus is complex. Many inflammatory factors are created inside the digestive system; however, their contribution to the pathophysiology of the disease has not been well studied. Analyzing cellular events in this illness is essential for a further understanding of the disease pathogenesis as well as the development of novel therapies (5). Therefore, we examined IL-33 in this study. Tissue levels of IL-33 were evaluated in patients with ERD and NERD, and the final results showed a significant difference between the ERD and control groups, which is consistent with an earlier study (9,17). Additionally, the results revealed a significant difference between the NERD and control groups. Furthermore, the level of IL-33 differed between the ERD and NERD groups, demonstrating the use of IL-33 as a distinguishing factor between these diseases, which exhibit differing presentations. Moreover, in agreement with previous reports (9,10,17), we confirmed a significant increase in the expression of IL-33 in biopsies from ERD patients compared with controls. Interestingly, unlike ERD patients, the esophageal mucosa of NERD patients exhibits a moderate increase in the level of IL-33. This finding may also be predictive of prognosis and could help discriminate between ERD and NERD patients.

IL-33 expression was significantly increased in the mucosa of patients with heartburn compared with that of the control group. IL-33 is a tissue-derived cytokine and a member of the IL-1 cytokine family, playing a crucial role in various biological processes, including immune and inflammatory responses, tissue homeostasis, balance, growth, and repair (10-12). IL-33 is involved in the damage caused by inflammatory diseases. Upregulation of IL-33 was seen in heartburn patients (10). IL-33, in addition to common features of IL-1 cytokines, has some unique features and has two intra- and extracellular roles (13). IL-33 is produced by tissue cells and is involved in the durability and maintenance of cells and tissues. In these cells, IL-33 is located in the nucleus and plays a crucial role in regulating gene expression. Additionally, the nuclear IL-33 regulates the production of pro-inflammatory cytokines, including IL-6 and IL-8. On the other hand, the nuclear IL-33 is stored as a warning that is released in response to cell damage. Extra nuclear IL-33, as a cytokine, moderates immune responses and repair mechanisms, and also begins the differentiation of T helper cells (Th cells) (13). IL-33 mRNA levels were significantly correlated with IL-6 and IL-8.IL-33 acts on IL-1 receptors (ST2) to activate the NF-κB nuclear factor or directly with NF-κB, releasing Th2 cytokines such as IL-13, IL-4, and IL-5; therefore, it can play a role as a pro-inflammatory factor (10, 11, 14).

These findings show that the IL-33 levels in both groups of patients are higher than those in control samples, indicating that in patients with ERD and NERD, the levels of pro-

inflammatory factors are high. Additionally, levels of IL-33 were higher in patients with ERD compared with the NERD group. The present finding aligns with the results of investigators, who reported that IL-33 was upregulated in the esophageal mucosa of patients with reflux esophagitis (9,17). Therefore, IL-33 may be an important factor in the development of GERD. Upregulated IL-33 messenger RNA expression was correlated with IL-8 and IL-6 expression in samples of patients with reflux esophagitis (RE) compared with control samples (17). Nuclear IL-33 is upregulated in erosive mucosa of RE patients and is correlated with IL-8 and IL-6 levels. (17). GERD is a long-term illness identified by difficulties that appear from the reflux of stomach contents, which significantly lower the quality of life, increase morbidity, and increase medical costs, conjoined handling the condition (18).

A positive association between IL-33 levels and the development and progression of GERD has been noted, suggesting that additional studies are needed to establish the importance of IL-33 in GERD. We believe that this will contribute to the development of future research in the area of pathogenesis and management of GERD, ultimately improving the quality of life for patients with NERD and ERD. The mechanism by which IL-33 plays a prognostic role in GERD is not well-understood. The role of Il-33 needs to be tested in a much larger population. Therefore, further basic and clinical studies are needed to determine its mechanism.

Limiting the findings

This study had several limitations. Firstly, the limited sample size of patients. Secondly, we did not have post-treatment data of patients with ERD and NERD. The patients' drug history was not evaluated, which should be covered in future research.

CONCLUSION

There was a significant difference in tissue levels of IL-33 between ERD and NERD patients and control subjects. Therefore, the IL-33 assay could be used to diagnose and differentiate between ERD and NERD patients and controls. The higher IL-33 levels in patients with ERD and NERD compared with the control group suggest a Th2 response dominance in the patient groups. IL-33 levels are positively correlated with GERD progression, which may be predictive of prognosis as well as to discriminate between patients with ERD and NERD. Therefore, in the future, by knowing the role of IL-33, finding drugs to interact with IL-33 may be beneficial to prevent the change of NERD and ERD to a normal status.

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AUTHORS' CONTRIBUTION:

FM, DQ, FN, SM, MK, and KH conceived the study, KH performed data analysis, DQ wrote the manuscript, FM collected data and wrote the manuscript, MK and SM interpreted the results and wrote the manuscript, DQ designed the study, wrote, and edited the manuscript.

CONFLICT OF INTERESTS:

The authors have no conflicts of interest to declare related to this work.

DATA AVAILABILITY:

The dataset presented in the study is available at the request of the corresponding author during submission or after its publication.

ETHICAL APPROVAL:

All protocols involving patients and control subjects were confirmed by the Ethics Committee of Babol University of Medical Sciences with the code number of (IR.MUBABOL.HR.REC.1397.089).

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