

Systemic Inflammatory Response in Patients with Gastroesophageal Reflux Disease

Vladimir Ivashkin¹, Yulia Evsyutina^{1*}, Alexander Trukhmanov¹, Svetlana Lyamina², Igor Malyshev²

¹Department of Internal Disease Propaedeutics, Gastroenterology and Hepatology, Sechenov First Moscow State Medical University, Moscow, Russian Federation

²Department of Pathophysiology, Moscow State University of Medicine and Dentistry, Moscow, Russian Federation

*Corresponding author: uselina@mail.ru

Abstract Aim. To characterize the systemic inflammatory response in patients with various forms of GERD. **Materials and methods.** The prospective clinical study included 45 patients with GERD and 10 healthy volunteers. An analytic survey of all participants was carried out which included, the collection of complaints and anamnesis, the identification of risk factors for GERD; esophagogastroduodenoscopy and a 24-hour esophageal pH-impedance monitoring. Using the method of flow cytometry, the levels of 7 cytokines were determined: two anti-inflammatory - IL-4 and IL-10, three pro-inflammatory - IL-8, IFN- γ and TNF- α , and two cytokines which may manifest as anti-inflammatory as well as pro-inflammatory activity depending on the circumstances (bivalent) - IL-2 and IL-6. **Results.** In patients with erosive and ulcerative esophagitis when compared to patients with NERD, Barrett's esophagus and healthy individuals, there was an increased expression of pro-inflammatory cytokines. Whereas in patients with Barrett's esophagus, when compared to other patients and healthy individuals, there was a resultant overexpression of anti-inflammatory cytokines. The levels of TNF- α and IL-8 correlates with the total number of acid reflux and acid bolus exposure, whereas the levels of IL-4 and IL-10 correlate with the total number of weakly alkaline reflux and weakly alkaline bolus exposure. The high level of IL-8 was associated with an increased incidence of recurrence of erosive esophagitis, despite the ongoing therapy for 2 years. **Conclusions.** In patients with erosive and ulcerative esophagitis in comparison with patients with NERD and Barrett's esophagus, there is a predomination in the production of pro-inflammatory cytokines such as IL-8, IFN- γ and TNF- α , indicating the development of Th1 immune response. In patients with Barrett's esophagus, there was an increased expression of anti-inflammatory cytokines such as IL-4 and IL-10, indicating the formation of the Th2 immune response.

Keywords: gastroesophageal reflux disease, systemic inflammatory response, cytokines

Cite This Article: Vladimir Ivashkin, Yulia Evsyutina, Alexander Trukhmanov, Svetlana Lyamina, and Igor Malyshev, "Systemic Inflammatory Response in Patients with Gastroesophageal Reflux Disease." *American Journal of Clinical Medicine Research*, vol. 3, no. 4 (2015): 6469. doi: 10.12691/ajcmr-3-4-2.

1. Introduction

Gastroesophageal reflux disease (GERD) is one of the most common diseases, and according to a recent epidemiological study, clinical and endoscopic signs of GERD can be found in 8-25% of the population depending on the continent, race and gender [1]. In the Russian Federation, the prevalence of GERD is 12 to 18% [2,3,4].

Despite vast improvements in the diagnosis and treatment of GERD, there are still many unresolved issues, mostly being patients with refractory GERD. These patients may experience recurrence of erosive and ulcerative lesions of the esophageal mucosa and develop complications such as peptic stricture of the esophagus, bleeding and Barrett's esophagus [5,6]. The number of patients who did not respond either partially or completely to a standard dose of a proton pump inhibitor (PPI) which was given once a day over an 8 week period (patients with refractory GERD) were 40-50% [7,8].

Over time, the major causes of the refractory GERD were considered as the following: lack of patients' adherence to treatment, the existence of a «nocturnal acid breakthrough», and a genetically determined polymorphism of isoenzymes CYP2C19 and CYP3A4 of cytochrome P450. [9]

In the recent years the problem of refractory GERD is being studied at the tissue and cellular level. The rate of damage of the esophageal mucosa, depending on the nature of reflux (acid, alkaline or mixed) is mainly focused. One of the most perspective interest in this area is the study of the cytokine profile in such patients. Cytokines, being a peptide signaling molecules, play an important role in the damage and can demonstrate pro-inflammatory as well as anti-inflammatory activity [10]. There are many scientific works which show the changes in immune response that in patients with GERD which occur in the form of an imbalance between cellular (Th1) and humoral (Th2) immunity, which in turn is possibly determined by the expression of the cytokines [11,12]. Most of the studies were focused on determining the level of cytokines in the tissues of the esophagus (due

to local immune response). There practically does not exist any scientific work which studies the systemic immune response that occurs in the body of the patients with GERD.

2. Aim of the Study

To study the systemic inflammatory response in patients with different forms of GERD.

3. Materials and Methods

The prospective study included 45 patients with GERD: 20 with nonerosive reflux disease (NERD) (11 men and 9 women, mean age 37.7 ± 12.0 years), 20 with erosive and ulcerative esophagitis (13 men and 7 women, mean age 38.3 ± 12.5 years) and 5 with Barrett's esophagus (5 men, mean age 34.2 ± 9.8 years). The control group consisted of 10 healthy volunteers, matching with the age and gender of the patients and not having any endoscopic and clinical signs of GERD.

A clinical examination was carried out on all persons taking part in this study, which included the collection of complaints and anamnesis, identification of risk factors for GERD esophagogastroduodenoscopy and a 24-hour esophageal pH-impedance monitoring. Using the flow cytometry method (Beckman Coulter FC500, USA) were determined the levels of 7 cytokines: two anti-inflammatory - IL-4 and IL-10, three pro-inflammatory - IL-8, IFN- γ , and TNF- α , and two other cytokines - IL-2 and IL-6, which can demonstrate both anti-inflammatory as well as pro-inflammatory activity, depending on the circumstances (bivalent cytokines). Analysis of the data was carried out licensed software developer FlowCytomix Pro ver. 3.0. Since cytokines are biological mediators, the levels of which may be influenced by external and internal factors, we were very serious about the criteria of inclusion and exclusion of patients in the study. In particular, we excluded patients with comorbidities or with acute illnesses that occurred in the previous 14 days

before the study; patients with severe concomitant somatic diseases (cardiac, vascular, pulmonary, renal, pancreatic, hepatic, intestinal (inflammatory bowel disease - Crohn's disease and ulcerative colitis)), systemic connective tissue diseases. All patients with GERD received proton pump inhibitors previously (majority, pantoprazole), but 14 days prior to carrying out all research it was discontinued therapy with PPI.

The clinical part of this scientific work was carried out at the Clinic of Internal Disease Propaedeutics, Gastroenterology and Hepatology, First Moscow State Medical University. The laboratory part of the study (the determination of the level of circulating cytokines) was conducted at the department of pathophysiology, Moscow State University of Medicine and Dentistry, laboratory of cell biotechnology. The protocol for the study was approved by the local Ethics Committee. A written informed consent was received from all patients before their inclusion in the study.

The statistical analysis of the results was done using the statistical data processing program R (Bell Laboratories, USA). The qualitative characteristics were described using $M \pm SD$ (mean \pm standard deviation). To ensure the statistical significance of the differences between the groups, were used the Student's t-test for independent and dependent samples (normal character of distribution), Wilcoxon test (if different from the normal), Fisher's exact test for data count (uni or bilateral). Statistically significant difference was considered as the probability of at least 95% ($p < 0.05$). For the correlation of the analysis was used τ -Kendall correlation (ranking indicators). In this case, it was assumed if the module correlation: $|\tau| \leq 0.25$ - the correlation is weak; $0.25 < |\tau| < 0.75$ - the correlation is moderate; $|\tau| \geq 0.75$ - the correlation is strong.

4. Results

Table 1 summarizes the levels of cytokines in patients with various forms of GERD and healthy volunteers.

Table 1. The level of circulating cytokines in patients with NERD, erosive and ulcerative esophagitis and Barrett's esophagus

Cytokines	Nonerosive reflux disease	Erosive and ulcerative esophagitis	Barrett's esophagus	Healthy volunteers
IL-2, pg/mL	6.13 \pm 0.72	9.18 \pm 1.33	12.89 \pm 1.76	5.39 \pm 1.19
IL-4, pg/mL	4.48 \pm 0.39	7.71 \pm 3.16	16.29 \pm 1.44	2.52 \pm 0.32
IL-6, pg/mL	4.42 \pm 0.84	5.04 \pm 0.77	5.42 \pm 0.38	7.44 \pm 0.70
IL-8, pg/mL	7.58 \pm 0.36	18.10 \pm 0.97	3.62 \pm 0.37	2.49 \pm 0.31
IL-10, pg/mL	3.62 \pm 0.37	5.88 \pm 0.84	8.90 \pm 0.79	1.99 \pm 0.24
IFN- γ , pg/mL	34.17 \pm 28.32	76.12 \pm 81.83	13.77 \pm 12.23	24.81 \pm 10.88
TNF- α , pg/mL	8.11 \pm 0.39	16.38 \pm 1.29	7.20 \pm 0.30	2.06 \pm 0.28

The levels of the cytokine IL-2 were significantly higher in patients with Barrett's esophagus than in patients with NERD ($p=0.003$), erosive and ulcerative esophagitis ($p=0.016$), and healthy volunteers ($p=0.0007$). IL-2 is a bivalent cytokine which is able to exhibit both anti-as well as pro-inflammatory activity. In this study IL-2 has shown most probably its anti-inflammatory properties. The fact to be noted is that its levels positively correlated with level of two other anti-inflammatory cytokines - IL-4 ($\tau=0.81$) and IL-10 ($\tau=0.72$). In addition to IL-2, IL-6 is also a

bivalent cytokine, however, unlike IL-2, which showed anti-inflammatory activity, the levels of this cytokine was significantly higher in healthy volunteers than in group of patients with Barrett's esophagus ($p=0.0004$), with erosive and ulcerative lesions of the esophagus ($p < 0.0001$), and patients with NERD ($p < 0.0001$).

The level of anti-inflammatory cytokine IL-4 was significantly higher in patients with Barrett's esophagus than in healthy volunteers ($p=0.0005$), patients with NERD ($p=0.001$), and erosive and ulcerative esophagitis

($p < 0.0001$) (Figure 1A). It should be noted that the correlative analysis revealed moderate positive correlation ($\tau = 0.48$) between the levels of IL-4 and the total number of weakly alkaline reflux and weakly alkaline bolus

exposure ($\tau = 0.50$) (Figure 2). An interesting fact to be noted is that patients with Barrett's esophagus were more often diagnosed with weakly alkaline reflux than patients with NERD and erosive and ulcerative esophagitis ($p = 0.01$).

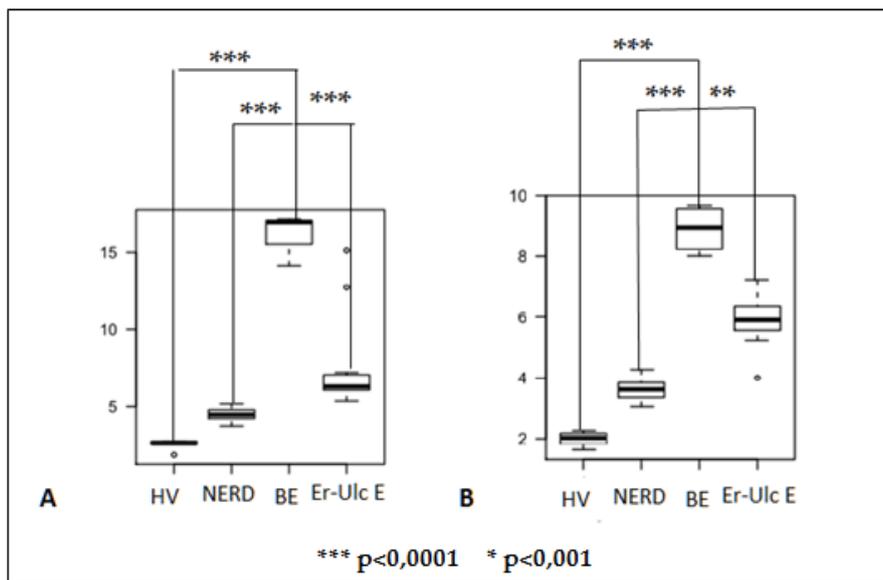


Figure 1. Levels of IL-4 (A) and IL-10 (B) in patients with different form of GERD

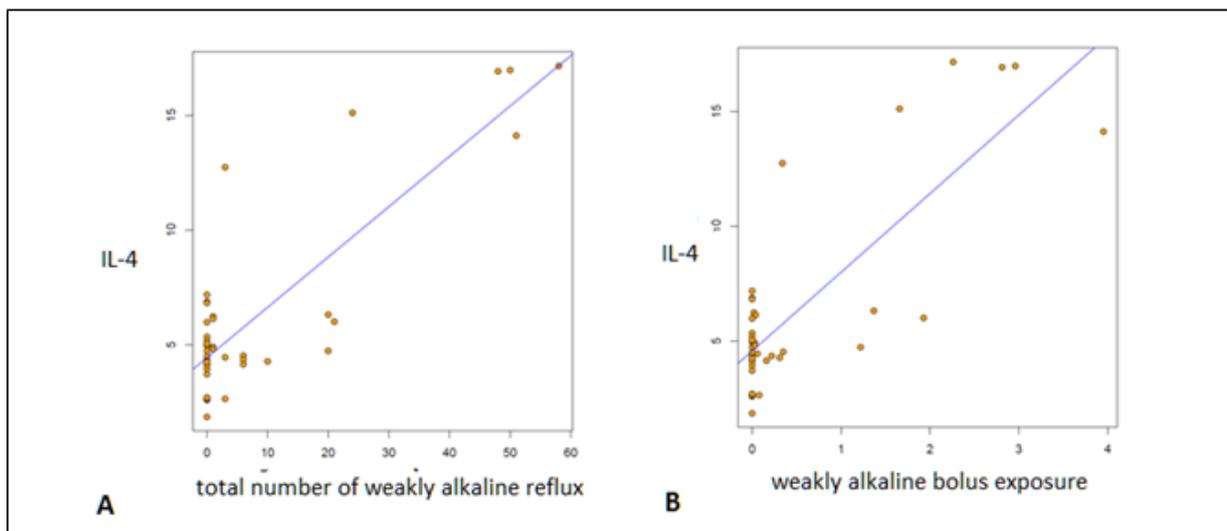


Figure 2. Correlations between the levels of IL-4 and the total number of weakly alkaline reflux (A) and with weakly alkaline bolus exposure (B)

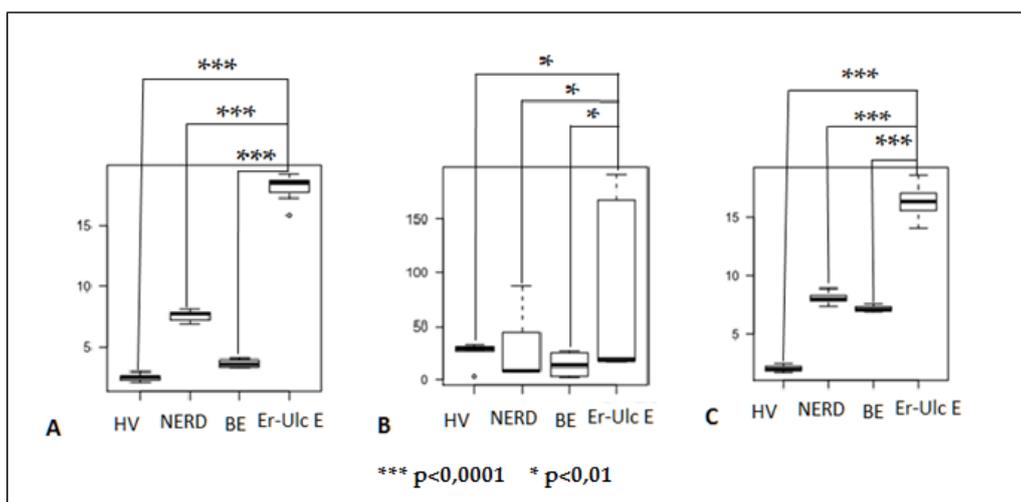


Figure 3. Levels of IL-8 (A), IFN- γ (B), and TNF- α (C) in patients with different forms of GERD

The levels of one of the key pro-inflammatory cytokines- IL-8 were significantly higher in patients with erosive and ulcerative esophagitis than in patients with NERD ($p<0.0001$), Barrett's esophagus ($p<0.0001$) and healthy volunteers ($p<0.0001$) (Figure 3A). We found a strong correlation ($\tau=0.76$) between the level of IL-8 and

the total quantity of acid reflux, and a moderate correlation between the level of IL-8 and acid bolus exposure ($\tau=0.42$) (Figure 4). Now it makes sense whilst taking into account the fact that in patients with erosive and ulcerative esophagitis, the quantity of acid reflux was significantly higher ($p<0.0001$) in comparison to the other groups.

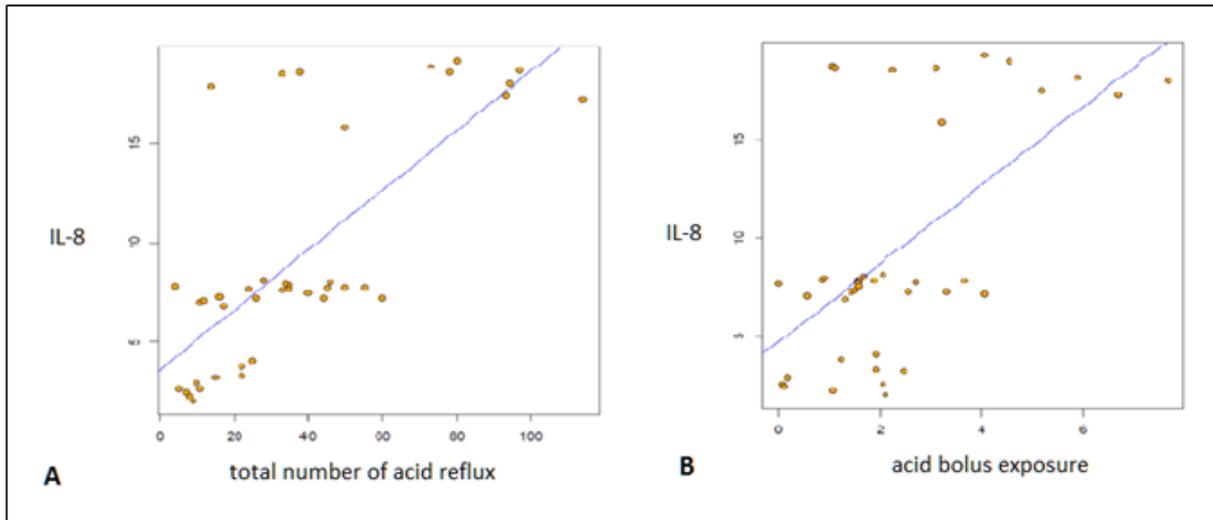


Figure 4. Correlations between the levels of IL-8 and the total number of acid reflux (A) and acid bolus exposure (B)

The analysis showed that the level of anti-inflammatory cytokine IL-10 was significantly higher in patients with Barrett's esophagus than in healthy volunteers ($p=0.0005$), patients with NERD ($p<0.0001$), and erosive and ulcerative esophagitis ($p=0.015$) (Figure 1B). The results of the analysis revealed a correlation between the levels of IL-10 and the total number of weakly alkaline reflux ($\tau=0.50$)

and weakly alkaline bolus exposure ($\tau=0.51$), which, as in the case with IL-4 confirms the relationship between Barrett's esophagus, the prevalence of weakly alkaline reflux and Th2 immune response in patients. This direct connection also confirms and reveals a strong correlation between the levels of the two major anti-inflammatory cytokines - IL-4 and IL-10 ($\tau=0.85$) (Figure 5).

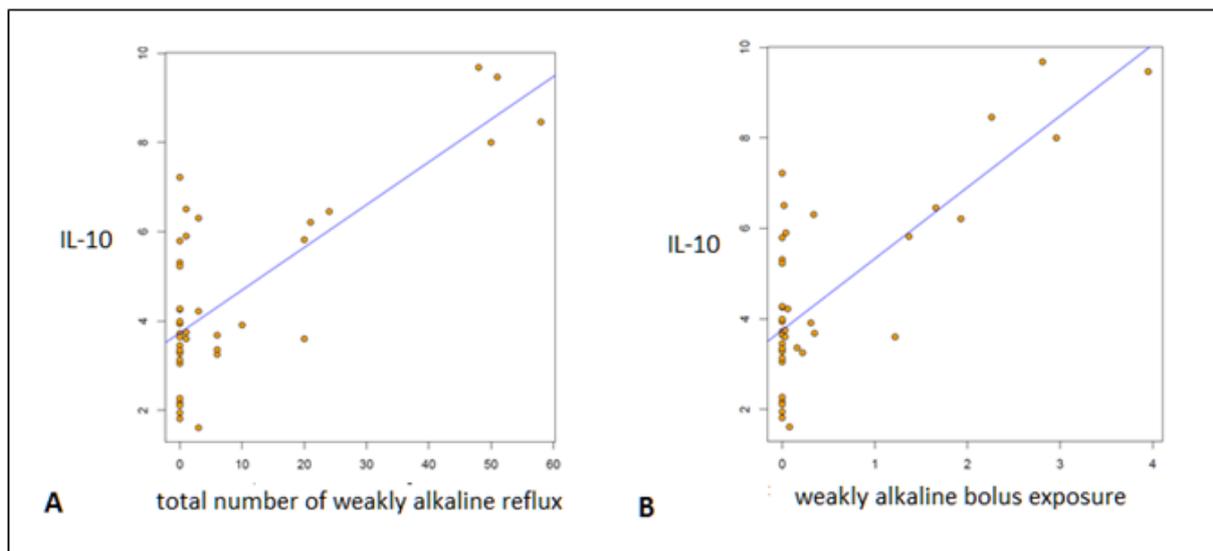


Figure 5. Correlations between the levels of IL-10 and the total number of weakly alkaline reflux (A) and weakly alkaline bolus exposure (B)

The level of IFN- γ was significantly higher in patients with erosive and ulcerative esophagitis than in patients with Barrett's esophagus ($p=0.03$) and healthy volunteers ($p=0.046$) (Figure 3 B).

The level of pro-inflammatory cytokine TNF- α , as shown in the analysis, was significantly higher in patients with erosive and ulcerative lesions of the esophagus than with the control group ($p<0.0001$), patients with NERD ($p<0.0001$), and those who had Barrett's esophagus ($p<0.0001$) (Figure 3 B). The analysis revealed a positive

correlation ($\tau=0.69$) between the level of TNF- α and the total number of acid reflux and acid bolus exposure ($\tau=0.48$), which, as noted earlier, was significantly higher in patients with erosive and ulcerative esophagitis. And, given the fact that the levels of TNF- α and IL-8 correlate with each other ($\tau=0.88$), which in turn is graphically confirmed by a positive association between the prevalence of pro-inflammatory response in these patients and increase in the quantity of acid reflux (Figure 6).

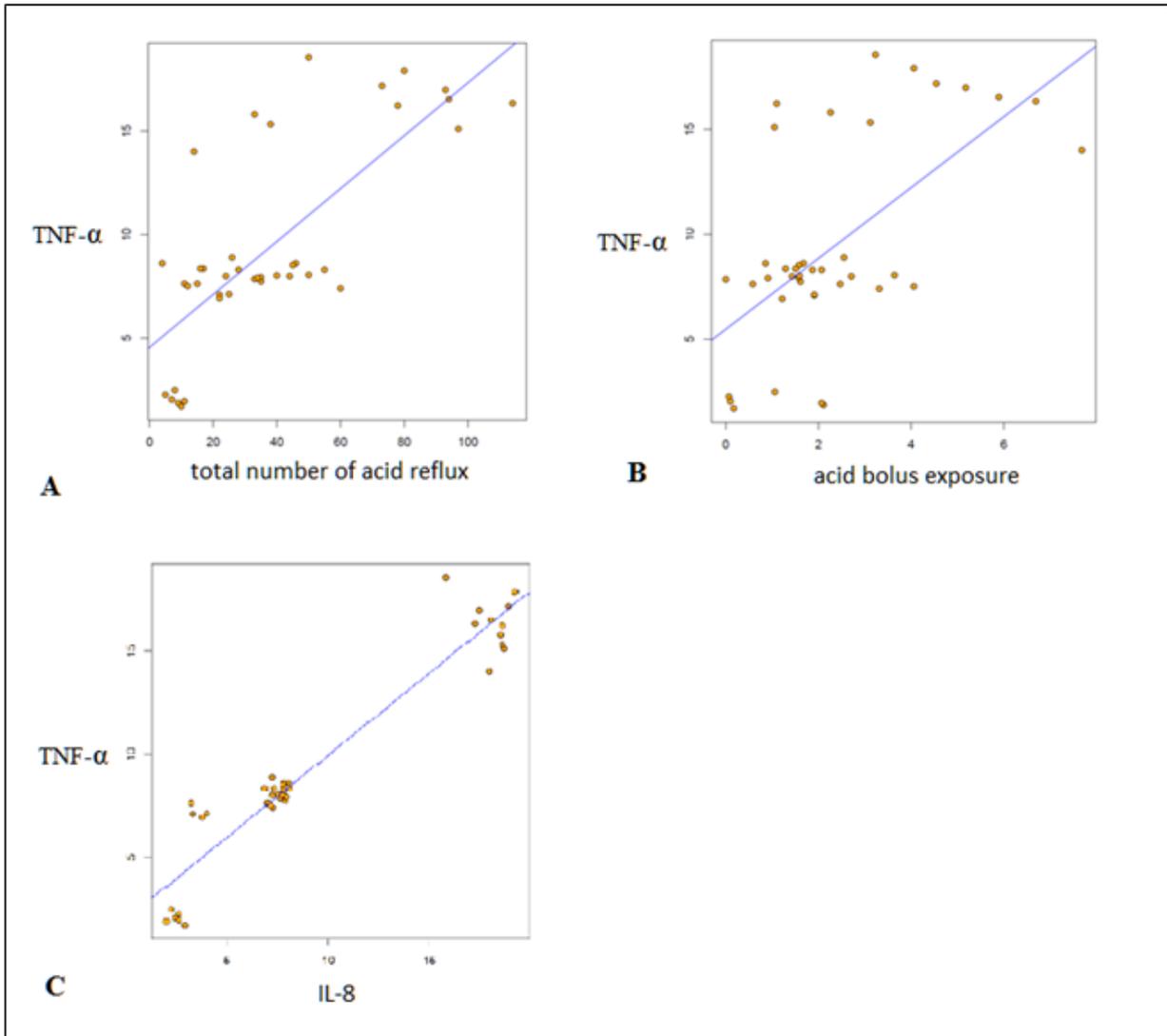


Figure 6. Correlations between the levels of TNF- α and the total number of acid reflux (A), acid bolus exposure (B), and the levels of IL-8 (C)

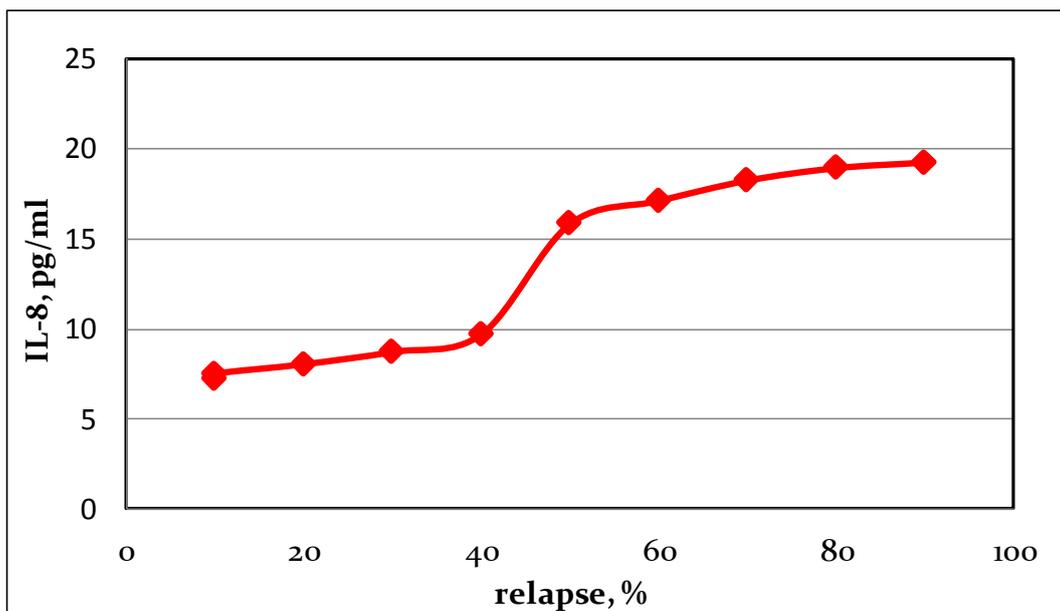


Figure 7. Association between the level of IL-8 and relapse of erosive esophagitis within 2 years

On comparing the cytokine markers with the clinical picture of the disease, a very peculiar point could be noted, there was a significant correlation between high levels of IL-8 and the frequency of relapse of erosive esophagitis,

despite the ongoing therapy for 2 years. The analysis showed that with increased levels of IL-8 there was an increased frequency of recurrence of erosive esophageal mucosal lesions ($p < 0.001$). Thus, when the concentration of IL-8 was 5 to 10 pg/mL, recurrence was observed in 10-40% of cases, whereas at a level of IL-8 was 15 to 20 pg/mL, the recurrence rate was 80-90% (Figure 7).

5. Discussion

The purpose of this present study was to investigate the systemic immune response in patients with GERD. For this, we determined the serum concentrations of cytokines: pro-inflammatory (IL-8, IFN- γ , TNF- α), anti-inflammatory (IL-4, IL-10), and bivalent (IL-2, IL-6). The analysis showed that in patients with erosive and ulcerative lesions of the esophagus, compared to patients with Barrett's esophagus and NERD, there prevails a production of pro-inflammatory cytokines (IL-8, IFN- γ and TNF- α), that indicates the development of Th1 immune response in these patients.

In contrast, in patients with Barrett's esophagus, when compared to patients with erosive and ulcerative esophagitis, NERD and healthy people, there was significant higher production of both the anti-inflammatory cytokines (IL-4 and IL-10) which in turn indicates the prevalence of Th2 immune response in these patients with Barrett's esophagus.

Performing a correlation of the analysis revealed a relationship between the systemic immune response in patients with GERD and the 24-hour intraluminal impedance pH monitoring. It was found that the level of pro-inflammatory cytokines (IL-8 and TNF- α) correlated with the total number of acid reflux and acid bolus exposure, whereas the level of anti-inflammatory cytokines (IL-4 and IL-10) correlated with total weakly alkaline reflux and weakly alkaline bolus exposure. On referring the literatures, we did not find any new information regarding scientific works which focused on the comparison of the expression of cytokines, indicators of the 24-hour esophageal pH-impedance monitoring and various forms of GERD. Thus, we first carried out a correlative analysis between the data and identified the above correlation.

The study found a relationship between the levels of the pro-inflammatory cytokine IL-8 and the frequency of recurrence of erosive esophagitis. It has been shown that an increase in serum IL-8 causes an increase in the frequency of relapse of erosive esophagitis, despite the prescribed therapy for 2 years. On referring the literature we found only one study in which it was shown that in patients with high levels of IL-8 (measured in the mucosa of the esophagus), there occurred a recurrence of erosive esophagitis within 3 years [10]. Thus, on basis of the results of the present study, it was first demonstrated that IL-8 which being determined in the systemic circulation, could be used as a marker for predetermining the progress of GERD.

6. Conclusion

1. There is the dependence between the levels of cytokines and the different forms of GERD. In patients with erosive and ulcerative esophagitis in comparison with patients with NERD and Barrett's esophagus, there is a predomination in the production of pro-inflammatory cytokines such as IL-8, IFN- γ and TNF- α , indicating the development of Th1 immune response. Whereas in patients with Barrett's esophagus, there was an increased expression of anti-inflammatory cytokines such as IL-4 and IL-10, indicating the formation of the Th2 immune response.
2. High level of IL-8 is associated with an increased incidence of recurrence of erosive esophagitis, despite ongoing therapy for 2 years.
3. The levels of TNF- α and IL-8 correlate with the total number of acid reflux and acid bolus exposure, whereas the levels of IL-4 and IL-10 correlate with the total number of weakly alkaline reflux and weakly alkaline bolus exposure.

References

- [1] El-Serag H.B., Ergun G.A., Pandolfino J., Fitzgerald S., Tran T., Kramer J.R. Obesity increases oesophageal acid exposure // *Gut*. - 2007. Vol.56. p. 749-755.
- [2] Ivashkin V.T., Trukhmanov A.S. Diagnosis and treatment of gastroesophageal reflux disease. Manual for physicians. 2010.
- [3] Lazebnik L.B., Bordin D. S., Masharova A.A., et al. «Restoring the quality of life and preventing heartburn elimination alginate»: results of a multicenter study VIA APIA // *Experimental and Clinical Gastroenterology*. 2010. №6. p. 70-76.
- [4] Maev I.V., Vyuchkova E. S., Chekina M.I. Gastroesophageal reflux disease - a disease of the XXI century // *The Physician*. 2004. №4. - p. 10-14.
- [5] Trukhmanov A.S. Gastroesophageal reflux disease: clinical options, prognosis, treatment: the thesis on scientific degree of Doctor of Medical Sciences.- 2008.
- [6] Evsyutina Yu.V., Trukhmanov A.S. Lack of response to therapy with proton pump inhibitors: the causes and tactics of patients // *Ther archive* 2015. 2. p. 85-89.
- [7] Bytzer P., van Zanten S.V., Mattsson H., Wernersson B. Partial symptomresponse to proton pump inhibitors in patients with nonerosive reflux disease or reflux oesophagitis - a post hoc analysis of 5796 patients // *Aliment Pharmacol Ther*. 2012. Vol.36. p. 635-643.
- [8] Cicala M., Emerenziani S., Guarino M.P., Ribolsi M. Proton pump inhibitor resistance, the real challenge in gastro-esophageal reflux disease // *World J Gastroenterol*. 2013. Vol.19. № 39. - p. 6529-6535.
- [9] Bulgakov S.A. The phenomenon of nocturnal acid breakthrough during treatment with proton pump inhibitors and therapeutic correction // *Pharmateka*. 2012. № 13. p. 62-66.
- [10] Isomoto H., Inoue K., Kohno S. Interleukin-8 levels in esophageal mucosa and long-term clinical outcome of patients with reflux esophagitis // *Scand J Gastroenterol*. 2007. Vol.42. p. 410-411.
- [11] Kohata Y., Fujiwara Y., Machida H., Okazaki H., Yamagami H., Tanigawa T, Watanabe K., Watanabe T., Tominaga K., Wei M., Wanibuchi H., Arakawa T. Role of Th-2 cytokines in the development of Barrett's esophagus in rats // *J Gastroenterol*. 2011. Vol.46. №7. p. 883-893.
- [12] Zhong Y.Q., Lin Y., Xu Z. Expression of IFN- γ and IL-4 in the esophageal mucosa of patients with reflux esophagitis and Barrett's esophagus and their relationship with endoscopic and histologic grading // *Dig Dis Sci*. 2011. Vol.56. №10. p. 2865-2870.