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Macrophage phenotype after human refluxate exposure, esophageal dysmotility and their correlation with gastroesophageal reflux disease

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Aim of the study. To investigate the esophageal dysmotility, changes in the esophageal mucosa and the immune response depending on the type of refluxate in gastroesophageal reflux disease (GERD) patients.
Material and methods. 68 patients with GERD were recruited: 28 (14 men; mean age, 45.74 ± 2.23 years) non-erosive reflux disease (NERD), 22 (15 men; mean age, 45.0 ± 3.24 years) erosive reflux disease (EE), 18 (13 men; mean age, 47.22 ± 2.95) Barrett's esophagus (BE). GERD patients underwent esophageal high-resolution manometry (HRM) with a 22-channel water-perfused catheter and Solar GI system (Medical Measurements Systems, Enschede, the Netherlands), 24-hour impedance and pH monitoring using the Ohmega Ambulatory Impedance pH Recorder (Medical Measurements Systems). We analyzed receptor characteristics of monocyte-derived macrophages in all groups of patients.
Results. On HRM examination, we showed that DCI (distal contractile integral) in NERD patients was higher than in EE ($p = 0.088$) and BE ($p = 0.076$), also LES RP (lower esophageal sphincter resting pressure) in NERD patients was higher than in EE ($p = 0.039$) and BE ($p = 0.012$). The analysis of reflux characteristics showed that the total reflux time with pH < 4 for BE patients was longer than that for NERD and EE patients. An analysis of receptor characteristics of monocyte-derived macrophages showed the prevalence of CD25 and CD80 expression in all groups of patients.
Conclusion. An analysis of the phenotype of macrophages derived from blood monocytes of GERD patients revealed a prevalence of M1 macrophages that was typical for the Th1 type of immune response. The degree of esophageal dysmotility was correlated with GERD severity and type.
Keywords: gastroesophageal reflux disease, immune response, blood monocytes, esophageal dysmotility
Conflict of interests. The authors declare no conflict of interest.

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Корреляции изменений фенотипа макрофагов крови после воздействия на них гастроэзофагеального рефлюктата и нарушений моторики пищевода с формой гастроэзофагеальной рефлюксной болезни

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Цель. Исследовать двигательную функцию пищевода, изменения слизистой оболочки пищевода и иммунный ответ в зависимости от характера рефлюктата у пациентов с гастроэзофагеальной рефлюксной болезнью (ГЭРБ).

Материалы и методы. Были обследованы 68 пациентов с ГЭРБ: 28 с неэрозивной рефлюксной болезнью (НЭРБ), 22 пациента с эрозивной рефлюксной болезнью (ЭЭ) и 18 пациентов с пищеводом Баррета (ПБ). Всем пациентам, включенным в исследование, была выполнена манометрия пищевода высокого разрешения 22-канальным водно-перфузионным катетером (Medical Measurements Systems, Enschede, the Netherlands), 24-часовая pH-импедансометрия (The Ohmega Ambulatory Impedance pH Recorder Medical Measurements Systems). Во всех группах пациентов мы оценили фенотип макрофагов крови после выделения их из моноцитов.
Результаты. В ходе анализа результатов манометрии пищевода высокого разрешения интегральная сократимость дистального сегмента пищевода у пациентов с НЭРБ была выше, чем у пациентов с ЭЭ (p = 0,088) и ПБ (p = 0,076). Давление покоя нижнего пищеводного сфинктера в группе пациентов с НЭРБ также было выше, чем у пациентов с ЭЭ (p = 0,039) и ПБ (p = 0,012). Процент времени с pH < 4 единиц у пациентов с ПБ был больше, чем у пациентов с НЭРБ и ЭЭ. Анализ CD поверхностных рецепторов макрофагов показал преобладание CD25 и CD80 поверхностных рецепторов у пациентов с ГЭРБ.
Выводы. Анализ фенотипа макрофагов крови пациентов показал преобладание поверхностных рецепторов M1 макрофагов, характерных для провоспалительного Th1 типа иммунного ответа. Нарушения двигательной функции пищевода были более выражены у больных с ПБ, чем у пациентов с НЭРБ и ЭЭ.
Ключевые слова: гастроэзофагеальная рефлюксная болезнь, иммунный ответ, моноциты крови, нарушение двигательной функции пищевода
Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

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Introduction

Gastroesophageal reflux disease (GERD) symptoms can be identified in 10 % to 20 % in the adult population in the Western countries and less than 5 % in Asia [1], and endoscopic findings of GERD have been observed in more than 10 % of all patients who have undergone upper gastrointestinal endoscopy [2–4]. Unfortunately, observational studies have shown that the overall prevalence of partial- and nonresponse to proton pump inhibitor (PPI) therapy was 45 % [5, 6]. The disease rather progresses with an increase in erosion size, ulceration, and development of esophageal complications such as peptic stricture, bleeding and Barrett's esophagus. There are many mechanisms involved in the pathogenesis of GERD, including impaired resting pressure (RP) of the lower esophageal sphincter (LES; LES RP), hiatal hernia, disturbed esophageal clearance, and gastric dysmotility [7–10]. Therefore, high-resolution manometry (HRM) is an essential diagnostic test for determining esophageal motor function disorders [11]. These problems are arousing increased interest, especially considering the findings from studies of the immune response in patients with GERD.

Cytokines are produced mainly by macrophages. Macrophages can change their phenotype under the influence of various factors. They produce inflammatory and anti-inflammatory cytokines, as well as growth factors and reactive oxygen species [12].

GERD is characterized by the impaired immune response manifesting as an imbalance of the cellular (Th1) and humoral (Th2) immunity, which may be caused by the difference in macrophage phenotypes: M1 (pro-inflammatory) or M2 (anti-inflammatory). M1 macrophages arise following stimulation with IFN-γ and M2 macrophages are stimulated by IL-4 or IL-13 [13–15].

Previous studies have found that activation of the Th1-mediated immune response leads to erosive GERD, while activation of the Th2-mediated immune response can cause Barrett's esophagus [16, 17].

Recently, refractory GERD at the tissue and cellular levels has become the subject of increased interest, and the possible effects of the immune response to GERD are being actively discussed [18]. Increased secretions of interleukin (IL)-1β [19], IL-8, tumor necrosis factor (TNF), and IL-10 have been observed in patients with GERD. Furthermore, experimental studies have shown that Th2-type immune response cytokine (IL-4, IL-10, IL-13) levels are significantly higher in BE patients [19]. However, no difference in pro-inflammatory cytokine levels has been observed. The most recent studies of BE have shown that it is characterized by IL-4 secretion and activation of the Th2-type immune response [18, 20, 21]. IL-4 induces metaplasia of esophageal squamous epithelial cells; therefore, IL-4 causes the development of BE in GERD patients [20].

According to published data, the type of esophageal damage and the response of the mucous membrane largely depend on the type of refluxate, as well as the patient's immune profile. Th2-immune response was shown to cause Barrett's esophagus and decreased acidity and the alkaline duodenal content in the esophagus may increase risk of this condition [22].

We hypothesized that different types of refluxate may in some way have a different effect on the macrophage phenotype, thus affecting the immune profile that determines the type of GERD. This study was aimed to investigate the damage to the mucous

membrane of the esophagus depending on the type of refluxate.

The study was conducted to assess the role of motor dysfunction and the burden of on immune cells caused by various types of refluxates. It has the potential to contribute to the future investigation of prokinetics use in GERD and to refine the treatment strategies for GERD.

Methods

Data Sources

This prospective, experimental study was conducted at Sechenov First Moscow State Medical University (Sechenov University) and Yevdokimov Moscow State University of Medicine and Dentistry. Clinical study protocol was approved by the local ethics committee of Sechenov University version 2.0 from 10.02.2016 and posted on ClinicalTrials.gov with study identifier NCT02699060 before enrollment of patients.

The following inclusion criteria in the study were identified:

1. Signed informed consent.
2. Male and female patients from 18 to 65 year old.
3. Clinically and/or endoscopically confirmed diagnosis of GERD.

Exclusion criteria were as follows:

1. Treatments with PPIs and/or H2RA were stopped at least 1 week prior to study inclusion.
2. Female patients who was pregnant or planning to become pregnant or lactating.
3. Any acute diseases or conditions, exacerbations of concomitant chronic diseases at the inclusion phase of the study.
4. Participation in a clinical trial in the past 3 months.
5. Any condition which, in the opinion of investigator, makes the patient unsuitable for participation in the study.

Patients with non-erosive gastroesophageal reflux disease (NERD), erosive esophagitis (EE) and Barrett's esophagus (BE) were enrolled. Patients were classified based on clinical evidence and the results of an upper gastrointestinal endoscopy. Written, informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

NERD: GERD patients with minimal mucosal changes by endoscopic examination. The changes included vascular injection or vascular spots above the Z-line. When the impedance-pH monitoring recordings were analyzed the symptom index (SI) and the symptom association probability (SAP) (heartburn) resulted in a positive SI and SAP in 28 patients with NERD during our study.

BE: patients with long segment (>3 cm) and short segments (<3 cm) of the epithelial column located between the upper border of the gastric folds and the proximal part of the Z line and the presence of intestinal metaplasia was histopathologically confirmed in biopsies of the Barrett epithelium segment.

EE: Patients with GERD symptoms with erosions or disruptions of the esophageal mucosa of different degrees by Los Angeles Classification as follows: Grade A, one (or more) mucosal break less than 5 mm that does not extend between the tops of two mucosal folds; Grade B, one (or more) mucosal break greater than 5 mm long that does not extend between the tops of two mucosal folds; Grade C, one (or more) mucosal break that is continuous between the tops of two or more mucosal folds but involves less than 75 % of the circumference; and Grade D, one (or more) mucosal break that involves at least 75 % of esophageal circumference [8].

Frequency Scale for the Symptoms of GERD (FSSG)

The intensity of gastroesophageal reflux symptoms was assessed using the Frequency Scale for the Symptoms of GERD (FSSG) questionnaire, in which a score of 4 indicated the highest frequency of symptoms and a score of 0 indicated an absence of symptoms. The FSSG questionnaire consists of 12 questions related to 7 acid reflux symptoms and 5 dysmotility-like symptoms, was used to assess the GERD symptoms [23].

Blood sampling

We determined type of Th-immune response by analyzing blood monocyte/macrophage phenotype in patients with different forms of GERD. Monocytes were isolated from the patients' blood samples and cultured to macrophages in standard conditions: RPMI1640 medium, 10 % FBS, 37 °C, 5 % CO_2. Pooled analysis of macrophage and monocyte derived macrophages included typical M1/M2 surface macrophage CD markers (CD25, CD80 and CD163, CD206) performed by flow cytometry (Beckman Coulter FC500). CD25, CD80 (M1 markers) and CD163, CD206 (M2 markers) are receptor characteristics Th1 and Th2 immune response, respectively.

Endoscopy and biopsy

All the patients underwent upper gastrointestinal endoscopy to confirm and grade the type of the disease (NERD, EE, BE). The examination included analysis of esophageal mucosa, expression of inflammatory changes, location, size, number of mucosal defects, as well as the appearance of the gastric and duodenal mucosa. Biopsy was performed of the four quadrants at each 2 cm and 2 cm above the Z-line [24, 25]. Microscopic features were based on hematoxylin and eosin (H&E) staining (original magnification x40).

Esophageal high-resolution manometry and esophageal pH monitoring

GERD patients underwent esophageal high-resolution manometry (HRM) with a 22-channel water-perfused catheter and Solar GI system (Medical Measurements Systems, Enschede, the Netherlands) and 24-hour impedance and pH monitoring using the Ohmega Ambulatory Impedance pH Recorder (Medical Measurements Systems). The 24-hour impedance and pH monitoring recording were uploaded onto a portable storage card and for computer-assisted manual analysis, using a specialized software program.

In GERD patients forms we analyzed following HRM parameters: the distal contractile integral (DCI) and the lower esophageal sphincter resting pressure (LES RP). Standard measuring parameters were collected: percentage of total time when pH was <4, longest reflux event, number of reflux events longer than 5 minutes, and number of reflux episodes in 24 hours. Reflux episodes were classified as acid, weakly acidic and weakly alkaline reflux. Heartburn was considered related to a reflux episode if they occurred after/within 2 minutes or during of a reflux episode.

Statistical analysis

For statistical analysis, we used descriptive statistics for numerical variables and frequencies, $p < 0.05$ was considered to be statistically significant. The analysis was performed by using SPSS-17.0 statistical analysis software (SPSS Inc., Chicago, IL, USA).

Results

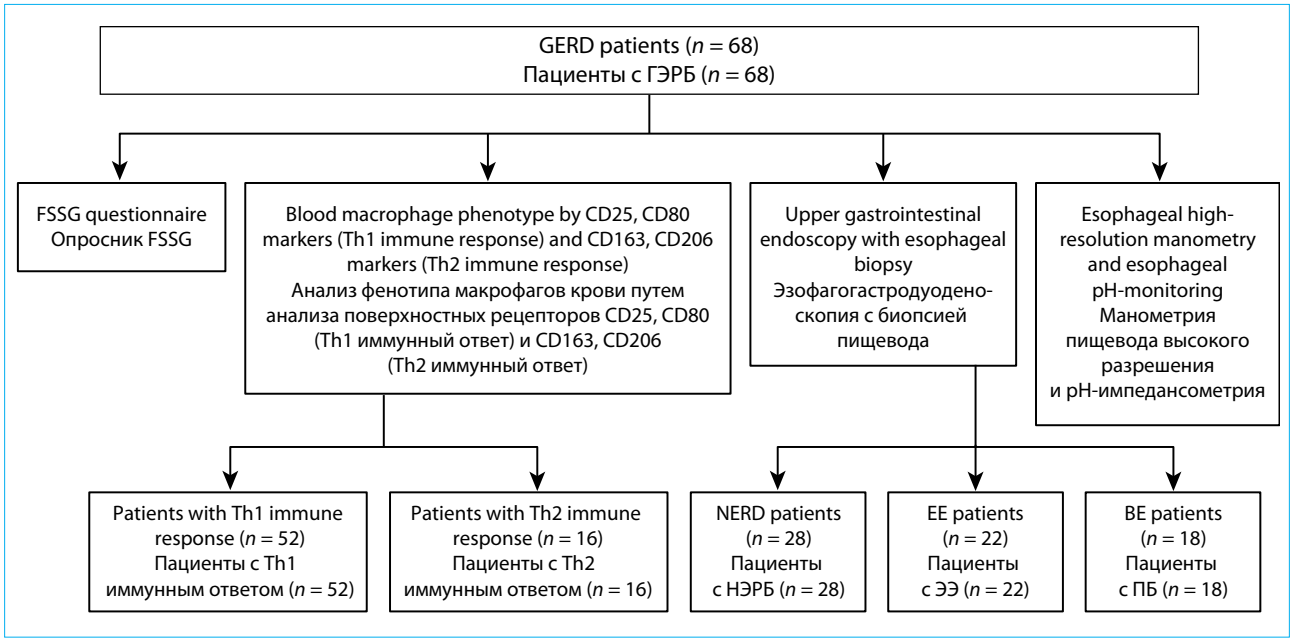
28 NERD patients (14 men; mean age, 45.74 ± 2.23 years), 22 EE patients (15 men; mean age, 45.0 ± 3.24 years) and 18 BE patients (13 men; mean age, 47.22 ± 2.95) were recruited at the V.H. Vasilenko Clinic of Internal Diseases Propaedeutics, Gastroenterology and Hepatology of Sechenov University (Figure 1).

FSSG questionnaire results

The FSSG questionnaire was used to assess GERD symptoms in this study. The total FSSG scores of the patients ranged between 1 and 21 points. 61 % of NERD patients had prevalent symptoms associated with acid reflux and 39 % of NERD patients had prevalent symptoms related to dysmotility. 27 % of EE patients had prevalent symptoms associated with acid reflux and 73 % of EE patients had symptoms related to dysmotility. 33 % of BE patients had prevalent symptoms associated with acid reflux and 67 % of BE patients had symptoms related to dysmotility.

Esophageal HRM

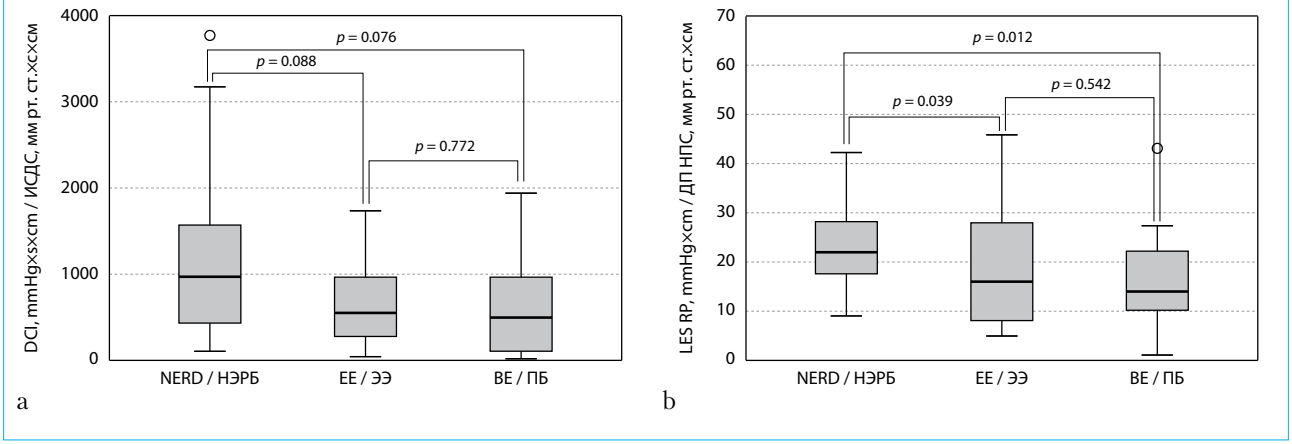
On HRM examination, we showed that DCI in NERD patients was higher than in EE ($p = 0.088$) and BE ($p = 0.076$), also LES RP in NERD patients was higher than in EE ($p = 0.039$) and BE ($p = 0.012$) (Figure 2). For NERD patients, the DCI



GER (ГЭР) — gastroesophageal refluxate (гастроэзофагеальный рефлюктат); GERD (ГЭРБ) — gastroesophageal reflux disease (гастроэзофагеальная рефлюксная болезнь); NERD (НЭРБ) — non-erosive reflux disease (неэрозивная рефлюксная болезнь); EE (ЭЭ) — erosive esophagitis (эрозивный эзофагит); BE (ПБ) — Barrett's esophagus (пищевод Баррета)

Fig. 1. Scheme of experimental study and patients' distribution

Рис. 1. Схема экспериментального исследования и распределения пациентов

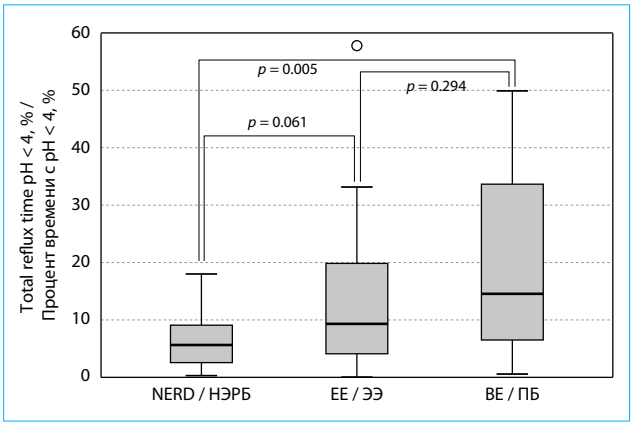


NERD, non-erosive reflux disease (неэрозивная рефлюксная болезнь); EE, erosive reflux esophagitis (эрозивный рефлюкс-эзофагит); BE, Barrett's esophagus (пищевод Баррета),
a) DCI, distal contractile integral (ИСДС, интегральная сократимость дистального сегмента), mmHg×s×cm
b) LES RP, lower esophageal sphincter resting pressure (ДП НПС, давление покоя нижнего пищеводного сфинктера), mmHg
DCI and LES RP for BE patients were less than that for NERD and EE patients.
ИСДС и ДП НПС у пациентов с ПБ были ниже, чем у пациентов с НЭРБ и ЭЭ.
Difference the LES RP for NERD patients and EE patients and BE patients was significant.
ДП НПС у пациентов с НЭРБ статистически значимо отличалось по сравнению с ДП НПС у пациентов с ЭЭ и ПБ.

Fig. 2. HRM parameters in patients with GERD

Рис. 2. Параметры МВР у пациентов с ГЭРБ

was 1140.7 mmHg×s×cm (104.0–3759.0); the DCI values were 695.7 mmHg×s×cm (41.0–2462.0) for EE patients and 682.0 mmHg×s×cm (14.0–1933.0) for BE patients. The LES RP for NERD patients was 23.48 mmHg (9.0–42.0); the LES RP values were 18.36 mmHg (5.0–45.5) for EE patients and 15.7 mmHg (1.0–43.0) for BE patients. Therefore, significant difference was observed between the LES RP for NERD patients and EE patients and BE patients.



NERD, non-erosive reflux disease (неэрозивная рефлюксная болезнь); EE, erosive reflux esophagitis эрозивный рефлюкс-эзофагит; BE, Barrett's esophagus (пищевод Барретa); total reflux time with pH <4, % (процент времени с pH<4)
The total reflux time with pH <4 for BE patients was longer than that for NERD and EE patients
Процент времени с pH<4 у пациентов с ПБ был больше, чем у пациентов с НЭРБ и ЭЭ

Fig. 3. The total reflux time with pH<4 % for GERD patients

Рис. 3. % времени с pH<4 в пищеводе у пациентов с различными формами ГЭРБ

Twenty-four hour pH-impedance Metrics

The total reflux time, the number of reflux episodes, the number of long reflux periods > 5 minutes, the longest reflux period were analyzed by 24-hour impedance and pH monitoring (Tables 1, 2). The AET of NERD patients was lower than that of EE and BE patients. As for the reflux episodes, NERD patients had a significantly decreased number of acid reflux episodes when compared with EE and BE patients. The minimal change in NERD patients may be due to the fact that PPI treatment was stopped at 1 week prior to study inclusion.

The analysis of reflux characteristics showed that the total reflux time with pH <4 for BE patients was longer than that for NERD and EE patients (Figure 3).

Morphological characteristics of esophagus

Neutrophilic and eosinophilic infiltration of the esophageal mucosa and submucosa and dilated intercellular spaces were observed in all GERD patients. Furthermore, neutrophilic infiltration and eosinophilic infiltration of the esophageal mucosa and submucosa were more commonly observed in NERD patients than in EE patients. Some EE patients had gastric metaplasia. BE patients had intestinal metaplasia and low-grade dysplasia of the esophageal mucosa.

Table 1. Main parameters of 24-hour esophageal pH monitoring for patients with different forms of GERD

Таблица 1. Основные параметры 24-часовой pH-импедансометрии пищевода у пациентов с различными формами ГЭРБ

Types of GERD patients Группы пациентов с различными формами ГЭРБ	Total reflux time <4 (%) % времени с pH менее 4	Median scores of reflux episodes Медиана числа эпизодов гастроэзофагеальных рефлюксов	Median scores of long reflux periods >5 minutes Медиана числа эпизодов ГЭР продолжительностью более 5 минут	Median scores of the longest GER, minutes Медиана самого длительного ГЭР, минуты
NERD patients Пациенты с НЭРБ	5.6 (0.4; 18.0)	39.0 (8.0; 90.0)	4.0 (0.0; 14.0)	13.0 (1.0; 99.0)
EE patients Пациенты с ЭЭ	11.25 (0.0; 57.8)	66.0 (2.0; 361.0)	5.0 (0.0; 30.0)	30.5 (0.8; 11.6)
BE patients Пациенты с ПБ	14.5 (0.6; 49.9)	81 (12.0; 211.0)	8.0 (0.0; 29.0)	39.0 (3.0; 350.0)

NERD — non-erosive reflux disease (неэрозивная рефлюксная болезнь); EE — erosive reflux esophagitis (эрозивный рефлюкс-эзофагит); BE — Barrett's esophagus (пищевод Баррета); AET — acid exposure time <4 % (процент времени с pH менее 4); GER — gastroesophageal reflux (гастроэзофагеальный рефлюкс).

Table 2. Assessment of the type of gastroesophageal refluxate in GERD patients

Таблица 2. Оценка характера гастроэзофагеального рефлюктата у пациентов с ГЭРБ

Types of GERD patients Группы пациентов с различными формами ГЭРБ	Median score of weakly acid reflux episodes Медиана количества слабокислых гастроэзофагеальных рефлюксов	Median score of acid reflux episodes Медиана количества кислых гастроэзофагеальных рефлюксов	Median score of non-acid reflux episodes Медиана количества слабощелочных гастроэзофагеальных рефлюксов
NERD patients Пациенты с НЭРБ	53.1 (28.8–99.0)	33.6 (0.0–79.0)	0.0 (0.0–2.0)
EE patients Пациенты с ЭЭ	34.4 (12.0–1000.0)	42.8 (2.0–111.0)	0.2 (0.0–2.0)
BE patients Пациенты с ПБ	31.8 (4.0–100.0)	58.6 (7.0–279.0)	0.2 (0.0–2.0)

NERD — non-erosive reflux disease (неэрозивная рефлюксная болезнь); EE — erosive reflux esophagitis (эрозивный рефлюкс-эзофагит); BE — Barrett's esophagus (пищевод Баррета); GER — gastroesophageal reflux (гастроэзофагеальный рефлюкс).
Patients with NERD predominantly had weakly acid GER, whereas EE and BE patients had predominantly acid GER.
У пациентов с НЭРБ преобладали слабокислые ГЭР, по сравнению с пациентами с ЭЭ и ПБ, у которых преобладали кислые ГЭР.

Blood sampling (monocytes/macrophages)

An analysis of receptor characteristics of monocyte-derived macrophages showed the prevalence of CD25 and CD80 expression in all groups of patients, which indicated M1 macrophages. For NERD patients, the median expression levels of surface M1/M2 macrophage CD markers were as follows: CD25, 39.4 (6.1–69.4) mg/l; CD80, 19.6 (2.8–49.3) mg/l; CD163, 15.7 (2.7–37.2) mg/l; and CD206, 10.5 (0.4–21.6) mg/l. For EE patients, the median expression levels of surface M1/M2 macrophage CD markers were as follows: CD25, 34.7 (13.1–59.6) mg/l; CD80, 22.8 (6.1–43.6) mg/l; CD163, 17.4 (0.6–37.2) mg/l; and CD206, 12.6 (0.3–30.6) mg/l. For BE patients, the median expression levels of surface M1/M2 macrophage CD markers were as follows: CD25, 39.2 (16.3–67.0) mg/l; CD80, 21.9 (8.3–46.1) mg/l; CD163, 14.9 (1.8–34.0) mg/l; and CD206, 8.9 (0.5–25.9) mg/l. The prevalence of CD25 and CD80 expression characterized the prevalence Th1 immune response (Table 3).

Discussion

We studied the esophageal dysmotility and type of immune response of GERD patients. In our study, we showed the prevalence of esophageal exposure to acid GER in patients with EE and BE, and the prevalence of weakly acidic GER in NERD development. We also demonstrated that all GERD patients had esophageal motor disorders. The progression of esophageal dysmotility leads to a prolonged exposure to reflux of the esophageal mucosa. BE patients had a greater prevalence of esophageal dysmotility than EE and NERD patients.

Table 3. Type of immune response by CD markers expression by blood samples in GERD patients
Таблица 3. Тип иммунного ответа у пациентов с ГЭРБ, определяемый путем анализа экспрессии CD маркеров макрофагов крови

Type of immune response Тип иммунного ответа	Number of GERD patients with CD25 and CD80 markers expression typical for Th1 immune response and CD163 and CD206 markers expression typical for Th2 immune response Число пациентов с ГЭРБ с экспрессией CD25 и CD80, характерных для Th1 типа иммунного ответа, и CD163 и CD206, характерных для Th2 типа иммунного ответа		
	NERD	EE	BE
Th1 immune response Th1 тип иммунного ответа	20	17	15
Th2 immune response Th2 тип иммунного ответа	8	5	3

NERD — non-erosive reflux disease (неэрозивная рефлюксная болезнь); EE — erosive reflux esophagitis (эрозивный эзофагит); BE — Barrett's esophagus (пищевод Баррета).

Patients with GERD may have not only acid refluxate, but also weakly acid and non-acid refluxate. In our study, we demonstrated that NERD patients had a significantly increased number of weakly acid reflux episodes when compared with EE and BE patients. On HRM examination, we showed that DCI and LES RP in NERD patients was higher than in EE and BE patients. It is important to supplement PPI treatment with prokinetics because it can improve esophageal motility and increase LES RP. For example, prokinetics mosapride and itopride enhance esophageal contractions [26] and decrease the number of episodes of transient LE relaxation, which are important causes of GERD [27]. Itopride has been shown to help decrease the duration of the highly acidic period in the esophagus (pH < 4) [28]. For patients with GERD, the addition of prokinetics to standard PPI therapy demonstrated better clinical results [29, 30].

GERD is characterized by disorders of the immune response and imbalanced cellular and humoral functions of the immune response. We hypothesized that the different types of refluxate may somehow differentiate the macrophage phenotype and the immune profile of GERD patients.

Interleukins are the ligands to the surface receptors on macrophages (CD25, CD80, CD163, CD206). The pro-inflammatory IL-2 is a ligand for CD25 and CD80 receptors [13, 31]. Anti-inflammatory IL-10 and IL-6 are ligands for the CD163 receptor [12], whereas anti-inflammatory IL-4 and TNF-β are ligands for the CD206 receptor. We supposed that the development of different types of GERD may be associated with inflammatory and anti-inflammatory cytokines, which, as mentioned, define the immune response of the whole body. An analysis of the monocyte-derived macrophage phenotype showed the prevalence of the expression of CD25 and CD80 in all groups of patients, which is the characteristic of Th1 immune response.

Limitations of the current study are as follows: 1) No control comparisons. However, further testing could be performed to evaluate healthy people without GERD as controls (to determine the immune status of healthy people). 2) The number of patients examined needs to be increased. 3) In the current study, we analyzed monocytes/macrophages isolation from venous blood GERD patients. However, we have not studied monocyte/macrophage phenotype in biopsy material of distal esophagus. Further studies are needed to research monocyte/macrophage phenotype assessment biopsy material of distal esophagus.

Conclusion

There is a relationship between the characteristics of the refluxate and the type of immune response of GERD patients. An analysis of the phenotype of macrophages derived from blood monocytes of GERD patients revealed a prevalence of M1 macrophages that was typical for the Th1 type of immune response.

The degree of esophageal dysmotility was correlated with GERD severity and type (NERD, EE, BE). In other words, GERD is a multifactorial disorder involving the deterioration of the motor function of the upper gastrointestinal tract. Differences in the immune response that were dependent on the pH of the refluxate emphasized the need for more detailed investigations of the immune profile of GERD patients.

In the current research, we studied the blood monocyte/macrophage phenotypes of GERD patients and the motility of the esophagus in patients with different forms of GERD. We demonstrated the presence of esophageal motility disorders in all GERD patients.

These data emphasize the need of conducting further studies of the immune status of patients with different forms of GERD. Consequently, study of immune response in patients with different GERD forms may lead to further development of new methods of treatment of GERD. Understanding the pathophysiological basis for GERD will pave way for well-targeted therapies and successful outcomes.

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