



Immunological Features of Mixed Saliva in Patients with Gastrointestinal Disease

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Abstract: The aim of this article was to study the immunological features of mixed saliva in patients with gastrointestinal tract disease. 140 patients with gastrointestinal pathology were examined. Pro- and anti-inflammatory cytokines (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 and TNF-a) and alpha-defensin 1-3 were studied. When studying the content of cytokines and antimicrobial peptide in the mixed saliva of patients with GIT disease, the predominance of proinflammatory cytokines over anti-inflammatory ones and the decrease of antimicrobial peptide level were noted, which may activate bone resorption?

Keywords: mixed saliva, patients with GI disease, cytokines and antimicrobial peptide.

Introduction. Numerous studies have shown the incidence of concomitant diseases at the dental appointment [Peshkova E.K., 2016; Tytyuk S.Yu. et al., 2019; Tytyuk S.Yu., Iordanishvili A.K., 2019, etc.]. It is also indicated that the oral cavity plays an important role in the digestive process, as it is here that the physical and chemical processes of food preparation for digestion begin. At the same time, the state of the teeth and periodontium significantly affects the functional state of the gastrointestinal tract. Similarly, gastrointestinal disorders can also affect oral tissue health. It should be noted that the absence of changes in the oral cavity in diseases of the gastrointestinal tract is rare [Robakidze N.S. et al., 2017; Mamaeva M.I., 2017; Raikov B.S., 2018]. Changes in the oral cavity can reveal such nosological forms of gastrointestinal tract diseases as chronic gastritis, gastroesophageal reflux or intestinal inflammation [Tsimbalistov A.V. et al., 2013].

Against the background of inflammatory bowel disease there is an increase in dental caries and non-carious dental lesions in 50% of young and middle-aged people from 18 to 35 years [Tytyuk S.Yu. et al., 2019; Tytyuk S.Yu., Iordanishvili A.K., 2019]. Oral manifestations in gastroesophageal reflux GERD correlate with tooth enamel erosion and its prevalence among adults and children with GERD reaches from 47.5% to 83.3% .

G.I. Lukina [2011] found that among all pathologies of the gastrointestinal tract, the lowest salivary pH values (pH=5.2) are detected in the oral cavity during GERD disease. GERD may also be the cause of pathologies in the soft tissues of the mouth and salivary gland dysfunction [Uspensky Y.P. et al., 2015].

Research G.I. Lukin (2011), indicated that among all diseases of the gastrointestinal tract, chronic gastritis accounts for 70% to 80%). Combined inflammation of pyloric mucosa of the stomach and duodenum in clinical practice is diagnosed as gastroduodenitis. In this case, the motor function of the stomach and peristalsis of the duodenum are disturbed that leads to delay of chyme in the initial parts of the intestine [Maev I.V., Samsonov A.A., 2005]. This contingent of patients complain of bad breath, a sense of bitterness, sourness and burning, and the frequency of gingivitis, angular cheilitis, geographic tongue, enamel hypoplasia, dental caries higher compared to healthy people [Janushevich O.O. et al., 2008].

As can be seen from the presented literature sources, the spectrum of oral cavity lesions in various comorbidities is wide. Therefore, the present work is aimed at expanding the professional knowledge of dentists for successful work with patients with concomitant diseases, in particular, with the prospect of early diagnosis, improving the quality of dental care and prevention of pathology in patients with diseases of the gastrointestinal tract.

Objective of the study: study of immunological features of mixed saliva in patients with diseases of the gastrointestinal tract.

Material and methods: 140 patients with gastrointestinal pathology, including 98 men (70%) and 42 women (30%), mean age 51,9 years were examined in the outpatient conditions of TGSI in the period 2020-2022. According to endoscopic examination different gastrointestinal lesions (chronic gastritis; peptic ulcer - gastric and duodenal ulcer) were revealed in the patients. The control group included 25 healthy subjects.

Diagnostics of various gastrointestinal tract parts lesions was based on classical criteria [Vasilenko V.Kh. Andersen et al., 1987, Ivashkin V.T. et al., 2001] and was performed taking into consideration clinical-endoscopic, functional and morphological data. The verification of chronic gastritis was carried out according to classification signs proposed by International Association of Gastroenterologists [Sydney, 1990, Houston, 1996], with consideration of traditional for Russia views [Aruin L.I. et al., 1998] on basis of endoscopic and morphological criteria. Patients and healthy controls were monitored according to a unified program, which included a general clinical examination, esophagogastroduodenoscopy (EGDS). Biomaterial was collected in the morning, on an empty stomach, in graded tubes. In all patients the collection of mixed saliva samples was performed initially before the drug was prescribed. Before starting the procedure, the patient rinsed his mouth with distilled water for 30 seconds, followed by 5 minutes of rest. The patient then swallowed all accumulated saliva, after which direct collection of material began for 15 minutes. Upon completion, the tube was tightly sealed with a lid, placed in a container with ice, and taken to the laboratory within an hour and a half. In the laboratory, the tubes were centrifuged at 3000 rpm for 10 minutes at 4°C, after which the saliva sample was frozen and stored at -80°C until examination. Pro- and anti-inflammatory cytokines (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 and TNF- α) and alpha-defensin 1-3 were determined in blood and oral fluid by solid-phase immunoassay using test systems manufactured by Vector-Best (Novosibirsk, Russia). Statistical data processing was performed on a personal computer using standard software package for applied statistical analysis (Statistica for Windows v. 7.0). The $p < 0.05$ value was used to assess the reliability of differences.

Research results and discussion

As is known, the spectrum of oral cavity lesions in various concomitant diseases is wide. At the same time, concomitant diseases contribute to the development of pathological conditions in the oral tissues, and on their background there is the reception of various drugs for their correction. Gerontological population, which is the main consumer of drugs, should not be ignored. When analyzing nosologies of gastrointestinal diseases, atrophic and chronic gastritis prevailed. According to the data obtained, 63% of patients with gastrointestinal pathology noted the presence of bad breath (halitosis). Dry mouth was mentioned by 28% of respondents. Changes in the color of the tongue and gums were noted by 52% of respondents. A burning sensation in the mouth was noted by 8% of respondents, and 12% of patients reported hyper-salivation. To determine the intensity of the carious process in patients with GIT pathology the KPI index was used. The KPI index in the patients was 8.48 ± 0.91 , which corresponds to the average level of caries intensity. The number of filled teeth ranged from 1 to 6. The number of extracted teeth was insignificantly lower than the number of filled teeth; it ranged from 1 to 24 teeth. CPU index values were significantly positively related to the diagnosis of GI nosology. The lowest values of the CPU index were in patients with chronic gastritis, and the highest values were in patients with IBDD.

In addition to clinical examination of oral tissues, patients with GIT pathology underwent examination of mixed saliva, which reflects changes occurring in the oral cavity.

The analysis of the results revealed some features of the cytokine profile in the mixed saliva of patients with gastrointestinal tract disease (Table 1).

It is known that cytokines, being able to regulate proliferation, differentiation, functional activity of cells, apoptosis, hematopoiesis, angiogenesis, as well as to perform intercellular and intersystem interaction, to determine the type, strength and duration of immune response, can have both pro- and anti-oncogenic effects. Their mechanism of action is realized in the extra- and/or intracellular way through binding to specific receptors located on the cytoplasmic membrane of cells or circulating in the soluble form. IL-1a and IL-1r are produced by activated macrophages, keratinocytes as inactive protein precursor molecules and are converted to active cytokines by either caspase-1 protease or IL-1-converting enzyme (1SE). The ability of IL-1r to inhibit gastric acid production is realized both directly, through its effect on receptors of parietal cells, and indirectly, through stimulation of PG E2 synthesis, which is a strong inhibitor of hydrochloric acid secretion, and through activation of receptors in the central nervous system located in the anterior hypothalamic region in the paraventricular nucleus.

Our studies demonstrated an average 3.6-fold increase in concentrations of IL-1a in mixed saliva in patients with gastrointestinal diseases compared to healthy patients. High blood values of IL-1a in patients with gastrointestinal tract disease most likely indicate the development of a chronic inflammatory process. This condition is characterized by increased production of cytokines, which exhibit pro-inflammatory effects, i.e. cytokines in these pathological processes play the role of both aggressive and protective factors.

Table 1. Cytokine system parameters in mixed saliva in patients with GI pathology

Indicators	Comparison group (p=25)	Patients with gastrointestinal pathology (p=140)
TNF- α (pg/ml)	12,54 \pm 1,43	19,63 \pm 0,98*
IL-1R (pg/ml)	13,58 \pm 1,24	48,61 \pm 3,68*
IL-6 (pg/ml)	23,14 \pm 1,15	55,78 \pm 0,38*
IL-8 (pg/ml)	816,24 \pm 18,64	2552,61 \pm 191,54
IL-4 (pg/ml)	10,71 \pm 1,04	8,21 \pm 0,72
IL-10 (pg/ml)	12,21 \pm 0,78	6,03 \pm 0,45*
Alpha -defensins 1-3 (ng/ml)	984,2 \pm 16,71	547,46 \pm 16,53*

Note: * $p < 0,05$ in relation to data of control group

Herewith their content depends on etiological factor, variant of course, stage, duration of chronic inflammatory and destructive disease. In response to chronic inflammation of gastrointestinal mucosa, there is induction of secretion of interleukin-8 by macrophages. It is known that by activating neutrophils, IL-8 leads to their degranulation, release of lysosomal enzymes, leukotrienes, which have a damaging effect on the mucosa of the gastrointestinal tract. In addition, increased levels of IL-8 are also found in the peripheral blood regardless of localization. In our studies, we observed a 3-fold increase of IL-8 level in mixed saliva in patients with gastrointestinal disease as compared to healthy individuals. Pro-inflammatory cytokine tumor necrotizing factor α (TNF- α) plays an important role in the formation of the inflammatory reaction. TNF- α is considered the strongest stimulus for IL-1 β production and is synthesized by T lymphocytes and macrophages. This cytokine is multifunctional and plays a predominant role in the formation of local and general pathological processes, in particular it activates the synthesis of pro-inflammatory interleukins, stimulates T- and B-lymphocytes, regulates the intensity of inflammation, increases phagocytic activity of monocytes, nitric oxide formation, also involved in the physiological processes and inflammatory response in the gastroduodenal mucosa. As can be seen from the presented results of the research, the level of tumor necrotizing factor α increased relative to the parameters of the comparison group by 1.6 times. Prolonged and pronounced elevation of TNF-a in mixed saliva in patients with GIT disease may contribute to the imbalance between the osteoblast osteogenesis and osteoclast osteodestructive function towards hyperactivation of the latter in the dental system. In the works of Akbiev D.S.[2017] it was noted that in patients with ulcer disease there is pro-inflammatory

cytokinemia along with increased activity of anti-inflammatory cytokine IL-4. The authors believe that the increased level of IL-4 in this situation probably has a compensatory nature in relation to pro-inflammatory cytokines and acts as a factor stabilizing the course of the disease. In addition, it is indicated that the levels of TNF- α and IL-6 can be indirectly judged about the activity of the inflammatory process in the gastroduodenal zone. The analysis of the obtained results, presented in Table 1, shows that the level of interleukin-4 in the mixed saliva of gastroduodenal disease patients is 23% lower than in the comparison group. At the same time, the level of IL-6 in the mixed saliva of the examined patients exceeded the initial level by 2.4 times. IL-6 is known to be an inducer of inflammatory reaction and triggers synthesis of acute phase proteins in the liver (C-reactive protein, serum amyloid A, etc.), as well as reduces production of fibronectin, albumin and transferrin in the liver. The key anti-inflammatory factor IL-10, is known to inhibit the production of TNF α , IL-1 β and IL-6 and inhibit the expression of the major histocompatibility complex class II. One of the markers of inflammatory process activation is considered to be the level of antibacterial proteins defensins. We investigated the content of α -defensins (HNP1-3) in the saliva of patients with GIT diseases. As can be seen from the presented findings, the content of α -defensins 1-3 in the oral fluid of patients of the main group and healthy individuals presented in Table 1 indicates a decreased secretion of α -defensins 1-3 in the oral fluid of the main group relative to the indicators of the comparison group. The revealed factual material indicates inactivation of α -defensins, which can lead to an increase in microbial colonization and increase the risk of viral and bacterial infections in the oral cavity. Low levels of defensins in the mixed saliva of patients with GI disease contribute to a decrease in IL-8 secretion and progression of the process of inflammation in the oral mucosa. Thus, the study of cytokine and antimicrobial peptide content in mixed saliva of patients with GIT disease showed predominance of pro-inflammatory cytokines over anti-inflammatory ones and decrease of antimicrobial peptide level, which can activate bone tissue resorption. In general, the identified changes may lead to an imbalance in the local immune response of mucous membranes and the development of both autoimmune and inflammatory diseases of the oral cavity.

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