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Research Article

Salivary Changes in Helicobacter Pylori-Positive (HP+) Chronic Gastritis

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Abstract

Introduction: Diagnosis and monitoring of chronic gastritis are most commonly by invasive endoscopic-biopsy technique. Finding non-invasive laboratory markers would save money and inconvenience for the patient. Saliva, with its essential protective role for the digestive system, is increasingly recognised as a good non-invasive diagnostic material.

Aim: To study several biochemical parameters in the saliva of patients with chronic gastritis (HP+ and HP-), to compare their levels with those of healthy subjects and to establish correlation relationships between their serum and salivary concentrations with a view to their possible use as a diagnostic tool.

Material and method: 140 subjects were studied, of which 60 patients (mean 58.73±12.08y) with chronic gastritis (44HP+ and 16HP-) with disease activity assessed by subjective complaints, serological and endoscopic findings. The control group consisted of 80 healthy- non-smoking volunteers (mean 56.86±8.67y). Unstimulated saliva and serum were used for analysis. The parameters we study are Uric Acid (UA), Total Protein (TPro), Albumin (Alb) and Secretory IgA (sIg A). They are analysed with B.Coulter ready-made kits with an adaptation of the oral fluid methods of an Olympus AU 640 biochemical analyser and ELISA Reader DiaMetra Italy.

Results: Significantly higher values of sIgA($p<0.0001$), Alb($p<0.0001$) and TP($p=0.0434$) were observed in HP+ patients compared to the control group, but not for UA. We found a correlation between saliva/serum values for UA only ($r=0.3389$, $p=0.011$). A moderate negative correlation existed between UA and endoscopic inflammatory changes ($r=-0.4203$, $p=0.016$). These changes are thought to be compensatory for the gastric inflammation, increased oxidative stress and altered salivary flow rate.

Conclusion: The data indicate significant changes in salivary parameters in HP+ chronic gastritis. Saliva has its weaknesses as a biological material, but it reflects well the pathological processes in the digestive system, especially with HP+ infection.

Keywords: Albumin; HP+ Chronic Gastritis; Saliva; Sig A; Total Protein; Uric Acid

Introduction

Chronic gastritis is a long-term non-specific inflammatory disease of the gastric mucosa, which is accompanied by impaired secretion, motor, and incretory functions. Eating habits, stress, multiple medications, poor oral hygiene, and contamination with H. pylori, smoking, and alcohol abuse are the reasons for its widespread in the modern urban world. The frequent need for health care makes it a significant public health problem. H. pylori infection is associated with some extradigestive diseases such as diabetes, coronary, and ischemic diseases [1]. Diagnosis of chronic

gastritis is based mainly on invasive endoscopic methods. Because of the unpleasant procedure and fear, patients often underestimate their complaints and condition. The mucosa covering the lumen of the digestive system is a barrier with a mechanical and protective role against hydrochloric acid, proteolytic enzymes, and antigenic structures (food antigens, antigens of comorbid or pathological microorganisms) in the gastrointestinal tract [2]. The colonization of the stomach by H. pylori causes permanent inflammation of the gastric wall, triggering a series of immunological reactions of the host. The oral cavity makes constant contact with the external environment and is closely linked to the entire digestive tract. Saliva is the first fluid that comes into direct contact with food, beverages, bacteria, chemicals, and volatiles and is a major protector of the digestive system. Impairment in oral homeostasis is a major cause of many systemic infections [3].

The saliva is a fluid, viscous fluid produced by three pairs of salivary glands (parotid, submandibular, and sublingual glands) and numerous smaller glands located in the oral cavity [4]. It is a complex system containing 99% water and 1% various low molecular weight substances, enzymes, hormones, antibodies, antimicrobials, and growth factors [5,6]. Some are synthesised locally by the salivary glands, while others are transported from the bloodstream by diffusion processes, active transport, or ultrafiltration. The saliva is a mirror of the functional, metabolic, hormonal and emotional state of the body [5].

The oral cavity is "peacefully" inhabited by a huge number of different microorganisms, many of them being a natural and healthy component supporting oral homeostasis. Saliva, with its various constituents, directly and indirectly, affect oral bacteria [7]. A humid environment with a relatively constant temperature (from 34 to 36 ˚C) and a pH near the neutral point are favourable conditions for the growth of a wide variety of microorganisms. The antimicrobial activity of the saliva, provided by a variety of proteins and peptides (mucins, lactoferrin, lysozyme, lactoperoxidase, staterin, histatin, and secretory immunoglobulin A), maintain in a symbiotic state the host-microflora relationship and counteract the pathogens [7].

Variation in salivary secretion and composition has been observed under various physiological stimuli and a number of oral and systemic pathological conditions. Oral fluid (OT) testing is becoming increasingly important as an opportunity to avoid unpleasant and risky manipulations, such as endoscopy, biopsy, in the follow-up of relapse and remission. Its easy, non-invasive extraction with a patient-friendly procedure makes it an increasingly preferred biological material.

Aim: To study several biochemical parameters in the saliva of patients with chronic gastritis (HP+ and HP-), to compare their levels with those of healthy subjects and to establish correlation relationships between their serum and salivary concentrations with a view to their possible use as da diagnostic tool.

Material and Method

The patient group included 60 people with chronic gastritis at an average age of 57.93±12.08 (range 30-78) years admitted to the Gastroenterology Unit of Military Hospital - Varna with pain and dyspeptic syndrome. The Control Group (CG) consists of 80 healthy, non-smoking volunteers who undergo routine annual prophylactic examinations. Mean age of CG was 56.86±8.67 (range 30-72) years. All participants in the study signed informed consent and passed general dental status. The study was approved by the local ethics committee (Minutes No 64/13.07.2017). Patients were selected according to the following inclusion criteria: disease activity, including subjective complaints, endoscopically confirmed chronic gastritis and HP infection. Exclusion criteria are the presence of a malignant complication and/or recent surgery. Patients who had oral inflammation or had undergone dental surgery within 48-72 hours before the study were not included in this study. Both groups were determined laboratory parameters, including CRP as a marker of inflammation. Venous blood is taken according to a standard preanalytical procedure. The blood serum was removed by centrifugation for 10 minutes at 3500 rpm. We study serum levels of antibodies to H. pylori (IgG) (LIAISON®DiaSorin), and confirm the infection with a fecal qualitative HP antigen test. In serum, Uric Acid (UA), Total Protein (TP), Albumin (Alb) and IgA are determined. We apply routine, standardised laboratory methods for measuring serum parameters analysed with an Olympus AU 640 biochemical analyser. The oral fluid is collected in the morning from 8 to 10 o'clock, in special graduated sterile conical-bottomed containers, by passively repeatedly removing the saliva collected in the mouth. 2-3 ml was obtained within 5 minutes. For reliable results, patients were instructed to comply with the following conditions: More than 30 minutes elapsed since the last meal, drink (coffee and other tonic drinks), chewing gum or the latter brushing teeth with paste and brush. Five minutes before the test, the mouth was rinsed twice for 10 seconds with saline or mineral water.

Material processing

The material is quickly processed within 30-60 minutes. The hypotonic nature of the saliva and the presence of healthy microflora lead to lytic processes of many biomolecules. On the graduated scale of the container, we count the amount of saliva collected. We centrifuge the biological samples at 3000 rpm for 10 minutes. The supernatant was carefully pipetted and aliquoted into Eppendorf type micro containers. They are stored at -20 ˚C until salivary parameters are determined. UA, TP and Alb are determined by Beckman Coulter kits (Olympus AU 640), with an adaptation of the oral fluid method. Secretory IgA was determined using a DiaMetra kit (ELISA Strip Reader, Italy).

Methods used

Salivary protein and albumin values are too low compared to serum, which requires the use of sensitive methods to determine

them. For salivary protein analysis, we apply the dye pyrogallol red, which alters its spectral absorption upon protein binding. Read spectrophotometrically at 570 λ. This method is sensitive in the low protein concentration range <3.0 g/L. The salivary values of albumin are 100 to 1000 times smaller than serum levels. To determine it, we use an immunological turbidimetric test for microalbumin. UA was tested by enzymatic c colourimetric method (Uricase-POD-PAP method) using a B. Coulter kit on an Olympus AU 640. For the purpose of calibration, we have created a series of calibrators with an appropriate matrix (Artificial saliva for medical and dental research) with the addition of certified standard solutions (TP 1.0 g/dL, Alb 0.2 g/dL, UA 8 mg/dL). The concentration of total SIgA in each sample was determined by immunosorbent assay (ELISA). Samples were diluted 1: 1000 with appropriate buffer before being added to the microplate. The wells of the plate are coated with monoclonal anti-IgA (alpha-chain specific, DiaMetra Italy). The second polyclonal antibody is conjugated to peroxidase. After incubation, the solid-phase bound free antibodies were separated by washing. Hydroperoxide (H_2O_2) and TMB were used as the substrate of the enzyme-linked immune reaction, resulting in a blue colour reaction and the colour-changing to yellow after the addition of Stop solution. The colour intensity is proportional to the concentration of sIgA in the sample.

Statistical Methods

Data analysis was performed using Graph Pad Prism v software. 6.0 using standard statistical methods (descriptive statistics, nonparametric T-test for mean comparison, analysis of variance and Spearman correlation analysis). Biochemical parametric data were presented as mean and standard deviation (mean±SD). Statistical significance was indicated at $p < 0.05$.

Results and Discussion

The patient group is divided into two subgroups depending on whether they have shown antibodies and antigen for Helicobacter pylori (Table 1).

persons studied	Man (N)	Woman (N)	Total (N)
HP Positive **Age(mean±SD)**	26 55.22±11.86	18 58.0±12.77	44 56.35±12.14
HP Negative **Age(mean±SD)**	11 63.55±10.88	5 58.0±12.23	16 63.77±10.05
Total patients group **Age(mean±SD)**	37 57.91±12.13	23 57.95±12.31	60 57.93±12.08
Control group **Age(mean±SD)**	40 58.2±8.52	40 55.52±8.77	80 56.86±8.67

Table 1: Demographics of the study groups.

According to the endoscopic characteristics of inflammatory changes and the topographic spread in the gastroduodenal mucosa, patients are allocated as follows (Table 2):

Endoscopic Dx	Regional (n=29)	Diffuse (n=31)
Erythematous gastritis	15	10
Erosive gastritis	14	17
Atrophic gastritis	0	4

Table 2: Endoscopic characteristics of change in the gastroduodenal mucosae of the total patient group study.

The parameters studied in saliva and serum are given in Table 3 and Table 4.

Parameters	HP+	HP-	Control group	P
Uric acid [umol/L]	210.6±56.68	219.8±58.45	222.9±36.8	0.4166
Total protein [mg/L]	891.0±354.7	788.8±237.6	725.0±393.6	0.0434
Albumin [mg/L]	89.69±62.92	67.56±25.21	50.83±19.87	<0.0001
sIg A [g/L]	139.9±33.24	98.33±18.44	108.3±47.69	<0.0001

Table 3: Salivary parameter values in patient HP +, HP- and control groups.

Parameters	HP+	HP-	Control group	P
Uric acid [umol/L]	374.41±93.19	336.94±81.72	342.82±73.56	ns
Total protein [mg/L]	70.40±7.10	72.22±4.35	72.51±8.68	ns
Albumin [mg/L]	43.77±6.20	45.33±4.20	46.56±4.26	ns
Ig A [g/L]	2.62±1.31	1.97±0.77	1.85±0.80	0.003
CRP [mg/L]	18.64±14.95	5.71±9.36	2.38±1.59	0.001

Table 4: Serum parameter levels in patient HP +, HP- and control groups.

The composition of the elements in the saliva can vary within quite wide limits depending on age, health, diet and bad habits (smoking, drugs, alcohol) [8]. The mucosal defense is accomplished through non-immune mechanisms (epithelial barrier integrity, commensal microorganisms, secretory activity, etc.) and immune defense factors implemented by the mucosal immune system (MALT). Secretory immunoglobulin A is a component of the adaptive immune system and works alongside other congenital mucosal protective factors (α-amylase, lactoferrin and lysozyme) [9]. Helicobacter pylori infects the gastrointestinal tract and is highly adapted to adverse environmental conditions. Integrating into the mucous layer covering the gastric epithelium, it results in cell-mediated immunity with inflammatory infiltrate of neutrophils, lymphocytes, plasma cells, macrophages and eosinophils in the gastric mucosa [9]. Despite Stimulated Local and Systemic Inflammation with the production of IgA antibodies, the host immune response is ineffective. The infection persists and chronicles [10,11].

Secretory IgA (S-IgA) is the predominant form of antibody that mediates specific immunological protection of mucosal surfaces. There are two forms of IgA - IgA1 (90%) and IgA2 (10%), which differ in structure. IgA1 is detected in serum and is a product of B-cell synthesis in the bone marrow. IgA2 is a product of B cells located in the mucosa and is a representative of the functioning mucosal immune system. Saliva contains approximately 60% of IgA1 in adults [12,13].

In our study, the HP + patient group showed significantly elevated mean sIgA values compared to HP and CG (Table 3). Similarly, elevated serum concentrations were observed for serum IgA (Table 4, Figure 1A, B). In the analysis performed between serum Ig A levels and salivary sIgA, we found no significant correlation (Table 5). Increased levels of sIgA in saliva may be due to its role in reducing bacterial density and protecting the gastric mucosa. Studies have shown that locally produced antibodies, especially IgA [14], can block H. pylori infection. sIgA prevents bacterial colonisation and adhesion and enhances bacterial opsonisation [10]. HP infection probably involves a number of adaptive oral immune mechanisms of the host to counteract the pathogenic microorganism. The secretory level of IgA also directly increases with age, even in healthy individuals [15]. The average age of the HP- group (although small) is highest, but the mean sIgA values are lowest compared to HP+ and CG. The classified as HP- are caused by medication (gastritis type C) or other comorbidities with ischemic changes in the GET. B-blockers, diuretics, antibiotics, NSAIDs, antiarrhythmics act on salivary flow and secretion, which may explain the lower values. To better assess the secretion of salivary immunoglobulin A, the HP- group should be expanded.

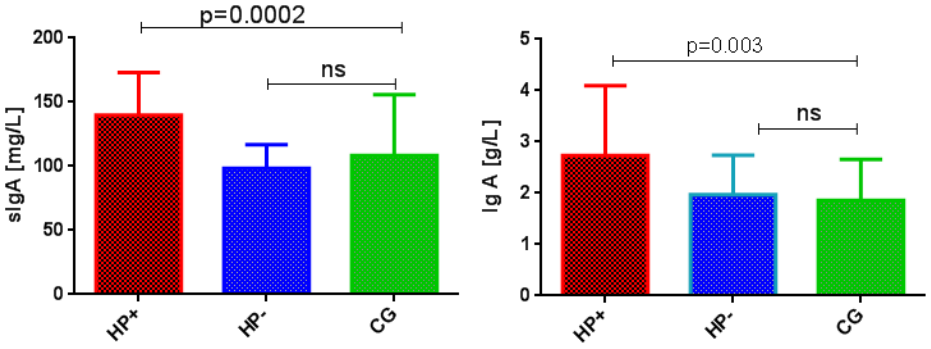

Figure 1: 1A. Salivary concentrations of sIgA in subjects studied in the three groups. **1B.** Serum IgA concentrations in the HP positive, HP Negative and control group.

Parameters	Saliva r	p	Serum r	p
TP saliva/serum	0.09266	ns	-0.02138	ns
sIgA saliva/serum	-0.04853	ns	-0.1569	ns
Alb saliva/serum	0.1777	ns	-0.06139	ns
UA saliva/serum	0.3389	0.0106	0.01374	ns

Table 5: Correlation relationships between parameters in unstimulated saliva and serum of the total patient and control group.

In their study, Necil Kutukculer, et al. [16] suggested that deficiency and reduced local defense mechanisms predispose to HP infection in children. The concentrations of sIgA and SC component in the saliva and gastric fluid of patients with HP infection show no significant difference compared to children who have no organic disease and HP infection. In this aspect, our study has the drawback of not measuring the level of sIgA in gastric juice. The data will give a full idea of the involvement of saliva and ora.l homeostasis in immune-inflammatory mechanisms and changes in the gastric mucosa [17].

The potential role of H. pylori in the pathogenesis of several oral diseases, such as recurrent aphthous stomatitis and periodontal disease, as well as squamous cell carcinoma, burning mouth syndrome and halitosis, has been demonstrated. The establishment of a bacterium in dental plaque and saliva appears as a reservoir for infection / reinfection of the stomach [17]. H. pylori infection has also been reported to be correlated with several systemic disorders such as dyslipidemia, hyperglycaemia and various cardiovascular diseases [18].

Total protein is a vital component of saliva. It is responsible for most of its functions such as lubrication, physical protection, cleaning, buffering, maintenance of tooth integrity, taste and digestion, and antibacterial activity. The major factors affecting the concentration of protein in saliva the are the rate of salivary flow, the secretion of the protein from the glands and the crevicular fluid [19]. The proteins originating in the salivary glands in the oral fluid are about 300, but their relative share in the protein component is approximately 83% (enzymes, mucins, cystatins, etc.). Dyspepsia and pain are leading syndromes in chronic gastritis activity and may affect salivary flow along the pathway of the sympathetic nervous system. As a result, mostly smaller amounts of protein, mucin and enzyme-rich saliva are excreted [20]. Xerostomia is a common symptom in patients with chronic gastritis because of the effect of medicines used to treat it. (Butylscopolamine, antibiotics, drotaverine hydrochloride, Omaprasol). Our study showed higher salivary protein values in the HP+ patient group and was statistically significant compared to CG (Table 3). While no such dependence has been observed between the HP- group and CG. No statistically significant difference was found in the values of serum TP levels. This increase in salivary protein is likely to indicate local glandular secretion of proteins involved in nonspecific immune defense in response to the host [19]. We found no correlation between salivary and serum protein levels in both the patient group and the control group.

Albumin is a carrier for molecules with low water solubility (lipids, hormones, unconjugated bilirubin) and maintains colloid-osmotic pressure. It represents more than 50% of all plasma proteins [21-22]. The factors that regulate albumin synthesis are nutrition, hormonal balance and osmotic pressure [21]. Albumin is an antioxidant protein and is found to be a minor component in whole saliva. It is considered as serum ultrafiltrate and can be used for the overall evaluation of oral mucosa function. Albumin is generally considered a reliable marker of mucositis or inflammation [23-24]. Therefore, a general dental examination was performed when selecting the control group and patients to rule out active local inflammation. The groups are in the age range of 30-78 years, and naturally, the tooth status changes over the years [25]. The tooths is reduced, which is corrected by bridges and partial dentures. A number of studies indicate an increase in salivary albumin with age. An increase was also observed in immunosuppression, diabetes and radiation therapy due to increased permeability of the basement membrane. In the HP + patient group, we observed a statistically significant increase in albumin compared to the HP- and CG groups (Figures 2,3).

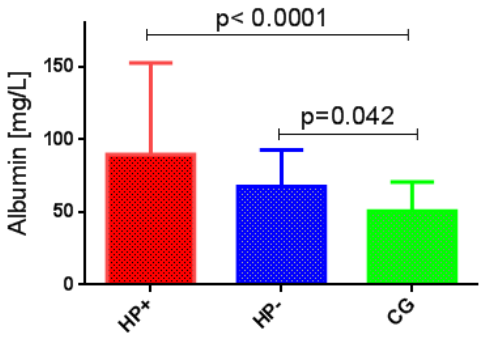

Figure 2: The albumin values of HP positive, HP Negative and control group.

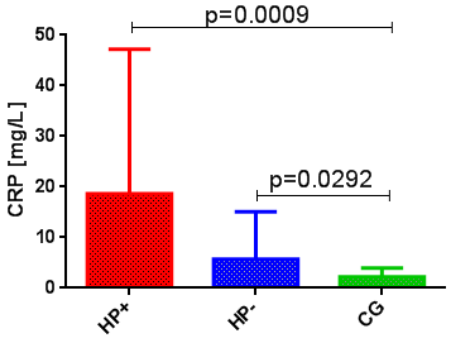

Figure 3: Values of the inflammatory marker CRP in serum of HP positive, HP Negative and control group.

The study of CRP as an inflammatory marker showed statistically significant high values in the HP+ group compared to HP- and CG. A similar relationship is observed between HP- and SG. Although the acute-phase protein is not specific and sensitive to chronic gastritis, a large range of reactivity is observed in HP+ patients (1.6-112 mg/l). We made a comparative analysis of CRP values with salivary and serum levels of the studied parameters. We found a slightly negative correlation with albuminin serum (r=-0.2283, p=ns) and saliva (r=-0.2319, p=ns) and with total protein, there was a moderate correlation in saliva (r=-0.3282, p=0.0154) and less in serum (r=-0.2491, p=ns). The inflammatory process alters the secretion a number of proteins in the liver and their distribution in the body. Albumin is a reverse-phase protein. Apparently, the inflammatory process, local oxidative stress, and variations in the filtration of albumin through the salivary gland capillaries explain the observed results, regardless of higher levels of salivary albumin [25] (Tables 6,7).

CRP vs. TP	CRP vs. IgA	CRP vs. UA	CRP vs. Alb
r=-0.2491	r=0.1012	r=-0.02183	r=-0.2283
p=0.0693	p=0.4667	p=0.8755	p=0.0968

Table 6: Correlation between the inflammatory marker CRP and the serum parameters tested.

CRP vs. UA saliva	CRP vs. TP saliva	CRP vs.Alb. saliva	CRP vs. sIG A
r=0.007703	r=-0.3282	r=-0.2319	r=-0.1979
p=0.9559	p=0.0154	p=0.0915	p=0.1515

Table 7: Correlation between the inflammatory marker CRP and the salivary parameters tested.

Oxidative stress plays an essential role in the pathogenesis of chronic inflammation and degenerative changes in gastric mucosa caused by HP. Uric acid is a non-enzymatic antioxidant (AOC) that neutralises free radicals and is a metal ion chelator [26]. It is a product derived from the oxidation of xanthine and hypoxanthine by the reductase of xanthine oxide [27]. About 70-80% of the AOC in the saliva is due to the action of UA. Its elevated values are associated with risk of cardiovascular disease, diabetes mellitus [28], metabolic syndrome [29], malignant neoplasms [30]. This may be partly explained by the pro-oxidants and pro-inflammatory properties of UA. Lyngdoh T, et al. documents the stimulating role of UA in the secretion of inflammatory cytokines produced by mononuclear cells such as TNF-α, IL-1b, and IL-6 [31]. In our study, no statistically significant difference was observed between the study groups, although the values in the HP+ patient group were the lowest. Probably, as an adaptive mechanism, its rise may be noted to cope with the occurring oxidative stress in the GET. Another explanation is probably the different disease duration, topography, and inflammatory activity. Our patients have recurrent complaints with an average duration of about 2.9 years (range 1-10 years). Some authors have found a good correlation between serum and salivary uric acid levels, especially in patients with gout. Based on these results, they suggest the use of UA levels in monitoring its treatment [32]. Fawaz Pullishery, et al. (2015) did not find any similar dependence in the study [33]. Our results showed a moderate correlation between UA saliva - serum in the patient group, whereas no similar dependence was observed in the CG group (Figure 4).

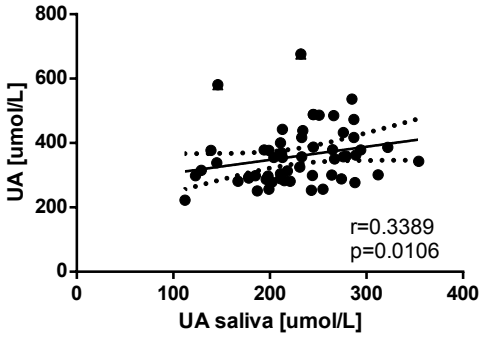

Figure 4: Correlation between serum and salivary uric acid levels.

Ndebi ME et al. describe an increase in serum uric acid in serum with chronic H. pylori infection. Our patient HP + group also showed higher UA values but no statistical significance [34]. The comparative analysis revealed that uric acid best correlates with the endoscopic inflammatory changes of the gastroduodenal mucosa. We observed a moderate negative correlation dependence (r=-0.4203, p=0.0016). We attribute this to the role of oxidative stress and the onset of imbalance with the reduction of AOC in chronic course and damage of the gastric mucosa (Table 8).

Endoscopic inflammation vs. UA saliva**	Endoscopic inflammation vs. TP saliva	Endoscopic inflammation vs. Alb. saliva	Endoscopic inflammation vs. sIG A
r=-0.4203	r=-0.07869	r=-0.01792	r=0.01821
p=0.0016	p=0.5717	p=0.8977	p=0.8960

Table 8: Correlation between degree of endoscopic inflammatory changes and salivary parameters studied.

Conclusion

Laboratory markers for the diagnosis of chronic gastritis are few and not sufficiently specific. Some indicators change as a result of the inflammatory response, clinical manifestations, and complications of their course. Helicobacter Pylori (HP) infection is the most common human infection. It is widespread worldwide, affecting over 50% of the population. HP-related illnesses, causing high morbidity and mortality, and treatment are associated with high financial costs. Saliva is an alternative biological material that is easily extracted by a non-invasive procedure. In our study, we

found significant changes in salivary parameters in HP + chronic gastritis. Oral diseases and impaired homeostasis in the oral cavity are closely associated with the penetration of pathogens and inflammation of the GET. Saliva has its weaknesses as a biological matrix. There are unresolved issues regarding the standardization of the procedure and the methods used to examine its indicators. But it reflects well the pathological processes in the digestive system, especially with HP + infection, and in the future, it will find increasing application in the diagnostic and monitoring process.

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