Change of Parietal Gastric Microbiota depending on the Stage of Mucous Coat Atrophy against the Background of Active Inflammation

L.V. Matveeva, R.Kh. Kapkaeva, L.M. Mosina, V.M. Kurusin, FSBEI of HPO (Federal State Budget Educational Institution of Higher Professional Education) Mordovsky State University n. a. N.P. Ogarev, Medical Institute, Saransk

Lyubov Vasilievna Matveeva – e-mail: MatveevaLjubov1@mail.ru

Summary
Damage to the stomach may contribute to an imbalance normomikrobiotsenoza with the development of inflammation, a violation of the trophic tissue and neuroimmune regulation.

Purpose – to study changes in parietal gastric microbiota on the background of an active inflammatory process and evaluate their dependence on the stage of atrophy. We studied 122 patients with chronic gastritis II-IV degree when compared with healthy individuals, and between themselves bacteriological method defined disbiotic change stomach, progressive with the increase atrophic process, in the form of increased colonization coccoid and rod-shaped bacteria, fungi genus Candida.

Key words: gastric microbiota, Helicobacter pylori, fungi of the genus Candida, dysbiosis, atrophic gastritis, an inflammation.

Introduction
Stomach of a healthy person is colonized by relatively large amount of microorganisms [1, 2]. Damage to stomach with the development of inflammatory atrophic process may be caused by a large amount of factors including persistence in the human organism of several microorganisms capable of breaking the balance of normal microbiocenosis, supporting chronic inflammation, disturbing trophic structure of tissues and neuroimmunoregulation.
In accordance with foreign sources, the most wide-spread phenomena in non-altered stomach are streptococci, micrococci, veilonella, actinomyces, fusobacteria, neisseria etc. [3–5]. Being present in the stomach, *Helicobacter pylori* (*H. pylori*) is dominant in microbiota composing up to 94% of total count [5].

In accordance with a number of authors [1, 6, 7], when chronic gastritis is in the acute form, biopsy material of the gastric mucous coat (GMC) has streptococci as predominant ones (*Streptococcus pyogenes*), as well as staphylococci, fungi of the genus *Candida*, *H. pylori*, *Escherichia coli* (*E. coli*).

Earlier it was discovered [2] that against the background of excessive growth of mucous microbiota of GMC reduction of *H. pylori* frequency occurs in the period of acute stage of chronic non-atrophic gastritis. Reduction of quantitative level of helicobacteria in biocenosis may be caused by change of the environment due to damage of GMC, as well as antagonist influence of the spreading conditionally pathogenic microorganisms [2, 8].

The data is available on the reduction in the quantity of *H. pylori* during atrophy of GMC and increase of variability of gastric microbiota against the background of the increased pH with increase in the quantity of *Prevotella* spp., *Rothia* spp. and *Streptococcus mitis* [4, 9]. Indication exists of the availability of direct connection between expression of dysbiotic changes in the stomach and the degree of seeding of *H. pylori* [10].

**The study goal** is studying differences in parietal gastric microbiota against the background of active inflammatory process and evaluation of the dependence of the mentioned processes on the stage of atrophy.

**Material and Methods**

The examination was carried out on the basis of Mordovia Republic Clinical Hospital, Microbiological Laboratory of Republic Clinical Hospital No. 4 of Saransk.
The control group based on random selection principle included 40 healthy volunteers (52.5% of men, 47.5% women, the average age of whom is 38.95±8.02 years), having at the moment of the examination no traits of acute stage of gastric pathology.

The patients with chronic gastritis of the II-IV degree (55.7% of men, 44.3% of women, the average age of whom is 43.9±7.5 years) were divided into groups depending on the stage of atrophy determined morphologically. The 1st group included 42 patients having chronic non-atrophic gastritis, the 2nd group – 40 patients having focal-atrophic (I–II degree) gastritis, the 3rd group – 40 patients with the spread atrophic (III–IV degree) gastritis.

In the course of EGDS (esophago-gastro-duodenoscopy) collection of gastobiopsy materials with parietal mucus was carried out with further microscopic, microbiological, histological study.

Occurrence and expression of seeding of GMC by *H. pylori* was determined at microscopic examination of the impression smear of gastobiopsy material, stained in accordance with Romanowsky-Giemsa.

Microbiota of the examined persons was studied by way of inoculation of the material diluted to nutritious media (blood, vitelline-salt, helicobacterial agar, Endo, Saburo medium etc.) and identification in accordance with standard methods. The number of microorganisms was determined by counting colony forming units in 1 g (lg CFU/g) with regard to the quantity of inoculation material and dilution.

At statistical processing of the results arithmetic average value (M) was calculated, as well as error of the arithmetic average value (m), Student criterion, degree of difference possibility in groups (p). Significant were differences at *p*≤0.05.

**Results and Their Discussion**

When gastrobiopsy materials with parietal mucus were inoculated for nutritious media, in the control group most often the following bacteria were discovered:
Staphylococcus epidermidis (in 60%), Streptococcus spp. (in 50%), Lactobacillus spp. (in 47.5%), Bifidobacterium spp. (in 45%), H. pylori (in 40% of cases), Escherichia coli (in 40%). Less often the following microorganisms were sown: Corynebacterium spp. (in 22.5%), Candida spp. (in 20%), Micrococcus spp. (in 17.5%). The least frequency of sowing was determined in the case of Actinomyces spp., Neisseria spp. – in 5% of cases, Staphylococcus aureus – in 2.5% of cases.

In the 1st group in comparison with healthy persons increase of GMC colonization by microorganisms was observed (fig. 1). Similar to the control group most often the following microorganisms were detected: Staphylococcus epidermidis (in 92.9%) and Streptococcus spp. (in 71.4%). The 3rd and 4th place was occupied by H. pylori (in 64.3% of cases) and Escherichia coli (in 54.8%). The frequency of sowing of Candida spp. increased up to 35.7% (the growth comprised 15.7%) against the background of sowing of Lactobacillus spp. (-11.8%) and Bifidobacterium spp. (-16.4%). The frequency of occurrence of Micrococcus spp. increased up to 23.8% (+6.3%), Staphylococcus aureus – up to 19% (+16.5%), Actinomyces spp. and Neisseria spp. – up to 14.3% (+9.3%), Corynebacterium spp. decreased to 14.3% (-8.2%).

In the second group occurrence of the following microorganisms prevailed: Staphylococcus epidermidis (in 87.5%), Streptococcus spp. (in 80%), H. pylori (in 60.0% of cases), Escherichia coli (in 52.5%). The frequency of sowing of Candida spp. increased up to 42.5% (the growth in relation to the control group comprised 22.5%, in relation to the 1st group – 6.8%) against the background of sowing of Lactobacillus spp. (up to 32.5%) and Bifidobacterium spp. (up to 25%). Occurrence of Staphylococcus aureus (27.5%) increased in relation to the control group by 25%, in relation to the 1st group– by 8.5%. The frequency of sowing Micrococcus spp. reduced to 20%, of Actinomyces spp., Neisseria spp., Corynebacterium spp. – to 12.5.
In the 3rd group the 1st place from the point of view of sowing frequency was taken by *Streptococcus* spp. (in 92.5%), the 2nd place – by *Staphylococcus epidermidis* (in 82.5%), the 3rd place remained occupied by *H. pylori* (in 57.5% cases). Occurrence of *Candida* spp. increased up to 50% (the growth comprised in relation to the control group 30%, in relation to the 1st group – 14.3%, in relation to the 2nd group – 7.5%). The frequency of sowing of *Escherichia coli* reduced to 42.5%, of *Lactobacillus* spp. – to 32.5%, of *Bifidobacterium* spp. – to 17.5%, of *Micrococcus* spp., *Actinomyces* spp., *Neisseria* spp., *Corynebacterium* spp. – to 10%. Occurrence of *Staphylococcus aureus* (32.5%) increased in relation to the control group by 30%, in relation to the 1st group– by 13.5%, of the 2nd group– by 5%.

Clinically healthy persons had the largest quantities of the following microorganisms observed: *Escherichia coli* (3.87 lg CFU/g), *Lactobacillus* spp. (3.71 lg CFU/g), *Streptococcus* spp. (3.68 lg CFU/g), *H. pylori* (3.65 lg CFU/g), *Corynebacterium* spp. (3.41 lg CFU/g), *Staphylococcus epidermidis* (3.27 lg CFU/g). In smaller quantities the following microorganisms were sown: *Candida* spp. (2.89 lg CFU/g), *Bifidobacterium* spp. (2.80 lg CFU/g), *Neisseria* spp. (2.78 lg CFU/g). The least quantities were determined in the case of *Micrococcus* spp. (2.45 lg CFU/g), *Actinomyces* spp. (2.39 lg CFU/g), *Staphylococcus aureus* (2.3 lg CFU/g). Sowing of *H. pylori* in quite a large quantity of 40.0% of healthy persons testifies of its representation in normal microbiocenosis of stomach. Traits of dysbiosis of gastroduodenal region (GDR) were not observed.

In the 1st group in the course of comparison with healthy persons increase in the quantity of the sown microorganisms was observed (Fig. 2). The biggest average quantities of the group were determined in the case of *Staphylococcus epidermidis* (3.32±0.37 lg CFU/g), *H. pylori* (2.86±0.30 lg CFU/g) and *Streptococcus* spp. (2.76±0.38 lg CFU/g), which were more than control group values by 69.4% (p < 0.01), 95.9% (p < 0.001), 50.0% (p < 0.05) accordingly. In the less quantities the following microorganisms were sown: *Escherichia coli*
(1.87±0.22 lg CFU/g) and Candida spp. (1.19±0.15 lg CFU/g), exceeding the values of healthy persons by 19.9% (p’ 0.05) and 101.7% (p’ 0.001). The quantity of Lactobacillus spp. (1.21±0.14 lg CFU/g) was less than the control group data by 27.5% (p’ 0.05), Bifidobacterium spp. (0.76±0.12 lg CFU/g) – by 39.7% (p’ 0.05), Corynebacterium spp. (0.55±0.07 lg CFU/g) – by 3.5% (p’ 0.05). Micrococcus spp. were sown in the quantity of (0.69±0.07 lg CFU/g), exceeding the healthy persons values by 50% (p’ 0.01), Staphylococcus aureus (0.50±0.08 lg CFU/g) – by 8.3 (p’ 0.001), Neisseria spp. (0.45±0.06 lg CFU/g) – by 3.2 (p’ 0.001) Actinomyces spp. (0.42±0.07 lg CFU/g) – by 3.5 (p’ 0.001). Comprehensive evaluation of population level of microorganisms discovered the I degree of gastroduodenal region dysbacteriosis (GRD) in the case of 26.2% of patients, the II degree – in 14.3% of patients with acute non-atrophic gastritis.

In the 2nd group the largest quantities in the group in the average were determined in the case of the following microorganisms: Staphylococcus epidermidis (3.44±0.41 lg CFU/g), Streptococcus spp. (3.26±0.40 lg CFU/g), H. pylori (2.99±0.32 lg CFU/g), which were higher than control group values by 75.5% (p’ 0.01), 77.2% (p’ 0.01), 104.8% (p’ 0.001) accordingly. Just like in the 1st group, the following microorganisms were sown in less quantities: Escherichia coli (1.72±0.20 lg CFU/g) and Candida spp. (1.51±0.18 lg CFU/g), exceeding healthy persons values by 10.3% (p’ 0.05) and 155.9% (p’ 0.001). The quantity of Lactobacillus spp. (1.03±0.11 lg CFU/g) was less than the control group data by 38.3% (p’ 0.01), Bifidobacterium spp. (0.57±0.09 lg CFU/g) – by 49.4% (p’ 0.01), Corynebacterium spp. (0.49±0.06 lg CFU/g) – by 14% (p’ 0.05). Staphylococcus aureus were sown in the quantity (0.84±0.12 lg CFU/g), exceeding the values of healthy persons 14 times (p’ 0.001), the values of the 1st group – by 68% (p’ 0.05). The quantity of Micrococcus spp. (0.61±0.05 lg CFU/g) was more than control group values by 32.6% (p’ 0.05), Neisseria spp. (0.42±0.06 lg CFU/g) – 3 times (p’ 0.001) Actinomyces spp. (0.38±0.06 lg CFU/g) – 3.2
times (p’ 0.001). As a result, the I degree of GRD was established in the case of 45% patients, II degree – in the case of 15% patients having acute stage of focal-atrophic gastritis.

In the case of wide-spread atrophic gastritis the biggest average quantity in the group was determined in the case of *Streptococcus* spp. (4.09±0.36 lg CFU/g), which was more than values of control and the 1st group by 122.3% (p’ 0.001) and 25.5% (p’ 0.05). *Staphylococcus epidermidis* (3.58±0.40 lg CFU/g), as well as *H. pylori* (2.84±0.28 lg CFU/g) sown in the quantities exceeding values of healthy persons by 82.7% (p’ 0.001) and 94.5% (p’ 0.001). The quantity of *Escherichia coli*, increased in the 1st and 2nd groups, reduced with the progression of atrophic process, reaching the level of 1.28±0.12 lg CFU/g, which was less than the data of control and the 1st group by 17.9% (p’ 0.05) and 31.6% (p’ 0.05). The opposite dynamics was noted in the course of *Candida* spp. cultivation, their quantity in the 3rd group (1.99±0.16 lg CFU/g) exceeded the values of healthy persons suffering from non-atrophic and focal-atrophic gastritis by 237.3% (p’ 0.001), 67.2% (p’ 0.01) and 31.8% (p’ 0.05). *Staphylococcus aureus* were sown in the quantity of (1.08±0.15 lg CFU/g), 18 times exceeding the values of healthy persons (p’ 0.001), of the 1st group – by 116.0% (p’ 0.001), of the 2nd group – by 28.6% (p’ 0.05). The quantity of *Lactobacillus* spp. (0.94±0.09 lg CFU/g) was less than control group data by 43.7% (p’ 0.001), *Corynebacterium* spp. (0.40±0.05 lg CFU/g) – by 29.8% (p≤0.05), *Micrococcus* spp. (0.33±0.04 lg CFU/g) – by 28.3% (p’ 0.05). *Bifidobacterium* spp. were sown in the quantity of (0.34±0.05 lg CFU/g), which was less than in the control, the 1st and the 2nd groups – by 73% (p’ 0.001), 55.3% (p’ 0.01) and 40.4% (p’ 0.05) accordingly. Quantity level of *Neisseria* spp. (0.36±0.05 lg CFU/g) and *Actinomyces* spp. (0.35±0.05 lg CFU/g) exceeded values of healthy persons 2.6 and 2.9 times (p’ 0.001). In the course of comprehensive evaluation of population level of microorganisms the I degree of GRD disbacteriosis was
established in 57.5% of patients, II degree – of 27.5% of patients having spread atrophic gastritis.

Conclusion

Patients with chronic gastritis of the II-IV degree compared to clinically healthy persons showed change in frequency of sowing mucous microbiota of GMC in the form of extension of colonization by coccoid and rod-shaped bacteria and fungi of the genus *Candida*. General traits for comparison groups included increase of occurrence of *Staphylococcus aureus* – by 7.6–13 times, *Actinomyces* spp. and *Neisseria* spp. – by 2–2.9 times, *Candida* spp. – by 1.8–2.5 times, *Streptococcus* spp. – by 1.4–1.9 times, *H. pylori* – by 1.4–1.6 times, *Staphylococcus epidermidis* – by 1.4–1.5 times with the reduction of sowing of *Lactobacillus* spp. – by 1.3–1.5 times, *Bifidobacterium* spp. – by 1.6–2.6 times, *Corynebacterium* spp. – by 1.6–2.3 times. In the course of inflammatory process GMC compared to the control group showed prevailing of coccoid forms of microorganisms. With the increase of spread and expressiveness of atrophic process in GMC, reduction of the rate of ray fungi sowing occurs, as well as that of colon bacillus with normal saccharolytic activity, *H. pylori*, *neisseria*, *Staphylococcus epidermidis* – with retaining of the increased occurrence, as well as bifidus bacteria, coryneformic bacteria, lactobacilli – with the reduction of relatively morphologically unchanged GMC, increase of sowing of *Candida* spp., *Staphylococcus aureus*, streptococci.

In the case of the examined patients when comparing with healthy persons quantitative changes of mucous microbiota are observed as well. General traits for the comparison groups are increase in the quantity of *Staphylococcus aureus* – 8.3–18 times, *Actinomyces* spp. –2.2–3.5 times, *Candida* spp. and *Neisseria* spp. –2–3.3 times, *H. pylori* – 1.9–2 times, *Streptococcus* spp. –1.5–2.2 times, *Staphylococcus epidermidis* – 1.7–1.8 times, reduction of the quantity of *Bifidobacterium* spp. – 1.7–3.7 times, *Lactobacillus* spp. – 1.4–1.8 times.
Thus, increase of spread and expressiveness of atrophic process in GMC against the background of the active inflammation is accompanied by the development and increase of dysbiotic changes of GRD. In accordance with provision IV of Maastricht treaty [11], in the course of gastric hypochlorhydria developed in the case of atrophic gastritis, colonization of GMC with non-helicobacteria species is increased, capable of producing cancer causing metabolites. Taking into account the obtained and available scientific data, determination of the content of gastric parietal microbiota at the II-IV stage of chronic gastritis with further individual correction of gastric microbiocenosis may potentially contribute to regression of atrophic process and cancer prevention, which requires additional dynamic studies.
Fig. 1. Frequency of occurrence (in %) of microorganisms from gastrobiopsy material of the patients having chronic gastritis.
Fig. 2. Change of the quantity of microorganisms (in %) in gastrobiopsy materials of the patients having chronic gastritis.

Notes: average values of the control group are taken as 100%;
* – statistically significant changes when compared to the control group