Saudi Journal of Pathology and Microbiology (SJPM)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Study of Molecular-Genetic Characteristics of Helicobacter Pylory Infection in Uzbekistan

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Original Research Article

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Article History
Received: 15.04.2018
Accepted: 26.04.2018
Published: 30.04.2018

DOI:
10.21276/sjpm.2018.3.4.7

Abstract: This article presents results of the study on 100 patients with pathology of gastrointestinal tract (erosive bulbitis, nonspecific ulcerative colitis, chronic gastritis, ulcer disease and gastric cancer) in order to investigate association of these pathologies with different genotypes of Helicobacter pylori in Uzbekistan. The researches showed, that the most frequent association with Cag+ was noted in the patients with ulcer disease and gastric cancer, and in a less degree with erosive bulbitis and nonspecific ulcerative colitis.

Keywords: pathology, erosive bulbitis, ulcerative colitis, chronic gastritis.

INTRODUCTION

The modern medicine, as gastroenterology, develops on the basis of consensuses and agreements in the various fields of medicine. Concerning diseases associated with Helicobacter pylori (HP) this document is presented by Maastricht's Consensuses, where the principles of HP, diagnosis, indications and principles of eradication therapy are shown clearly. However these protocols should not be understood as dogma, because medicine is continuously developing, new facts appear and, it is interesting, every region has its own peculiarities in relation to prevalence of HP infection, her pathogenic characteristics and antibiotic resistance. Meanwhile Uzbekistan with other countries of our region is related to the countries with high level of contamination of the population with HP, achieving in some regions 60-80% and correlating with high frequency of gastric cancer.

At present time it is noted that in spite of high rate of infection with HP in many countries, not all have associated with this infection diseases, such as gastritis, gastric and duodenum ulcer, gastric cancer. Probably, that development of these diseases is connected with presence of genes of virulence in bacteria. That is, it is necessary to separate those genotypes of bacteria which induce development of gastric diseases and to define not dangerous and less dangerous genotypes. The full eradication of HP in the region where infection accounts for more than 80%, is practically impossible, because probability of the recurrent infection is high. Therefore, determination of HP virulence presenting in the stomach of patients seems to be one of methods for prevention of development of gastric diseases, particularly gastric cancer.

Purpose of our researches is determination of HP virulence in the patients with gastroenterological diseases in Uzbekistan.

MATERIALS AND METHODS

Investigation included 100 samples of biopsy from patients receiving stationary treatment at the department of gastroenterology of Joint-stock Company "RSSPMC and MP". Of them 4 patients were with erosive bulbitis 16 – with nonspecific ulcerative colitis, 14- chronic gastritis B, 30-gastroduodenal ulcer disease (GDUD) and 36 – gastric cancer, which were divided into 5 groups, according to nosology. From every biopsy sample there was selected genome DNA, with use of kits of reagents Diatom ™ DNA Prep 200 (manufacture of OOO "Laboratory IzoGen). The DNA isolation was performed under the standard protocol of DNA isolation for a set of reagents Diatom ™ DNA Prep 200 with updating of a stage of lysing till 16 hours at 37^{o}C. Supernatant with DNA underwent directly to genotyping by PCR-amplification. PCR analysis was performed with use of kit of reagents for PCR amplification of DNA GenePak ^{TM} PCR Core (manufacture of OOO "Laboratory IzoGen"). PCR amplification was performed by modified protocol. Typing of the DNA samples by genes CagA, VacA and IceA was made with use of specific oligonucleotide primers, presented in Table 1. The products of PCR amplification were visualized in 2% agarose gel within 1,5-2 hours at voltage 120 watt; were painted with

ethidium bromide, were visualized in ultraviolet-light and photographed in gel-documentary system.

Table-1: Typing of the DNA samples by genes CagA, VacA and IceA

Gene	Primer	Primer sequence (5' -- 3')	The size and site
Cog A	CAGAF CAGAR	GATAACAGGCAAGCTTTTGAGG CTGCAAAAGATTGTTTGGCAGA	349 (1228-1576)
Gene	Primer	Primer sequence (5' – 3')	the size and site
Cag A	CAGAF CAGAR	GATAACAGGCAAGCTTTTGAGG CTGCAAAAGATTGTTTGGCAGA	349 (1228-1576)

RESULTS

We genotyped 100 samples of biopsy by genes of virulent CagA, VacA, IceA, of them 57 samples were isolated in Khorezm province and Republic of Karakalpakstan, 43 samples in patients from Tashkent-city and Tashkent province. The method of direct genotyping of HP from biopsy material has been developed, its efficacy has been shown for 100 samples of biopsy.

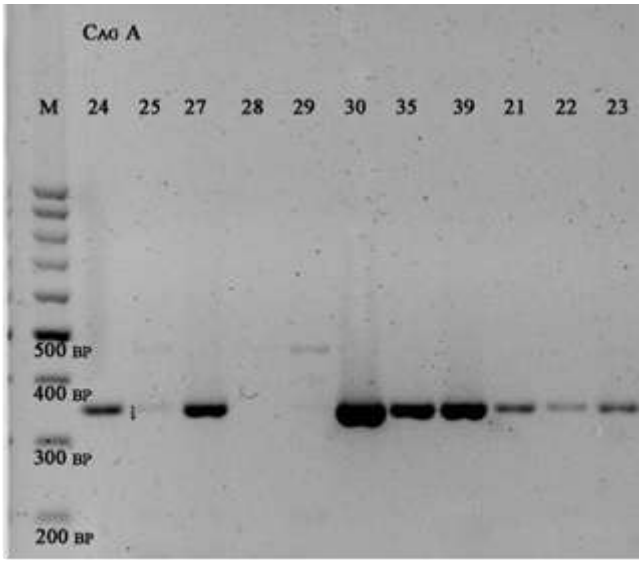

Fig-1: A gene of virulence CagA.

As the received results of genotyping show (Table-2) in 85 (85 %) from 100 samples the gene CagA was found out. Practically in all samples - 99 (99 %) there was verified gene VacA s.

Table-2: Distribution of genotypes CagA, VacA and IceA of H.Pylolri in general samples.

CagA	**Отриц (-)**	**Полож (+)**		
	15	85		
	15%	85%		
VacA s	**s1**	**s2**	**s1,2**	**s-**
	68	14	17	1
	68%	14%	17%	1%
VacA m	**m1**	**m2**	**m1,2**	**m-**
	24	58	14	4
	24%	58%	14%	4%
IceA	**A1**	**A2**	**A1,2**	**A-**
	22	9	60	9
	22%	9%	60%	9%

Each of 5 studied groups reflects a growing degree of severity of disease of the gastrointestinal tract, the heaviest of which is the gastric cancer. In the literature there are data, showing about association of CagA gene with various gastroduodenal pathologies.

The carried out researches shown, that the gene CagA had the following distribution: in group of the patients with erosive bulbitis (n=4) it was met in all samples, in group of the patients with nonspecific ulcerative colitis (n=16) it was determined in 11 samples, in group of the patients with gastritis (n=14) -

in 13 samples. In groups of sufferings from gastric and duodenal ulcer (n=30) and gastric cancer (n=36) CagA + genotype was verified in 28 and 29 samples, respectively (Fig-2).

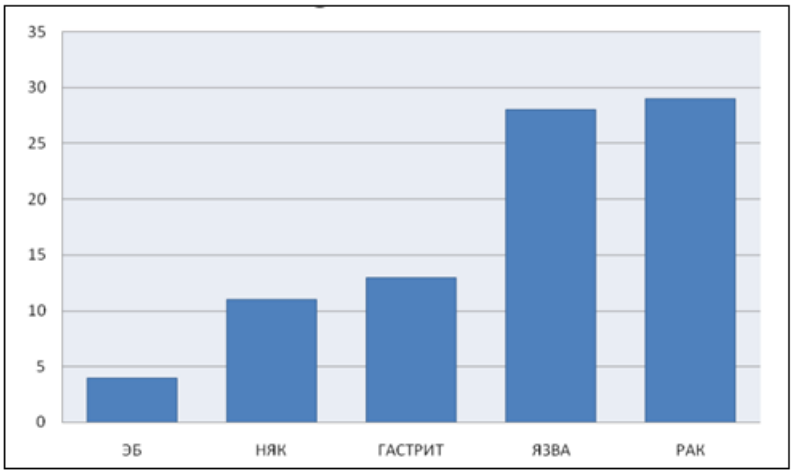

Fig-2: Distribution of CagA genotype in general sample in relation to groups with various diseases. (Erosive bulbitis; nonspecific ulcerative colitis; gastritis; ulcer; cancer)

It was impossible to count up reliability of distribution of so various in number samples for everyone separate pathology, because in the general sample there was absent control group of the healthy people, or not infected, but suffering from some gastroduodenal disorder. However Fisher's test allowed to count up reliability for general sample. As a zero hypothesis the probable association of CagA positive genotypes with gastroduodenal pathologies (for five in the given work) was considered. The value p has appeared high enough - $p = 0.206$ in $p \geq 0,05$, that accounted for 85% of reliability. The result of χ^2 - test – $\chi^2 = 6,67$ (degrees of freedom 4), under the table of quantiles we calculate probability, which is equal 0,154, that confirms reliable distribution in this research.

DISCUSSION

According to the literary data, the importance of notions about distribution of HP genotypes at various diseases can be caused by the following facts: strains of CagA have more significant influence on the prognosis of disease, than strains without CagA [1-3]; strains with a type s1VacA are more often associated with diseases of a stomach, than strains s2VacA [4]; BabA2 strains of HP are strictly connected with duodenal ulcer disease (P=0.0002) and adenocarcinoma (P=0.033) as against VacAs1 and CagA strains, which are associated only with duodenal ulcer disease (P=0.004); and at last, the efficiency of treatment also in many respects depends on a genotype of HP [5]. So, there are data showing, that some factors of H. pylori virulence can affect on the sensitivity of bacteria to antibacterial therapy. In particular, it is established, that CagA-positive strains are more susceptible to antibiotics in comparison with CagA-negative. Besides the presence of allele VacA s1m1 also increases HP sensitivity in comparison with allele VacA s2m2. Such вариабельность of sensitivity of the microorganism to antibiotics can be related to the more active division of CagA-positive and VacA s1m1 strains of bacterium.

The received data considerably differ from distribution of CagA in the Northern and East Europe - 71 %, but are comparable to results received in France and Portugal - 95 and 100 %, accordingly.

Thus, positive CagA strain is noted in 70 % of the patients with gastroduodenal pathology, more often at the patients from Tashkent-city. Opposite negative CagA strain is found two times more often at the patients of Khorezm province, in comparison with the patients from Tashkent. It is necessary to take into account the received results, while prescribing antichelicobacter therapy.

CONCLUSIONS

- Uzbekistan is related to the regions with high degree of contamination of the population with pathogenic strains of Helicobacter pylori with regard the mutations of genes of CagA-positive strains of Hp with type s1VacA.
- At developing local protocols of eradication therapy in Uzbekistan it is necessary to taking into account regional features of HP strains.

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