PCR DETECTION OF HELICOBACTER PYLORI GENOME IN COLONIC MUCOSA: NORMAL AND MALIGNANT

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Abstract: Background and Aims. The aim of this study was to detect Helicobacter pylori (H. pylori) in colorectal cancer tissue specimens and relate the possible role of this microorganism in the etiology of colorectal cancer.

Patients & Methods. From February 2002 to April 2003 83 CRC patients (55 male, 28 female) and 40 control patients (19 male, 21 female) entered the prospective study. The biopsy samples of CRC tissue and normal mucosa were obtained during open surgery on CRC patients. In the control patients biopsy samples were taken during colonoscopy. Pathology confirmed adenocarcinoma in all the CRC patients. The existence of genetic material of H. pylori was determined by detection of the ureA gene by nested PCR. K-ras PCR was also performed on all patients.
Results. *H. pylori* PCR was positive in 1 case (1.2%) of CRC in the tumour tissue and in 5 samples (6.0%) of the normal colonic mucosa in the cancer patients. The control patients were PCR positive to *H. pylori* in 13 samples (32.5%). According to the Chi-square test, there is no statistical correlation between *H. pylori* infection and CRC ($x^2 = 2.9395; p > 0.05$), but there is a significant prevalence of *H. pylori* infection in controls compared to CRC ($x^2 = 15.5625; p < 0.01$). The K-ras PCR showed gene mutations in 19 tumour tissues of CRC (31.6%) and in 2 cases (3.4%) of normal colonic mucosa of CRC patients. In controls K-ras PCR showed 1 gene mutation (3.0%). There is a significant statistical correlation between K-ras mutation and CRC ($x^2 = 16.0694; p < 0.01$).

Conclusion. Our established PCR for *H. pylori* is feasible for CRC tissue as well. However, *H. pylori* is not considered to play an important role in the pathogenesis of CRC. The identification of K-ras mutations in routine PCR analysis correlates with the presence of CRC.

Key words: *Helicobacter pylori*, PCR, Colorectal carcinoma.

Introduction

*H. pylori* is a microaerophilic Gram-negative spiral-shaped bacterium that infects 50% of the world population. Its prevalence varies widely in different parts of the world with average rates of 40–50% in western countries, rising to >90% in the developing world [1]. This microorganism [2] is associated with chronic gastritis, peptic ulcer and gastric adenocarcinoma development [3–5]. There is a significant geographical relationship between gastric cancer mortality rates and the prevalence of *H. pylori* infection. It has been proved that subtypes of *H. pylori* might differ in pathogenicity [6], and also the prevalence of *H. pylori* expressing the cytotoxin-associated gene A (cagA) in gastric cancer patients is significantly higher in age- and gender-matched controls [7, 8]. Previous studies reported positive [9, 10] and negative [11, 12] association between *H. pylori* infection and colorectal neoplasia.

Since the development of CRC is a multi-step process characterized by the accumulation of genetic alterations, scientists Fearon and Vogelstein set the model that assumes the involvement of the APC (Adenomatous Polyposis Coli) gene in adenoma formation and the K-ras oncogene in the transition from intermediate adenomas to carcinomas in sporadic CRC [13], but the involvement of environmental factors, as well as infective agents, cannot be underestimated. Recent reports suggest that *H. pylori* may be an association factor involved in colorectal cancer (CRC) development in patients infected with *H. pylori* strains [14, 15]. However, it is far from clear whether *H. pylori* is present in CRC tissues and whether or not *H. pylori* plays a similar role in colorectal carcinogenesis as has been proposed for gastric cancer development. 16S rDNA sequ-
ence analysis has demonstrated considerable genomic diversity among \textit{H. pylori} clinical isolates, and numerous sequence-specific PCR assays, combined with 16S rDNA sequencing, have been developed to identify \textit{Helicobacter} species [16, 17]. The development of PCR established a new possibility to detect the presence of \textit{Helicobacter pylori} in extragastric tissues as well. We have recently detected \textit{H. pylori} in bile and tissue of patients with cholangiocellular carcinoma [18]. The Swedish group of Grahn et al. [19] was the first which previously described the potential molecular identification of \textit{Helicobacter} DNA in CRC biopsies by means of a 16S rDNA PCR amplification assay combined with pyrosequencing analysis. Furthermore, Maggio-Price et al. [20] proved that infection of SMAD3$^{-/}$ mice with \textit{Helicobacter} triggers colon cancer in 50\% to 66\% of those animals.

The aim of this study was to detect \textit{H. pylori} in colorectal cancer tissue specimens and relate the possible role of this microorganism in the etiology of colorectal cancer.

\textbf{Material and methods}

\textit{Patients}

The study population consisted of 83 consecutive CRC (55 male, 28 female) and 40 control patients (19 male, 21 female), who were referred from February 2002 to April 2003 to the Belgrade Institute for Digestive Diseases, University Clinical Centre, for endoscopic evaluation and surgical treatment of CRC. The control group consisted of patients with 1) ulcerative colitis in clinical remission, 2) a previous history of polypectomy and 3) normal endoscopic finding (n = 15, 15, 10, respectively). Informed consent for the study was obtained from all patients, after approval of this study by the local ethics committee. At the time of endoscopy patients completed a standardized questionnaire providing information about life-style variables. All patients completed a questionnaire soliciting information on life-style factors, including diet, smoking, alcohol consumption, and coffee drinking. For smoking, we asked about the quantity of cigarettes and the years of smoking to calculate pack/years; for diet, we asked about red meat, fibre, fish or alcohol consumed; for coffee consumption, the number of cups per day was requested; we considered a chronic NSAID uptake as well; daily habits such as physical activity or stress were evaluated too. The final clinical diagnosis of the patients was ascertained after endoscopy in both groups of patients and confirmed in the experimental group after surgery. Histopathology confirmed adenocarcinoma in 81 and squamous-cellular carcinoma in 2 CRC patients. For all 40 control patients, histopathology presented with normal findings.

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Surgery and endoscopy

The Dukes classification and localization of colorectal carcinoma among the patients of the experimental group is described in Tables 1 and 2. Endoscopy prior to surgery excluded a presence of any synchronous carcinoma. During surgical treatment of the CRC patients’ samples of mucosa were taken from malignant tissue and from normal tissue as well. The surgical samples formed 5x5 mm cubes. After conservation in sterile tubes, mixed with 1cc RNA, and later stabilization solution (RNA later, Ambion) they were deep-frozen at – 40°C till the final PCR analyses.

Table 1 – Tabela 1

<table>
<thead>
<tr>
<th>Dukes classification</th>
<th>Dukes stadium</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>CRC patients</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2 – Tabela 2

<table>
<thead>
<tr>
<th>Localization of tumour</th>
<th>Localisation на тумор</th>
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<tbody>
<tr>
<td>Localization tumor</td>
<td>CRC patients</td>
</tr>
<tr>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

The biopsy samples from the control patients were obtained randomly during standard total colonoscopy using a 7Fr biopsy forceps. We usually took 10 biopsies for PCR and pathohistology. Those for PCR analyses were stored in the same medium as surgical samples, as described previously.

Cytology and Histology

Endoscopic and surgical biopsies of the normal colonic mucosa and CRC tissue were processed for PCR analysis and histology. Histologic examination of H&E-stained sections was done by pathologists blinded to infection status using a standard Zeiss Axiophot microscope for the presence of malignant cells.

PCR Analysis

In order to prove the feasibility and applicability of the PCR analyses, we designed two different PCR models for potential risk factors of CRC pathogenesis (K-ras and *H. pylori*).

*H. pylori*

To exclude methodological bias, the samples were analysed blindly in two different laboratories employing several different PCR strategies to detect *Helicobacter* species. After removal of RNA later, the tissue was rapidly frozen and crushed to produce readily digestible pieces. The processed tissue was placed in a solution of proteinase K and digestion buffer and incubated until most of the cellular protein was degraded. High-molecular-weight DNA was prepared from samples according to a recently established protocol [21]. In brief, 1 mL of aqueous layer was mixed with 1 mL of buffered phenol, pH 8.0, vortexed, and centrifuged at 9000 rpm for 10 minutes. The aqueous phase was transferred to a fresh tube containing an equal amount of a 1:1 phenol:chloroform mixture, vortexed, and centrifuged. This second step was repeated until the solution was clear. DNA was precipitated with sodium acetate and recovered with ethanol. The genomic material was further resuspended in TE buffer and finally dissolved. The DNA was then used for *H. pylori*, and furthermore for K-ras analyses as well. Different PCR assays were performed, covering the *urease A* gene and the 16S gene with three assays each. For *urease A*, a nested PCR was performed as described previously [22]. From the sample DNA, 100–200 mg were used as a template in a 50-µL PCR reaction containing 200 µM dNTPs, primer concentration of 20 pmol, and 1 U Taq polymerase (all from Pharmacia, Uppsala, Sweden). The PCR conditions were as follows: 15 cycles at 94°C for 1 minute, 54°C for 30 seconds, and 72°C 30 seconds. One tenth (5 µL) of the first-round PCR product was used for second-round PCR in a fresh tube containing the new set of primers and otherwise unchanged PCR conditions. A second round nested PCR was performed for another 25 cycles [22]. To amplify *Helicobacter* species-specific 16S rDNA from CRC samples, two different PCR strategies were performed. PCR primers were designed on the basis of published sequences of *H. pylori* (*H. pylori* 26695, section 122 of 134 of the complete genome) or adapted from previous publications [12, 20, 21]. The first PCR was performed by using either the highly conserved *Escherichia coli* 16S rDNA primer pairs C93 and C94 to obtain a 1465-base-pair (bp) amplicon, covering nearly the whole 16S rDNA gene, or by using the more *Helicobacter* specific primer pairs C95 and C96 to generate a 519-bp gene fragment. Nested PCR was then performed using the *Helicobacter* species-specific primer pairs C97 and C98 to obtain a 398-bp gene product. In brief, 2 µL of sample DNA were used as the template and added to a 50 µL reaction mixture using standard PCR con-
ditions (AmpliTaq with GeneAmp; Perkin Elmer, Roche Diagnostics, Mannheim). Both rounds of PCR were performed with an initial denaturation at 95°C for 10 minutes and a 40-cycle temperature profile consisting of heating to 94°C for 30 seconds, annealing at 55°C for 1 minute and 72°C for 2 minutes. PCR products were visualized by agarose gels, and specific gene products were extracted and purified (QIAquick PCR purification kit; QIAGen, Hilden). All Helicobacter species specific amplicons were sequenced subsequently (ABI PRISM 377 DNA Sequencer; Applied Biosystems, Weiterstadt) and compared with published sequences using the basic BLAST search programme. All PCR assays were performed at least in duplicate. In addition to the conventional PCR protocols described above for 16S rDNA amplification, two nested LightCycler (Roche Diagnostics, Mannheim) PCR assays were performed using 2-µL aliquots of the sample DNA as a template. The previously described primer pair HP-1/HP-2 and nested primers HP-3/HP-4 were designed to amplify a characteristic segment within the urease A gene of H. pylori (see Table 3) [23]. The Helicobacter genus-selective primer pair HG-1/HG-2 and nested primers HG-3/HG-4 were designed to amplify a characteristic segment within the 16S rDNA of all Helicobacter species (see Table 3). First-round and nested PCR reactions were performed under standard amplification mixture conditions, as recommended in the instructions for the LightCycler FastStart DNA Master Hybridization Probes (Roche Diagnostics). After an initial denaturation at 95°C for 10 minutes, the 40-cycle temperature profile consisted of heating at 20°C per second to 95°C with a 10-second hold, cooling at 20°C per second to 50°C with a 10-second hold, and heating at 20°C per second to 72°C with a 20-second hold. PCR products were analysed in a 1.5% agarose gel. Specific amplicons were subsequently purified for sequencing using the High-Pure PCR Product Purification kit (Roche Diagnostics).

Table 3 – Tabela 3

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer sequences for conventional PCR (†)</td>
<td>Helicobacter pylori ureA (‡‡)</td>
<td></td>
</tr>
<tr>
<td>Outer primers</td>
<td>Sense: 5’ GCC AAT GGT AAA TTA GTT CC 3’</td>
<td>312</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’TTA CTC CTT AAT TTT TAC 3’</td>
<td></td>
</tr>
<tr>
<td>Inner primers</td>
<td>Sense: 5’ TTC TTT GAA GTG AAT AGA TGC 3’</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’ ATA GTT GTC ATC GCT TTT AGC 3’</td>
<td></td>
</tr>
</tbody>
</table>

**Helicobacter** spp. 16S rDNA

Outer primers (amplicons 6595–5131 in GenBank AE000644)

- HP C93: 5’ AGA GTT TGA TYM TGG CTC AG 3’ ($\S$)
- HP C94: 5’ TAC GGY TAC CTT GTT ACG ACT TC 3’ ($\S$)

Outer primers (amplicons 5880–6398 in GenBank AE000644)

- HP C95: 5’ GCA ATC AGC GTC AGT AAT G 3’ ($\S\S$)
- HP C96: 5’ GCT AAG AGA TCA GCC TAT GC T 3’ ($\S\S$)

Inner primers (amplicons 5945–6342 in GenBank AE000644)

- HP C97: 5’ GCT ATG ACG GGT ATC C 3’ ($\S\S$)
- HP C98: 5’ GAT TTT ACC CCT ACA CCA 3’ ($\S\S$)

Primer sequences for LightCycler PCR ($\S$,¶)

**Helicobacter pylori** urease A

Outer primers (amplicons 361–780 in GenBank X17077)

- HP-1: 5’ GCC AAT GGT AAA TTA GTT 3’
- HP-2: 5’ CTC CTT AAT TGT TTT TAC 3’

Inner primers (amplicons 459–655 in GenBank X17077)

- HP-3: 5’ GCC GAC AGA CCG GTT CAA ATC GG 3’
- HP-4: 5’ GCC TTA AAT CCA AAG ATT CT 3’

**Helicobacter** spp. 16S rDNA

Outer primers (amplicons 236–610 in GenBank AF177475)

- HG-1: 5’ TAT GAC GGG TAT CCG GC 3’
- HG-2: 5’ ATT CCA CCT ACC TCT CCC A 3’

Inner primers (amplicons 280–572 in GenBank AF177475)

- HG-3: 5’ CTG AGA CAG GGT CCA GAC TC 3’
- HG-4: 5’ CAA ATG CAG TTC TRY RGT TAA GC 3’

Abbreviations: bp, base pairs; PCR, polymerase chain reaction; ($\dagger$) Base codes are standard International Union of Biochemistry codes for bases and ambiguity; ($\dagger\dagger$) See Monstein et al. (25); ($\S$) Adapted and modified from Lazcano-Ponce et al. (26); ($\dagger$) See Holzinger et al. (27); ($\dagger\dagger$) See Myung et al. (28); (¶) See Schnell and Schubert. (29);

**K-ras**

To extend our PCR analyses in order to compare their feasibility and sensibility, we used previously prepared high-molecular DNA according to standard PCR protocols. The *K-ras* PCR was performed as described previously [24]. In brief, the codon 12 flanking region was spanned by two primers covering a 99-bp fragment. A modification on the 3’ end of the upstream (5’) primer...

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lies on the last base of codon 11, creating an artificial MspI restriction endonuclease site in case the wild-type K-ras gene is present [24]. A 20-µl aliquot of the 50-µl PCR reaction product was used in a MspI enzyme (Boehringer Mannheim) digest for 2 h. If the wild type is present, the reaction yields a 78-bp and a 21-bp fragment. If the K-ras gene is mutated in codon 12, a double band will result, representing the undigested original 99 bp and 78 bp in addition to the 21-bp fragment. The result is visualized on a standard 3% agarose gel (NuSieve; FMC).

Biostatistics

Chi square and Fisher’s exact test analyses were performed with the SPSS 11.0.0 statistical software package. The results were considered to be significant at the level P < 0.05.

Results

The experimental (CRC) and control group were stratified firstly into three subgroups involving patients with a positive history of ulcerative colitis, polypectomies or normal. Statistically there is no difference between the subgroups according to either epidemiological, dietary or molecular-biology parameters, so we analysed CRC and control groups thereafter as homogenous.

There were significantly more males among the CRC patients, especially older than 60 yrs. High fat diet, red meat, diet without fish, stress, alcohol and smoking are found to be significantly important risk factors, while fibre diet, coffee consumption, physical activity, a positive familial history to a presence of CRC are presented as statistically non-significant factors. Among the clinical signs blood in stool and diarrhoea significantly correlate to CRC. The presence of ulcerative colitis correlates highly with the development of CRC, while a previous history of polypectomy does not correlate with further CRC (see Table 4).

Among our experimental and control groups there was no statistical difference in Helicobacter pylori serologic testing for IgG antibodies (x² = 0.342, p > 0.05). H. pylori PCR was positive in 1 case (1.2%) of CRC in the tumour tissue and in 5 samples (6.0%) of a normal colonic mucosa in the cancer patients. All patients who were positive to the H. pylori ureA gene on PCR were previously positive to the serologic H. pylori test. The control patients were PCR positive to H. pylori in 13 samples (32.5%). According the Chi-square test, there is no statistical correlation between H. pylori infection and CRC (x² = 2.767; p > 0.05), but there is a significant prevalence of H. pylori infection in controls compared to CRC (x² = 15.145; p < 0.01).
We successfully detected the presence of the K-ras gene in 61 (73.4%) malignant and 63 (75.9%) normal mucosal samples in the CRC patient group. In the control group the PCR K-ras was positive in 33 (82.5%) samples. There is no statistical difference in PCR K-ras positivity between CRC malignant and/or normal mucosa compared to the control group ($x^2 = 1.215, p > 0.05$; $x^2 = 1.866, p > 0.05$, respectively). The K-ras PCR showed gene mutations in 19 tumour tissues of CRC (31.6%) and in 2 cases (3.4%) of normal colonic mucosa of CRC patients. In the controls K-ras PCR showed 1 gene mutation (3.0%). There is a significant statistic correlation between K-ras mutation and CRC malignant samples compared to the control group ($x^2 = 8.243; p < 0.01$), but also compared to CRC malignant and normal mucosa among CRC patients ($x^2 = 15.755; p < 0.01$).

Table 4

<table>
<thead>
<tr>
<th>Potential CRC risk factors</th>
<th>Cases (n = 83)</th>
<th>Controls (n = 40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SD)</td>
<td>61.2 ± 10.2</td>
<td>49.5 ± 15.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Females</td>
<td>28 (33.7%)</td>
<td>21 (52.5%)</td>
<td>0.052</td>
</tr>
<tr>
<td>High fat</td>
<td>49 (59.8%)</td>
<td>12 (30.0%)</td>
<td>0.0035</td>
</tr>
<tr>
<td>Red meat</td>
<td>75 (90.4%)</td>
<td>22 (55.0%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fish</td>
<td>16 (19.3%)</td>
<td>32 (80.0%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fibre</td>
<td>52 (62.7%)</td>
<td>30 (75.0%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Stress</td>
<td>63 (75.9%)</td>
<td>6 (15.0%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Physical activity</td>
<td>23 (27.7%)</td>
<td>13 (32.5%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Heredity</td>
<td>24 (28.9%)</td>
<td>6 (15.0%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Colon polyps</td>
<td>18 (21.7%)</td>
<td>15 (37.5%)</td>
<td>0.082</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>25 (30.1%)</td>
<td>15 (37.5%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>58 (69.9%)</td>
<td>15 (37.5%)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Alcohol</td>
<td>41 (49.4%)</td>
<td>3 (7.5%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Coffee</td>
<td>40 (48.2%)</td>
<td>27 (67.5%)</td>
<td>0.054</td>
</tr>
<tr>
<td>Smoking</td>
<td>25 (62.5%)</td>
<td>25 (62.5%)</td>
<td>0.034</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>22 (26.5%)</td>
<td>11 (27.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Constipation</td>
<td>26 (31.3%)</td>
<td>21 (52.5%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>55 (66.3%)</td>
<td>12 (30.0%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Blood in stool</td>
<td>63 (75.9%)</td>
<td>19 (47.5%)</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

Discussion

Previous studies based on seroepidemiological analyses have indicated that infection with *H. pylori* is a risk factor for gastric cancer and that antibodies against *H. pylori* are present in patients with colorectal polyps and adenocarcinoma.
nomas [4, 13]. However, even though measurement of circulating \textit{H. pylori} antibodies is commonly used and highly specific it may not always reflect a real-time \textit{H. pylori} infection [12]. On the other hand, the non-invasive \textit{H. pylori} stool tests including all three stool antigen tests (Premier Platinum HpSA, Amplified IDEIA HpStAR and ImmunoCard STAT!HpSA) in the post-treatment setting were slightly inferior to that of the UBT test and serology, with monoclonal antibody-based tests showing better results. It is still unclear as to whether or not \textit{H. pylori} infection is an association factor in colorectal cancer development [14, 15].

Shmuely \textit{et al.} [15] tested serum IgG antibodies against \textit{H. pylori} (ELISA) and CagA protein (Western blot assay) in patients with colorectal adenocarcinoma, gastric adenocarcinoma and with other malignancies (cancer controls). Among patients infected with \textit{H. pylori}, CagA+ seropositivity is associated with an increased risk of both gastric and colonic cancer. This finding should stimulate additional research into the role of cagA+ \textit{H. pylori} infection in the development of colorectal cancer.

The study of Grahn \textit{et al.} [19] considered PCR amplification and subsequent pyrosequencing analysis to reveal the presence of \textit{Helicobacter}-specific 16S rDNA variable V3 region sequences in CRC biopsy specimens. \textit{Helicobacter} DNA was identified in 11 out of 42 (26%) colon cancer and 10 out of 35 (29%) rectum cancer biopsies. The difference in \textit{Helicobacter} DNA prevalence between colon and rectum tumour biopsies was not significant. There was also no significant difference found in \textit{Helicobacter} DNA prevalence between Dukes’ classes A/B and C/D. Moreover, no significant difference in \textit{Helicobacter} DNA prevalence according to gender was observed. This study, despite the fact that that control group was not designed, firstly confirmed the potential of the \textit{Helicobacter}-specific PCR amplification assay used combined with real-time pyrosequencing to detect and subtype \textit{Helicobacter} DNA in biopsy specimens.

Our study detected only one positive sample of malignant tissue and 5 of normal tissue in CRC patients, compared to 13 positive control patients. The increased incidence of \textit{H. pylori} positive PCR among control patients could be explained with a large number of endoscopic biopsies during colonoscopy from 10 randomly taken from a different part of the large bowel, which could be responsible for the higher possibility of detection of \textit{H. pylori} genomic material.

The animal study of Maggio-Price \textit{et al.} [20], using real-time PCR, found that \textit{Helicobacter} organisms concentrate in the caecum, the preferred site of tumour development. Mucinous adenocarcinomas developed 5 to 30 weeks after infection. These results suggest that bacteria may be important in triggering colorectal cancer, notably in the context of gene mutations in the TGF-B signalling pathway, one of the most commonly affected cellular pathways in colorectal cancer in humans.
The study of Limburg et al. [31] found that smoking and CagA sero-positivity together could lead to an increased risk of CRC development. In our study it was found that smoking strongly correlates with CRC, as well as age, gender, high fat diet, red meat, a diet without fish, stress and alcohol consumption.

Since all our patients who were positive to \textit{H. pylori} ureA gene on PCR were previously positive to the serologic \textit{H. pylori} test, we can observe that there should be no possibility of contamination. On the other hand there were no PCR \textit{H. pylori} positive samples that showed a negative serologic test. In our study we combined the PCR analysis to a presence of \textit{H. pylori} and K-ras mutation in CRC patients and controls. Our established PCR for \textit{H. pylori} is feasible for CRC tissue as well. However, \textit{H. pylori} is not considered to play an important role in the pathogenesis of CRC. The identification of K-ras mutations in routine PCR analysis correlates with the presence of CRC.

REFERENCES


Р е з и м е

**PCR-ДЕТЕКЦИЈА НА ГЕНОМОТ НА HELICOBACTER PYLORI КАЈ МУКОЗА НА КОЛОН: НОРМАЛЕН И МАЛИГНЕН**

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Основа и цели: Целта на оваа студија беше да се открива Helicobacter pylori (H. pylori) во примероците на колоректално ткиво на канцер и да се поврзе можната улога на овој микроорганизам во етиологијата на колоректалниот канцер.

Пациенти и методи: Од февруари 2002 до април 2003 година, 83 пациенти со CRC (55 од мажки пол, 28 од женски пол) и 40 контролните пациенти (19 од мажки пол, 21 од женски пол) влегаа во оваа студија. Примероците од биопсните на ткивото на CRC и на нормалната мукоза беа добени за време на отворена хируршка интервенција на пациенти со CRC. Контролните пациенти примероците од биопсии беа добени за време на колоноскопија. Патологијата потврди аденокарцинома кај сите пациенти со CRC. Присуството на генетски материјал на H. pylori беше открено со откривање на генот на ureA со вгнездени PCR. Исто така, беше извршен K-ras PCR на сите пациенти.

Резултати: H. pylori PCR беше позитивен во eden случај (1,2%) од CRC, a примероците на CRC беше откривене во pet примероци (6,0%) од нормалната мукоза на колон кај пациентите со канцер. Контролните пациенти беа PCR позитивни на H. pylori кај 13 примероци (32,5%). Според тестот Chi-square, нема статистичка корелација меѓу инфекцијата H. pylori и CRC (x² = 2,9395; p > 0,05), но нема значителна преваленци на инфекцијата H. pylori кај контролните споредено со CRC (x² = 15,5625; p < 0,01). K-ras PCR покажа генетска мутација кај 19 примероци од CRC (31,6%) и во dva случаи (3,4%) од нормалната мукоза на колон пациенти со CRC. Контролните K-ras PCR покажа edna генетска мутација (3,0%). Има значителна статистичка корелација меѓу мутацијата K-ras и CRC (x² = 16,0694; p < 0,01).

Заклучок: Нашот констатиран PCR за H. pylori може да се примени и на CRC ткивото, макар и не се смее дека H. pylori има важна улога во патогенезата на CRC. Идентифицирањата на K-ras мутациите при рутинска PCR анализа е во корелација со присуството на CRC.

Клуви зборови: Helicobacter pylori, PCR, колоректално ткиво на канцер.

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