Attempts to detect Helicobacter pylori in atherosclerotic plaques

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Abstract

Cardiovascular and cerebrovascular diseases are regarded to be the main causes of mortality in developed countries, atherosclerosis being at their pathological base. During the recent years, attention was paid to the role of bacterial infections, including Helicobacter pylori, in the process of atherogenesis and coronary heart disease development. The aim of the study was an evaluation of H. pylori presence - by means of PCR technique - in atherosclerotic changes, obtained by endarterectomy, performed during coronary artery bypass grafting (CABG). In the analysed group of patients, the following risk factors were found: hyperlipidaemia, smoking, hypertension, obesity, diabetes mellitus, cardiac infarction. No DNA of the bacteria was traced in any of the patients.

Key words: Helicobacter pylori, PCR technique, atherosclerotic plaques.

Introduction

Inflammatory factors play an important role in the pathogenesis of atherosclerosis. In patients with coronary heart disease, increased levels of C-reactive protein, TNF-α, interleukin 1, 6 and fibrinogen are observed. Since, in some patients with coronary heart disease, no common risk factors are reported (such as: hypertension, smoking, overweight, hypercholesterolaemia, genetic predispositions) viral (Cytomegalovirus, Hepatitis A, Herpes simplex type 1 and 2) and bacterial (Chlamydia pneumoniae and Helicobacter pylori) participation has been suggested in the development of atherosclerosis. These microorganisms can directly invade the vascular endothelium, inducing inflammatory response, or secrete endotoxins with either local or systemic impact [1]. The influence of infection with Helicobacetr pylori on the development of vascular diseases has not yet been explained. The results of serological analyses indicate bacterial presence in 50% of the adult population. The occurrence of antibodies in blood serum is not to be interpreted as a continuous infection or as a constant exposition of the cardiovascular system to pathogenic factors [2, 3, 4].

Our study attempted at finding DNA of Helicobacter pylori in atherosclerotic plaques, collected by endarterectomy, performed during coronary artery bypass grafting (CABG).

Material and methods

The material for the study (atherosclerotic plaques) was collected from twenty-one (21) patients (20 male and 1 female) in age between 42-73 years (the mean age: 57 years) with coronary heart disease, diagnosed by coronarography, operated at the Clinic of Cardiosurgery, Medical University of Bialystok between the years 1998-2003. Operated patients were seropositive for H. pylori and were determined risk factors of coronary heart disease. All of the patients were qualified to implantation of aortal-coronary bypasses because of extensive atherosclerotic changes in the coronary arteries and disqualification for transcutaneous interventions. In eighteen (18) patients, CABG was performed within schedule, while in the other three (3), the surgery was accelerated because of unstable course of the coronary heart disease. The decision about endarterectomy was intraoperatively made, on the basis of the coronary artery morphology and at the planned spot of distal bypass grafting. Cylinder-shaped atherosclerotic change was obtained by preparation, performed from the side of the adventitia, following a longitudinal incision of the artery. The collected material was placed

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in 10% solution of buffered formalin and transported to the Department of Clinical Molecular Biology at the Medical University of Bialystok. After 12-hour fixing, the material was routinely handled and embedded into paraffin blocks. For DNA isolation, 10 paraffin sections (5 µm thick) were placed in 1.5 ml Eppendorf tubes and deparaffinated in room temperature, using xylene - a mixture of xylene with chloroform (1:1) and absolute ethanol. DNA was isolated and purified by the phenolic-chloroform technique. The purified and dried DNA sediment was dissolved in 30 µl of elution buffer, incubated through the night in room temperature and then stored in -20^{0}C until further assay. During DNA isolation, also negative control was set up. PCR reaction was performed for a fragment of vacA Helicobacter pylori gene (specific vacuolating gene) of 229 bp in length, using a pair of primers: VAC3624F and VAC3853R (the sequence of the primers: VAC3624F GAG CGA GCT ATG GTT ATG AC; VAC3853R ACT CCA GCA TTC ATA TAG A). The obtained reaction mixture of 10 µl in volume contained: buffer x 10, dNTP (40 mM), the VACF primer (12 pmol/µl) the VACR primer (12 pmol/µl), Red polymerase (1U/µl), H_20_{dest}, DNA solution - 2ml. The thermal profile of the reaction was carried out for 40 cycles (94^{0}C - 30 s, 51^{0}C - 1 min, 72^{0}C - 30 s). The effectiveness of PCR technique was determined on the material from gastric biopsy specimens, fixed and embedded into paraffin blocks, identically as the atherosclerotic plaques, in which the presence of H. pylori had been found. Those samples stood for positive control. The PCR reaction was twice repeated. In order to visualise the product of PCR, electrophoresis was performed in 1.8% agarose gel with an addition of ethydine bromide (room temperature, voltage = 120V).

Results

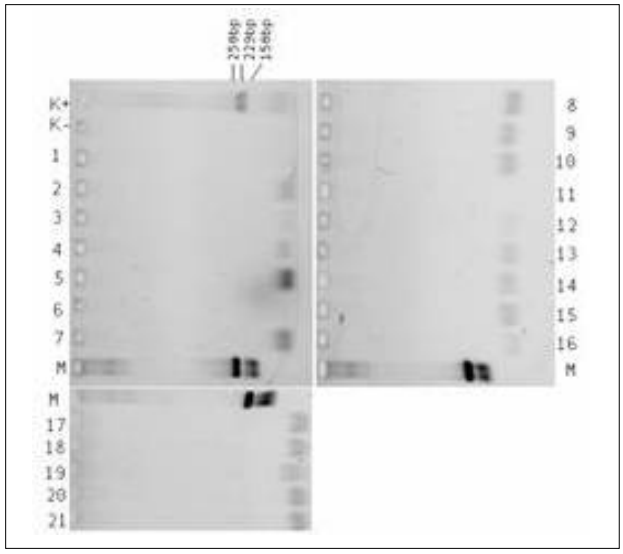
Demographic data demonstrated that hyperlipidaemia was the most frequent risk factor (67%) of coronary heart disease, followed by smoking (62%) and hypertension (52%). In 25% of the patients, diabetes mellitus was diagnosed, while 20% of them revealed obesity. Two thirds of the patients had cardiac infarction in history. The above factors occurred, regardless of the presence of Helicobacter pylori in atherosclerotic changes, as no genome of the bacteria was traced in the analysed material (Fig.1).

Discussion

Infection with H. pylori may be associated with a number of parenteral diseases, including - among others - autoimmunising diseases, skin diseases, hepatic encephalopathy, the sudden infantile death syndrome and atherosclerosis of the coronary arteries [5]. Bacterial DNA can be detected in host's tissues by means of a very sensitive method - PCR.

In the performed studies, no presence of H. pylori genome was observed in any of the evaluated atherosclerotic change. The obtained results were concordant with some of the earlier reports. Neither Blasi et al. nor Dore et al. found the genome of H. pylori in any of their studied samples, collected from the aorta, the abdominal aorta, the carotid artery and the femoral

Figure 1. Amplification products of H. pylori control and negative samples in agarose gel.



M - DNA molecular weight marker (250 bp and 150 bp),
K^+ positive control (229 bp),
K^- negative control, 1-21 negative samples.

artery [2, 3, 4]. Danesh et al. identified bacterial DNA in 1, out of 36 analysed cases only [6]. In turn, Farsak et al. obtained positive results in 37% of evaluated arteries [7].

Contradictory and discrepant results of studies, as presented in the reports, may have resulted from a focal localisation or periodical (transient) colonisation of tissues by H. pylori. The mechanism of blood vessel colonisation by this bacteria has not yet been unveiled. A finding of H. pylori DNA in the oral cavity, the liver, the bile tract or in atherosclerotic plaques may then turn out to be a false positive result. The bacteria is killed in blood serum, while free DNA in peripheral circulation may influence the false-positive results in tissues. Employing PCR for 16S rRNA and vacA genes, the presence of bacterial DNA in circulation was found in 65% and 50% of infected patients, respectively, while not a trace of that DNA was observed in the control group [8].

In the analysed group of patients (20 males and 1 female), we found the following risk factors of vascular diseases: hyperlipidaemia, smoking, hypertension, diabetes mellitus, obesity and cardiac infarction, while neither chronic peptide ulcer disease nor gastric carcinoma were noted in that reference. We cannot exclude an association of the above mentioned factors with H. pylori infection, although no bacterial DNA was found in any of the studied samples.

In 18 cross-sectional studies, each involving, at least, 500 persons, no unequivocal results were presented, either. The evaluation included, among others: systolic and diastolic blood pressure, the body mass index, plasma viscosity, total cholesterol concentration and the concentrations of glucose and C-reactive protein. The majority of the studied parameters did not show any statistically significant differences between the patients and the controls. Only a slight increase of plasma viscosity and glucose concentration was observed in the group of patients [9]. The participation of Helicobacter pylori in the ischaemic heart disease has not been unequivocally confirmed. The few reports,

which mention the presence of the bacterial genome in atherosclerotic changes, may not be regarded as evidence for a direct engagement of the bacteria in the pathogenic process - the bacteria in question may be, but a harmless commensal, localising itself in pathologically changed tissues.

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