Pancreatology 16 (2016) 865–868

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Helicobacter pylori is undetectable in intraductal papillary mucinous neoplasm

Birol Baysal [a, 1], Ali Tüzün İnce [a, *, 1], Bilge Gültepe [b], Zuhal Gücin [c], Fatma Ümit Malya [d], Mukaddes Tozlu [a], Hakan Şentürk [a], Pelin Bağcı [e], Çiğdem Ataizi Çelikel [e], Fügen Aker [f], Selvinaz Özkara [f], Esra Paşaoğlu [g], Nevra Dursun [g], Banu Yılmaz Özgüven [h], Deniz Tunçel [h]

[a] *Gastroenterology Department, Bezmialem Vakıf University, Istanbul, Turkey*
[b] *Microbiology Division, Bezmialem Vakıf University, Istanbul, Turkey*
[c] *Pathology Division, Bezmialem Vakıf University, Istanbul, Turkey*
[d] *Surgery Department, Bezmialem Vakıf University, Istanbul, Turkey*
[e] *Pathology Division, Marmara University, Istanbul, Turkey*
[f] *Pathology Division, Haydarpaşa Numune Education and Research Hospital, Istanbul, Turkey*
[g] *Pathology Division, İstanbul Education and Research Hospital, Istanbul, Turkey*
[h] *Pathology Division, Şişli Etfal Education and Research Hospital, Istanbul, Turkey*

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form
8 June 2016
Accepted 9 June 2016
Available online 13 June 2016

Keywords:
Hpylori
Intraductal papillary mucinous neoplasm
Pancreatic ductal adenocarcinoma

ABSTRACT

Background: About half of the world population is infected with *Helicobacter pylori* (*H. pylori*), a bacterium associated with gastric cancer and considered to be a risk factor for pancreatic ductal adenocarcinoma. Whether the bacterium is associated with intraductal papillary mucinous neoplasm, believed to be a precursor of pancreatic ductal adenocarcinoma, is unknown. The aim of this study was to investigate the presence of *H. pylori* DNA in tissue sections of intraductal papillary mucinous neoplasm.
Methods: The presence of *H. pylori* DNA was tested in a retrospective controlled study of formalin-fixed, paraffin-embedded pancreatic tissues from 24 patients who underwent surgery for intraductal papillary mucinous neoplasm. Histologically normal tissues surrounding neoplasms were used as control. *H. pylori* DNA was evaluated after deparaffinization, DNA extraction, and purification, and results were evaluated statistically.
Results: Samples were collected from 13 males and 11 females with mean age 59 years (range 44–77), and consisted of 19 cases of main-duct and three cases of branched-duct intraductal papillary mucinous neoplasm. Two patients were diagnosed with pancreatic cancer and main-duct intraductal papillary mucinous neoplasm. *H. pylori* DNA was not detected either in intraductal papillary mucinous neoplasm tissue, or in surrounding normal tissue.
Conclusions: Although *H. pylori* has been implicated in pancreatic ductal adenocarcinoma, it may not play a key role in the development of intraductal papillary mucinous neoplasm.

1. Introduction

Helicobacter pylori (*H. pylori*) is a motile, gram-negative microaerophilic organism that frequently infects humans worldwide [1]. The association between *H. pylori* infection and peptic ulcer disease, gastric cancer, and mucosa-associated lymphoid tissue lymphoma is well-established [2]. A growing body of research also now suggests a relationship between *H. pylori* and pancreatic cancer, acute, chronic, and autoimmune pancreatitis [3–17]. In particular, *H. pylori* is considered a risk factor for pancreatic ductal carcinoma, as has been shown in some demographic studies [3,4], but its association with intraductal papillary mucinous neoplasm has not been analyzed.

Intraductal papillary mucinous neoplasm, a cystic neoplasm with characteristic mucinous secretion [18,19], is believed to be a

* Corresponding author. Gastroenterology Clinic, Bezmialem Vakif University Medical Faculty, Istanbul, Turkey.
E-mail address: dralince@gmail.com (A.T. İnce).
[1] Ali Tüzün İnce and Birol Baysal contributed equally to this study.

http://dx.doi.org/10.1016/j.pan.2016.06.005
1424-3903/

precursor to pancreatic ductal carcinoma [18,20–22]. The neoplasm originates from the main or side branches of the pancreatic duct epithelium [18,19], and is classified accordingly into three types (main-duct, branched-duct, and mixed), and four histological subtypes (intestinal, pancreatobiliary, oncocytic, and gastric). Notably, intraductal papillary mucinous neoplasm accounts for 20–50% of all cystic neoplasms [22], but is observed in only about 2–20% of pancreatic cancers [23]. Nevertheless, the World Health Organization recommends that these lesions be classified by histological evaluation as benign, borderline, or invasive. The neoplasm is frequently observed in patients >60 years old [24], with age- and sex-adjusted cumulative incidence 2.04/100,000 (95% confidence interval 1.28–2.80) and point prevalence 25/100,000 [20]. The incidence continues to increase due to recent advances in diagnostic modalities, better understanding of pathology, and better surveillance [19].

The pathogenesis of intraductal papillary mucinous neoplasm is unclear [18]. We hypothesize that *H. pylori* may be involved. For instance, antral *H. pylori* colonization reduces D-cell numbers, suppresses somatostatin production, and triggers hyperacidity. In turn, hyperacidity increases production of pancreatic bicarbonate, and induces release of secretin, a hormone that stimulates growth in the rat pancreas [25] and DNA synthesis in ductal cells. This stimulation may ultimately result in the development of intraductal papillary mucinous neoplasm. Nevertheless, the causative relationship between *H. pylori* and intraductal papillary mucinous neoplasm has not been previously evaluated to the best of our knowledge. The purpose of this study was to investigate the presence of *H. pylori* DNA in paraffin-embedded pancreas tissues from patients with intraductal papillary mucinous neoplasm.

2. Materials and methods

2.1. Samples

A total of 24 formalin-fixed, paraffin-embedded pancreatic tissue samples were selected from specimens archived between 2012 and 2014 in pathology laboratories at Bezmialem Vakif University Faculty of Medicine (n = 10), Marmara University Faculty of Medicine (n = 4), Istanbul Education and Research Hospital (n = 4), Şişli Etfal Education and Research Hospital (n = 3), and Haydarpaşa Education and Research Hospital (n = 3). All laboratories have quality standard certificates, and tissue blocks were stored at the appropriate temperature and humidity. One pathologist first examined the blocks to confirm the diagnoses, and then sectioned the blocks for DNA analysis. Samples were obtained with approval from the Ethics Committee of the Faculty of Medicine, Bezmialem Vakif University (Ethical Committee ID: 71306642/050-01-04/259–17.09.2014).

Tissues were from patients who underwent surgery for intraductal papillary mucinous neoplasm, and who were diagnosed with *H. pylori* by endoscopic biopsy. Z.G., a pathologist, reviewed all samples before deembedding. Tissue samples were predominantly main-duct intraductal papillary mucinous neoplasm. Adjacent histologically normal tissues were used as control, so that a total of 48 samples, two from each patient on average, were analyzed.

2.2. Tissue preparation

Excess paraffin was trimmed from each tissue block using a scalpel, and samples were then cut into six sections 20 μm thick. Sections were then stored at 4 °C until further processing, in two 1.5 mL Eppendorf tubes with six serial sections each. Samples were deparaffinized over three cycles of immersion in 1 mL xylene and separation from the resulting supernatant by centrifugation at 12,000 rpm for 5 min. Specimens were then dehydrated in 500 μL graded ethanol (pure ethanol, 95% ethanol, and 70% ethanol), discarding each wash after centrifugation at 12,000 rpm for 5 min. Finally, samples were air-dried.

2.3. DNA extraction

DNA was isolated from deparaffinized samples on an automated QIAcube system (Qiagen, Germany), using QIAamp DNA FFPE Tissue Kit (Qiagen, Germany). Purified DNA was stored at −20 °C until analysis. The kit is especially designed for formalin-fixed, paraffin-embedded tissue sections, and is based on special lysis conditions that minimize formalin crosslinking of nucleic acids. The kit also uses QIAamp MinElute spin columns to obtain high-quality DNA in small volumes. Sample processing was validated using positive and negative controls supplied with the kit.

2.4. DNA amplification

Presence of *H. pylori* was tested using *H. pylori* RG quantitative real-time PCR Kit (Genome Diagnostics Pvt. Ltd., İndia), following the manufacturer's instructions. Assays were performed by an individual blinded to samples.

2.5. Statistical analyses

Mean, standard deviation, range, and percentage are reported, where appropriate. Data were analyzed in SPSS version 19 for Windows (SPSS Inc., Illinois, Chicago, USA), with *p*-values less than 0.05 considered to be statistically significant.

3. Results

We analyzed formalin-fixed, paraffin-embedded pancreatic tissue sections from patients (n = 24) with intraductal papillary mucinous neoplasm. The patient population consisted of 11 females and 13 males, with mean age 59 years and range 44–77 years. All patients had undergone potentially curative or palliative surgery. Most patients (n = 19) were diagnosed by histology with the main-duct form, while the branched-duct form was observed in three samples. Two patients had both pancreatic cancer and main-duct intraductal papillary mucinous neoplasm. *H. pylori* was not detected in tumors or adjacent normal tissues.

4. Discussion

H. pylori, the prototype species of the genus *Helicobacter*, has been the subject of great interest in the last 30 years. The bacterium causes acute and chronic gastritis, peptic ulcer [26], gastric cancer [1,2], and gastric lymphoma in humans [27]. Its possible link to pancreatic disorders is also being actively investigated, although such studies are very rare [14,15,25], because samples of pancreatic tissue and pancreatic juice are not readily available. The potential relationship between *H. pylori* and pancreatic cancer was first suggested by serology-based studies [3,4], in which individuals infected with *H. pylori* were found to be at two-fold higher risk of pancreatic cancer [3]. These results were eventually confirmed in a prospective cohort enrolled in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study [4].

In addition, ribosomal DNA from *H. pylori, H. bilis, H. hepaticus, H. sp. flexispira,* and *H. cineadi* was detected in tumor and/or surrounding tissues in 75%, 57%, 38%, and 60% of patients with pancreatic ductal carcinoma, neuroendocrine cancer, multiple endocrine neoplasia, and chronic pancreatitis, respectively [14]. In particular, *H. pylori* DNA was detected in the pancreas in 19% of