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Hepatitis B virus markers in hepatitis B surface antigen negative patients with pancreatic cancer: case series

HBV in Pancreatic Cancer Development

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Abstract

BACKGROUND

Background: Hepatitis B virus (HBV) is a known carcinogen that may be involved in pancreatic cancer (PC) development. Detection of HBV biomarkers (especially - expression of HBx) within the tumor tissue may provide direct support for this. However, there is still a lack of such reports, particularly in non-endemic for HBV infection regions.

Aim: to present two cases of patients with pancreatic ductal adenocarcinoma, without history of viral hepatitis in whom the markers of HBV infection were detected in blood and in the resected pancreatic tissue.

CASE SUMMARY

The results of examination of two patients with pancreatic cancer, who gave informed consent for participation and publication, were the source for this study. Besides standards of care, special examination to reveal occult viral hepatitis B infection was performed. This included blood tests for HBsAg, anti-HBc, anti-HBs, HBV DNA, and pancreatic tissue examinations with polymerase chain reaction for HBV DNA, pgRNA HBV, cccDNA and immunohistochemistry staining for HBxAg and Ki-67.

Both subjects were operated on due to pancreatic ductal adenocarcinoma and serum HBsAg was not detected. However, in both of them anti-HBc antibodies were detected in blood, although HBV DNA was not found. Examination of the resected pancreatic tissue gave positive result for HBV DNA, expression of HBx and active cellular proliferation by Ki-67 index in both cases. However, no pregenomic HBV RNA and covalently closed circular DNA HBV were detected in case 2.

CONCLUSION

These cases may reflect potential involvement of HBV infection in the development of pancreatic cancer.

Key Words: Pancreatic cancer; Pancreatic ductal adenocarcinoma; Hepatitis B virus; Previous hepatitis B; Anti-HBc; HBx antigen

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Core Tip: Hepatitis B virus (HBV) is a known carcinogen that may be involved in pancreatic cancer (PC) development. Detection of HBV biomarkers (especially - expression of HBx) within the tumor tissue may provide direct support for this. However, there is still a lack of such reports, particularly in non-endemic for HBV infection regions. In the report of case series, we present two cases of HBsAg-negative patients with pancreatic ductal adenocarcinoma, in whom the markers of HBV were detected in blood and in the tumor tissue. This reflects potential participation of the virus in the etiology and pathogenesis of pancreatic ductal adenocarcinoma.

INTRODUCTION

Pancreatic cancer (PC) is one of the most prevalent cancers worldwide and its incidence rate is growing [1]. Among different types of pancreatic cancer, pancreatic ductal adenocarcinoma (PDAC) represents 90% of cases [2]. Despite difference in epidemiology observed region by region (incidence rate 0.5-9.7 per 100 000 people), it causes about 4% of all deaths per year globally [2]. PC is known for its aggressive nature with low five-year survival rate that does not exceed 9% [3].

Early detection of PC remains a challenge. Therefore, stratification of risk factors, and identification of subjects at risk is actual. The known risk factors of PC are male sex, non-O (I) blood group, cigarette smoking, low physical activity, genetics and positive family history, presence of diabetes mellitus, obesity, dietary factors (high levels of red and processed meat, low fruits and vegetables consumption, alcohol intake) and history of pancreatitis [4]. Association of the PC with some infections, including hepatitis B virus

(HBV) infection has been described [5, 6]. However, the results of these reports are controversial, and mechanisms by which HBV may be involved in pathogenesis of PC are not fully clear.

Hepatitis B virus is a known carcinogen that causes up to 80% of cases of hepatocellular carcinoma in endemic regions [7]. Also, the virus may be involved in non-liver oncogenesis due to its ability to integrate into the genome of infected cells, to cause genomic aberrations, and enhance expression of oncogenes or inhibit tumor suppressors [8]. Several reports have shown that replication of the virus may occur not only in the liver, but also in other organs, including pancreas [9-11]. Moreover, pancreatic beta cells and hepatocytes develop from the ventral foregut endoderm during ontogenesis and thus may share characteristics, favorable for HBV-induced tumor development [12]. Markers of previous or current HBV infection are commonly found in patients with PC, while HBV DNA and viral antigens have been detected in the pancreatic tumor tissues, suggesting potential role of the infection in the etiology of this cancer [13-15]. However, most of these reports came from Asian countries, where HBV infection is prevalent, and most of subjects were HBsAg-positive. In contrast, uncertain results of the cohort studies performed in Europe (1 from Denmark, and 2 from Sweden) make an association of the PC and HBV infection questionable [5, 16-18]. Although the data of epidemiological studies are important, direct support of the involvement of HBV infection in PC development may be provided with the detection of HBV biomarkers (especially - expression of HBx) within the tumor tissue. However, there is still a lack of such reports, especially in non-endemic for HBV infection regions.

Here we provide the report of two cases of patients with no history of HBV infection, admitted to the Moscow Clinical Research Center n.a. A.S. Loginov for pancreatic cancer treatment, who gave their consent for special examination and the use of the obtained data for scientific purposes, including publication of images.

CASE PRESENTATION

Chief complaints

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History of present illness

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History of past illness

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Personal and family history

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Physical examination

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Laboratory examinations

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Imaging examinations

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Materials and Methods

Local Ethic Committee approved additional examinations of the subjects beyond standards of care. Special examination included detection HBV infection markers (HBsAg, anti-HBs, anti-HBc, HBV DNA) in blood and in resected pancreatic tissues (HBV DNA, pregenomic HBV RNA and cccDNA), and immunohistochemistry staining of pancreatic tissues for HBxAg and cell proliferation marker Ki-67.

Blood samples were obtained after overnight fasting and analyzed immediately in the local laboratory. Immunologic tests were performed using the Sunrise analyzer (Tecan GmbH, Austria) and specific immunoassays kits (Vector-Best Co., Russia).

Plasma HBV DNA was isolated using commercial AmpliSens Riboprep kit (AmpliSens Biotechnologies, Russia) according to manufacturer's instructions and quantified using polymerase chain reaction (PCR) assay AmpliSens HBV-FL (AmpliSens Biotechnologies, Russia) kit (lower limit of detection of 10 IU/mL). To isolate nucleic acids from biopsies, samples were first homogenized in the MagNA Lyser (Roche Diagnostics, Switzerland). HBV DNA was isolated by AmpliSens Riboprep kit (AmpliSens Biotechnologies, Russia) and quantified by AmpliSens HBV-FL (AmpliSens Biotechnologies, Russia) kit.

To quantify covalently closed circular DNA HBV (cccDNA), nucleic acids were first treated with T5 exonuclease (New England Biolabs, UK) at 37°C for 60 *min* and inactivation at 70°C for 20 *min* [19]. HBV cccDNA was quantified with specific sets of primers and probes and normalized to genomic β-globin.

To analyze pregenomic HBV RNA (pgRNA HBV) analysis, nucleic acids were treated with RNase-free DNase I (New England Biolabs, UK) for 30 *min* at 37°C, purified by using AmpliSens Riboprep kit (AmpliSens Biotechnologies, Russia), reverse transcribed by AmpliSens Reverta-FL (AmpliSens Biotechnologies, Russia), and quantified by AmpliSens HBV-FL (AmpliSens Biotechnologies, Russia) kit. CFX96 Real-Time System (Bio-Rad, USA) PCR machine was used for the analysis of plasma and pancreatic tissue samples. Gel-electrophoresis of PCR-amplified HBV DNA is shown in supplementary figure 1.

Immunohistochemistry of pancreatic tissues was performed after deparaffinization. Slides were fixed in 4% paraformaldehyde, washed 3 times in Tris-HCl (50 mM, pH 8.0) followed by incubation with a blocking buffer (0.02% of Triton X-100, 10% horse serum, and 150 mmol/L NaCl in Tris-HCl, 50 mmol/L, pH 8.0) for 30 *min* and 1 *h* staining with primary rabbit anti-HBx (ab39716) (Abcam, USA). Then, slides were washed 3 times for 5 minutes in a washing buffer (0.02% of Triton X-100 and 200 mmol/L NaCl in Tris-HCl, 50 mmol/L, pH 8.0), incubated for 1 *h* with secondary Alexa Fluor 594 goat anti-rabbit antibodies (ab150080) (Abcam, USA). After that, the slides were treated with primary tagged Alexa Fluor® 488 rabbit anti-Ki-67 (ab197234)

and Hoechst 33342 (ab228551) for 1 *h*, washed 3 times for 5 *min* in washing buffer and finally mounted with a Fluoroshield reagent (Abcam, UK). Images were captured using Thunder imaging systems (Leica Microsystems, Germany) with 10× objectives. Ki-67 and HBxAg staining was analyzed using LAS X (Leica Microsystems, Germany). Ki-67 index was counted as the percentage of Ki-67-positive cells [20].

Cases presentations

Case 1. A 61 y.o. white/Caucasian man, blood type O (I), with history of chronic pancreatitis and alcohol abuse was admitted for the planned surgery in June 2019. Previous repeated screening blood tests on HBsAg were negative.

At admission, blood tests revealed signs of previous hepatitis B, but no markers of current HBV infection (shown in Table 1).

He underwent laparoscopic distal subtotal pancreatic resection with resection of the splenic vessels by Warshaw). Histological assessment revealed ductal adenocarcinoma of the pancreas, pT1 G2 R0 N0 V0 Pn0 [21].

Special examination of the resected pancreatic tissue revealed markers of HBV replication and active cellular proliferation, as well as expression of the HBV regulatory X protein (HBx) (shown in tab. 1, Figure 1).

Case 2. A 60-year-old white/Caucasian man, blood type A (II), with known history of chronic pancreatitis, type 2 diabetes mellitus, obesity (body mass index 34.5 kg/m^2), alcohol abuse and smoking experience >20 years, was admitted in February 2020 for planned surgical treatment due to previously diagnosed pancreatic cancer involving superior mesenteric vein. Before surgery, he received seven courses of neoadjuvant chemotherapy according to the FOLFIRINOX scheme with no progression of the tumor.

At admission, no markers of current HBV infection were detected by blood tests. However, serum anti-HBc was positive, suggesting that the patient had a previous hepatitis B (shown in Table 1).

The patient underwent gastropancreatoduodenal resection. Morphological examination of the tissue identified pancreatic ductal adenocarcinoma with involvement of duodenal wall (pT2 R0 N0 V0 Pn1 TRS 3) [21, 22].

Examination of resected pancreatic tissue gave positive result for HBV DNA, with no other markers of active viral replication (shown in Table 1). Still, immunohistochemistry revealed expression of HBx and high level of cellular proliferation by Ki-67 index (shown in Table 1, Figure 1).

FINAL DIAGNOSIS

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TREATMENT

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OUTCOME AND FOLLOW-UP

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DISCUSSION

These two cases demonstrate the presence of HBV markers in HBsAg-negative patients with pancreatic cancer in non-endemic for the infection region.

Both of our patients had several known risk factors for PC development. We suppose that previous HBV infection could be additional risk factor in them. It is known, that HBV infection, even resolved, may present molecular basis for carcinogenesis. Carcinogenic mechanisms in HBsAg-negative persons with previous HBV infection may be realized through the involvement of episomal structure (cccDNA), which remains in the cell nucleus as a matrix for the life-long synthesis of new virions. In the case 1, detection of not only HBV DNA, but also cccDNA and pgRNA HBV (transcribed exclusively from cccDNA) suggests that this patient had a silent low-level replication of the virus in the pancreatic tissue. In the subject 2, pgRNA HBV and cccDNA were not

detected despite a significant amount of HBV DNA in the pancreatic tissue. While no HBV replication in this patient was found, integrated HBV DNA could evidently cause the expression of HBx, similar to hepatocellular cancer [23]. This protein, detected in pancreatic tissue of both of our subjects, is considered the most pro-oncogenic [24]. It is assumed that HBx plays a major role in pathogenesis of liver cancer through nuclear translocation, protein-protein interactions, influence on transcription regulation, induction of chromosomal instability, control of proliferation, transformation, invasion and metastasis of tumor cells even in cases when HBV replication is absent [23,24]. These mechanisms may also play role in extra-hepatic cancers development. To our knowledge, there are only two studies that described HBx expression in pancreatic cancer tissues, both performed in a cohort of Asian patients in HBV endemic region [11, 25]. Song C. *et al* reported that out of 10 subjects with PC and detected HBx expression, only three were HBsAg-negative.

Although the presence of HBV biomarkers in pancreatic adenocarcinoma tissue detected with PCR and immunohistochemistry does not allow proving causal relationship between two conditions, it reflects potential involvement of the virus in the etiology and pathogenesis of pancreatic cancer. It may be important, that Ki-67 proliferative index was more than 50% in both subjects. The literature data suggests that such values are relatively rare among PC patients (~12.4%), and associated with more aggressive grading and poor prognosis [20].

Together with data of the cohort studies, our cases may be important for clinical practice. It is not yet clear whether universal testing of all patients with PC for anti-HBc and HBV DNA is necessary. However, these tests are reasonable when chemotherapy is planned, and when blood transaminases flare on the mentioned treatment occurs [26-27].

Detection of cccDNA in pancreatic tissue in HBsAg-negative subject in our report may support the need for revision of the statements of the Taormina Workshop (2018), which defines occult HBV infection (OBI) as the presence of replication-competent HBV DNA in the liver and/or HBV DNA in the blood of people who test negative for HBsAg [28]. As extrahepatic replication of HBV DNA may occur in HBsAg-negative subjects (as

shown in a number of studies and in our case 1), skipping a mention of specific organ for HBV DNA (cccDNA) detection seems reasonable.

CONCLUSION

The described cases may reflect potential involvement of HBV infection in the development of pancreatic ductal adenocarcinoma. Larger studies are necessary to assess risks of pancreatic ductal adenocarcinoma in subjects with previous HBV infection and define HBV-associated mechanisms of cancerogenesis in them.

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PRIMARY SOURCES

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