Detection Of Hepatitis B Virus DNA In Moroccan Patients With Epithelial Ovarian Carcinoma (EOC) By Polymerase Chain Reaction

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Abstract: Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer; representing 90% of all ovarian cancers. The viruses are known as human malignancies agents. We tried to analyze the presence of Hepatitis B Virus infection in women with epithelial ovarian carcinoma. PCR-based detection of HBV infections was carried out on 50 tissue samples from patients with histologically proven EOC using consensus primers. The samples analyzed showed 8% (4/50) positivity for HBV-DNA in cancerous ovarian tissues. All of the positive patients had serous adenocarcinoma and advanced stage disease. The results of this study suggest that hepatitis B could play a major role in the etiology of ovarian cancer.

Keywords: HBV-DNA, epithelial ovarian cancer, fresh frozen tissue, PCR method.

1 INTRODUCTION

Despite significant advances occurred in both surgical and chemotherapeutic techniques, the epithelial ovarian cancer remains the leading cause of death from gynecological malignancy in the world with 5-year survival rates lower 50% for all stages[1]. Among the most common histopathological types, epithelial ovarian cancers (EOC) represent 80% to 90% of all ovarian cancers. Clearing up the etiologies of the EOC, may help to better its prognosis. Five to 10 % of ovarian cancers are attributable to genetic predisposition.It is now accepted that infectious factors, especially viruses, play an important role in the occurrence of cancers including those of the breast and gynecological sphere[3]. To date, in Africa more than 30% of cancers are caused by infectious disease. Several oncogenic viruses are involved in cancers etiologies, such as HBV, EBV, and HPV[4]. The hepatitis B virus is known to be involved in the development of liver cancer. However, several studies have shown that HBV may replicate in other extrahepatic cells[5]. Some studies revealed that the virus has extensive reservoirs of extrahepatic replication[5b]. HBV proteins and nucleic acids have been found in a number of non-hepatic tissues including lymph nodes, spleen, bonemarrow, kidney, colon, stomach, periadrenal ganglia, skin, thyroid, pancreas, testis, ovaries, brain, heart and lung tissue[6]. Other studies have failed to prove the presence of extrahepatic HVB, making controversial results in this context. We tried to analyze the presence of Hepatitis B Virus infection in women with epithelial ovarian carcinoma attending to oncology department of Ibn Rochd University hospital.

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2 MATERIALS AND METHODS

2.1 Study Population

In this study, we analyzed data of 50 patients with histological proven epithelial ovarian cancer, diagnosed and treated in Oncology Center in IbnRochd University Hospital center of Casablanca between 2011 and 2012. We considered only patients who had complete records. The fresh ovarian biopsies were collected after surgical intervention in the department of gynecology and obstetrics "A" IbnRochd University Hospital of Casablanca and immediately placed in cryotubes and stored in liquid nitrogen. Informed consent was obtained from all participants and the study protocol was approved by the local ethics committee of the University Hospital IbnRochd. Samples were transported to the laboratory of Virology, Microbiology and Quality / ETB, Faculty of Science and Technology, University Hassan II Mohammedia-Casablanca and were kept at -20 ° C until DNA extraction.

2.2 DNA extraction

Sections with a thickness of 10 microns were performed using scalpels, from biopsies frozen at -20 °C.Tissue samples were digested in a buffer (100 mmol/L NaCl, 10 mmol/L Tris-HCl pH 8.0, 25 mmol/L ethylenediamine tetra-acetic acid [EDTA] and 1% sodium dodecyl sulfate)

containing 20 mg/mL proteinase K at 37°C overnight. Total DNA was isolated by phenol–chloroform extraction and ethanol precipitation. The DNA pellets were suspended with 20 mL sterile water and stored at -20°C until PCR amplification. Nested polymerase chain reaction (PCR)-based detection of HBV infections with HBPr1/HBPr135 as outer primers (≈ 1000 bp) and HBPr2/HBPr3 as inner primers (388bp) was used for to amplify the préS1/AgHBS/HBpol region of HBV in EOC as previously describe by Naito et al.[7]. Amplifications were performed by adding 5 mL purified DNA to a mixture of 50 mmol/L KCl 2 , 10 mmol/L TrisHCl pH 8.0, 1% Triton X-100, 7mmol/L MgCl2 , 100 mmol/L of each dNTP (dATP, dCTP, dGTP and dTTP), 100 pmol of each primer and 1 unit of Taq DNA polymerase enzyme (Promega, Madison, WI, USA). Amplifications were performed by initial denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min and extension at 72°C for 1 min, followed by 7 min of final extension at 72°C in a programmable thermal cycler program Perkin Elmer 2400 GeneAmp PCR thermal Cycler (Scientific Support, Inc, Hayward, CA).

3 RESULTS

This study focused on a population of 50 women who underwent surgery for ovarian tumor in Ibn Rochd hospital in Casablanca (Morocco). The mean age was 48.64 years, ranging from 25 to 80 years. Epidemiological characteristics of the studied population and the histological diagnosis are shown in Table 1. HBV-DNA was found to be positive in four patients (8%) (Figure1). All of the positive patients had Serous adenocarcinoma (4/39, 10.3%) and advanced stage disease (Table 2).

Characteristics	Effective	Percentage
Age		
Mean±SD: 48.9±11.9		
<25	3	6
25-40	9	18
41-55	18	56
56-70	8	16
>70	2	4
Age of first mentruation		
≤13	46	92
>13	4	8
Menopausal		
Yes	22	44
No	28	56
Histological type (WHO)		
serous adenocarcinoma	39	78
Mucinous adenocarcinoma	6	12
Endometrioid carcinoma	3	6
Undifferentiated carcinoma	2	4
Clinocal stage (FIGO)		
I	4	8
II	11	22
III	32	64
IV	3	6

***TABLE 1**: Epidemiological characteristics of the studied population*

		HBV-DNA	
Histological type	Effective	Positive	Negative
Serous	39	4 (10.3)	35 (89.7)
Mucinous	6	0 (0)	6 (100)
Endometrioid	3	0 (0)	3 (100)
Undifferentiated	2	0 (0)	2 (100)
Total	50	4 (8)	46 (92)

***TABLE 2**: Histological type of positive HBV patients with EOC in the studied population*

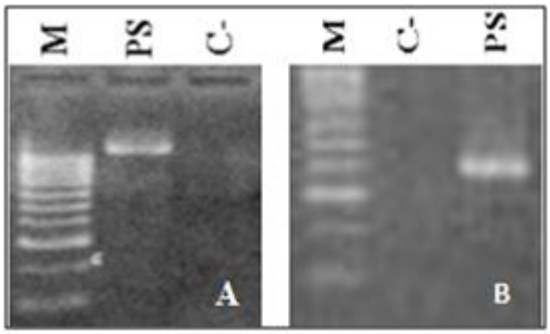

***Figure 1**: PCR products were analyzed on a 2% agarose gel stained with ethidium bromide and visualized by UV-trans-illumination. A)HBPr1/HBPr135 PCR products, B) HBPr2/HBPr3 PCR products.M = 100 PCR marker, C- = negative control and PS = positive samples.*

4 DISCUSSION

Ovarian cancer is among the most common cancers of the female genital tract, its prognosisis bad[8]. A true "silent killer", this cancer is often detected at an advanced stage of the disease due to the absence of early clinical symptoms[9]. It ranks first in the gynecological cancers mortality, more than 50% of women diagnosed die each year within 5 years[10]. To our knowledge, this study is the first to investigate the presence of hepatitis B virus in epithelial ovarian carcinoma in Casablanca area. Indeed, if the involvement of HBV in the occurrence of liver cancer[5a] is recognized by all, its implications in other extrahepatic cancers remain controversial[5b]. The scarcity of studies about illustrates the difficulty of research to prove formally that involvement in the image of human papillomavirus (HPV) in other cancer than cervical cancer[6].In this study, we tried to find the presence between HBV and ovarian cancer. Among the cases investigated four (8%) were HBV-DNA positive. All positive cases were serous cystadenocarcinoma (and advanced stage disease (stage II and III). Our results confirm the hypothesis of extrahepatic virus replication of HBV, particularly in ovarian cells[5a, 11]. Indeed, HBV has long been regarded as a specifically

hepatotropic virus, but it is now recognized as a pantropic virus capable of replicating in extrahepatic tissues and organs[5a, 12]. Chen et al study showed that HBsAg, HBcAg, and HBV DNA can be detected in ovarian tissues from patients with HBV infection[13].Around 30% of cancers in Africa are infectious[4a]. The results of this preliminary study once confirmed, could lead a change of mindset in the management of patients with ovarian cancer in the region of Grand Casablanca.

5 CONCLUSION

HBV may have a role in carcinogenesis of epithelial ovarian cancer. Future studies are necessary and it is useful to examine this possible relationship in large case-control studies using other more sensitive techniques.

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