Prevalence of Hepatitis B Virus Markers among the Women with Breast Cancer

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Abstract

Introduction: Breast cancer represents a formidable peril to the female populace on a worldwide level. The association between breast cancer and various factors, including viral infections, has been extensively investigated. Recently, the link between HBV infection and breast cancer patients has garnered attention. The present research aims to assess the prevalence of HBV markers among women diagnosed with breast cancer in Ahvaz city, Iran. Materials and Methods: Serum specimens were procured from 90 patients who had been clinically diagnosed with breast cancer. The age of the patients ranged from 29 to 80 years, with a mean age of 49.42±10.7. Histological examination of biopsy specimens revealed that 75 (83.33%) were ductal, 11 (8.88%) lobular, 2 (2.22%) mucinous, 1 (1.11%) medullary, and 1 (1.11%) was metastatic. The serum samples were subjected to initial HBsAg and anti-HBc testing via ELISA. Samples that tested seropositive (HBsAg + anti-HBc) were subsequently analyzed for the S region of HBV through nested PCR and DNA sequencing. Finally, a phylogenetic tree was constructed for positive HBV DNA tests. Results: Among the 90 breast cancer patients, it was found that 3 (3.33%) cases of ductal carcinoma and one (1.11%) lobular carcinoma displayed positivity for HBV markers (HBsAg, anti-HBc, HBV PCR). Notably, one (1.11%) patient with ductal carcinoma solely demonstrated anti-HBc positivity. The phylogenetic tree analysis of the S region revealed that all HBV strains identified were categorized as genotype D. Conclusion: The statistical analysis did not reveal any significant findings (p= 0.315) in the distribution of cancer types across different age groups. Among patients diagnosed with breast cancer, a notable prevalence of 5.5% was observed in HBV markers. The dominant HBV genotype among breast cancer patients was identified as genotype D.

Keywords: Hepatitis B Virus- carcinoma- ductal- breast- nested polymerase chain reaction

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Introduction

The chronic infection of HBV, which affects approximately 257 million individuals worldwide and results in 887,000 annual deaths [1], has been reported to be accountable for acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide [2-5]. Rezaei et al. [6] recorded a reduction in the occurrence of hepatitis B infection in Iran, whereby the frequency dwindled from 3.02% in 2000 to 1.09% in 2016. Furthermore, it was noted that the prevalence of Hepatitis B was more than 1.3 times higher in males than in females in 2016. Breast cancer can be a leading cause of death in women worldwide. It is estimated that there are approximately 2.3 million new cases of breast cancer and 685,000 deaths worldwide each year [7]. It is expected that one in nine women will develop breast cancer and one in 29 will die from the disease [8]. Several factors may be associated with developing breast cancer, including getting older, genetic mutations in certain genes (BRCA1 and BRCA2), family history of breast or ovarian cancer, chest radiotherapy, exposure to diethylstilbestrol (DES), excessive alcohol consumption, significant overweight, and hormone replacement therapy (HRT) after menopause [9-12]. The primary classifications of breast cancer consist of non-invasive (or in situ) or ductal carcinoma in situ (DCIS), invasive, and metastatic breast cancer [13, 14]. Invasive ductal carcinoma (IDC) represents approximately 80% of all breast cancers and is the most frequently occurring subtype (IDC). Invasive lobular carcinoma, or ILC, represents the second most prevalent form of breast cancer, which is associated with specific genetic mutations and is generally more aggressive than IDC.
cancer, accounting for 10 to 15% of all breast cancer cases. While ILC can affect women of any age, it typically manifests in older women. [13-15]. Metastatic breast cancer, also referred to as advanced or stage IV breast cancer, denotes a late-stage condition whereby the disease has spread to other organs in the body, including the lymph nodes, lungs, liver, bones, and brains [10, 13, 14].

According to Bonhoeffer et al. [16], Hepatitis B virus (HBV) falls under the classification of Hepadnaviridae. Recently, HBV has reclassified into 10 genotypes (A-J) based on more than 8% genetic variability [17]. It has estimated the nucleotide substitution rate of HBV to be about 1.4 – 5.0 × 10⁻⁵ per site per year [18].

Chronic Hepatitis B Virus (HBV) infection results in persistent harm to hepatocytes, thereby resulting in the impairment of estrogen inactivation and the circulation of elevated levels of unbound estrogen in the bloodstream, which has the potential to stimulate the development of breast cancer [19].

Occult hepatitis B infection (OBI) represents a condition characterized by the absence of detectable hepatitis B surface antigen (HBsAg) in the serum, while still exhibiting detectable levels of hepatitis B virus (HBV) DNA in both serum and/or intrahepatic compartments. The presence of mutations within the preS1, preS2, and S regions of the HBsAg gene can lead to the absence of detectable HBsAg [20].

Iran is considered to be an area with a low prevalence of HBV, with an estimated rate of HBsAg carriers being 2.2% [21]. In 2019, the coverage of 3 doses of the HBV vaccine reached 85% worldwide, compared to around 30% in 2000, resulting in a significant reduction in HBV carrier rates and hepatitis B-related morbidity and mortality [22].

The majority of cancer patients with asymptomatic hepatitis B virus are not conscious of their infection. Reactivation of HBV has been documented in breast cancer patients receiving chemotherapy [23, 24]. The outcome of HBV reactivation is acute fulminant hepatitis, liver failure, and death [25, 26]. Therefore, the aim of this study was to investigate the occurrence of HBV in breast cancer patients in Ahvaz city, capital of Khozestan, Iran.

Statistical analysis
Data analyses were conducted utilizing the Statistical Package for the Social Sciences 22.0 (SPSS Inc., Chicago, IL, USA). The Chi-Square test was employed for computation purposes. The acceptance of a significance level of P<0.05 was established.

Materials and Methods
Ninety female patients diagnosed with breast cancer were the subjects of this study. Serum samples were collected from Glestan and Apadana hospitals in Ahvaz cities during Feb 2020- Jan 2022. The study encompassed certain parameters for the inclusion criteria, namely the females who underwent surgical intervention within a hospital setting. Conversely, the exclusion criteria took into account patients with bilateral breast cancer and those afflicted with metastatic cancer.

ELISA screening was implemented on all serum specimens in order to identify the presence of HBsAg and anti-HBc. Serum specimens that exhibited positivity for both HBsAg and anti-HBc, as well as those that solely displayed positivity for anti-HBc, or demonstrated negativity for both HBsAg and anti-HBc, were subjected to nested PCR for the purpose of amplifying the partial S region of HBV DNA. The DNA that underwent amplification was subsequently subjected to sequencing. The findings of the breast cancer biopsies were subsequently verified by a pathologist.

Molecular testing
DNA was isolated from the serum of patients using a DNA extraction kit (Sinaclon, Iran) in accordance with the manufacturer’s instructions. The extracted DNA was stored at -20°C until further analysis was required.

Nestecd PCR
For the first round, the following outer primers of the S region, S1-F: 5’-CATCAGGATTCTCTAGGACCTC-3’ and S2-F: 5’-TTTGGTTAGAATCCTTACACA-3’ were used PCR test [27]. The reaction mixture utilized in the PCR process contained 5 µl of an isolated DNA sample, which was quantified at 400 ng and served as the template. Additionally, the mixture contained 2.5 µl of a 10x reaction buffer, 0.75 µl of MgCl2 (50 mM), 0.5 µl of forward/ reverse primer, 1 µl of dNTP (10 mM), 0.2 Cinna Gen Taq DNA Polymerase (5 u/µl), and double-distilled water up to 25 µl. The thermal cycler (TC-512, Teche, UK) was utilized for the reaction mixture, which underwent an initial denaturation at 94°C for 5 minutes, followed by 40 cycles at 94°C for 45°C, 52°C for 45s, 72°C for 45s, and a final extension at 72°C for 8 minutes. For the second round, the following inner primers were implemented, S3-R: 5’-AGGACAACCGGCAACATAC-3’ and S4-R: 5’-CCAACAAGAAGATGAGGACATGACACAATCCTACACA-3’ [27].

For the second round, 2.5 µl of the PCR product derived from the initial round was introduced to a PCR reaction mixture containing identical components as those stipulated for the first round and then subjected to a thermal cycler. The thermal cycler was programmed in the following sequence: initial denaturation at 94°C for 5 minutes, followed by 40 cycles at 94°C for 45°C, 52°C for 45s, 72°C for 45s, and a final extension at 72°C for 8 minutes. All reactions were conducted in the presence of both negative and positive controls.

Sequencing and phylogenetic analyses
The amplified products obtained from PCR that displayed affirmative outcomes were subjected to sequencing in both the forward and reverse directions. This sequencing process was conducted using the Applied Biosystem 3500 instrument, manufactured by ABI Scientific in the United States. Subsequently, the nucleotide sequencing analysis of the isolated partial “S” region of the Hepatitis B Virus (HBV) was aligned with the HBV database available on the National Center for Biotechnology Information (NCBI) website. This alignment was performed to determine the genotype of the HBV. The results obtained from the partial sequencing of the “S” region were then submitted to GenBank with the
Hepatitis B Virus Markers among the Women with Breast Cancer

The occurrence of breast cancer among a diverse range of ages exhibits a minimum occurrence of 1.11% in individuals under the age of 29, while the highest incidence of 27.77% is observed in the 40-49 age category for ductal carcinoma. Additionally, the prevalence of lobular carcinoma varies from 1.11% in individuals over the age of 80 to 5.55% in those belonging to the 50-59 age group. However, these findings did not yield statistically significant results (p=0.315) (Table 1).

The patients who had various forms of cancer and different stages of cancer displayed three cases (3.33%) of ductal carcinoma, with one case (histological grade III) and two cases (histological grade II). Additionally, one case (1.11%) of lobular carcinoma (histological grade II). All of these cases exhibited positive HBV markers, including HBsAg, anti-HBc, and HBV PCR. Interestingly, there was a single patient (1.11%) with ductal carcinoma of histological grade II who exclusively demonstrated anti-HBc positivity, as shown in Table 2.

The phylogenetic tree analysis

The findings of the phylogenetic tree analysis of the S region of HBV isolates unveiled that all four HBV isolates (OR021896-OR021899) obtained from Ahvaz city, which are denoted by black circles, are grouped together with genotype D2 from Russia (OP256019) and genotype D from Iran (JN040804). This is illustrated in Figure 1.
Table 1. Displays the Age Distribution among Breast Cancer Patients with Varying Cancer Types

<table>
<thead>
<tr>
<th>Age grouping Range</th>
<th>Ductal (29-80)</th>
<th>Lobular (29-80)</th>
<th>Cancer types</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>1 (1.11%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>30-39</td>
<td>13 (24.44%)</td>
<td>1 (1.11%)</td>
<td>2 (2.22%)</td>
<td>--</td>
</tr>
<tr>
<td>40-49</td>
<td>25 (27.77)</td>
<td>4 (4.44%)</td>
<td>1 (1.11%)</td>
<td>0.315</td>
</tr>
<tr>
<td>50-59</td>
<td>22 (24.44%)</td>
<td>5 (5.55%)</td>
<td>1 (1.11%)</td>
<td>--</td>
</tr>
<tr>
<td>60-69</td>
<td>11 (12.22%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>70-79</td>
<td>3 (3.33%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>&gt; 80</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 1 presents the prevalence of breast cancer across diverse age cohorts, with the minimum incidence of 1.11% noted in persons below the age of 29, while the maximum incidence of 27.77% was observed in the 40-49 age bracket for ductal carcinoma. Moreover, the prevalence of lobular carcinoma varied from 1.11% in individuals aged above 80 years to 5.55% in those belonging to the 50-59 age group. Nevertheless, these results were not statistically noteworthy (p=0.315).

Table 2. Distribution of HBV Markers in Patients with Various Type of Breast Cancer

<table>
<thead>
<tr>
<th>Categories Age 29-80</th>
<th>HBV markers</th>
<th>cancer types</th>
<th>histological grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBsAg</td>
<td>anti-HBc</td>
<td>HBV PCR</td>
</tr>
<tr>
<td>34</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>41</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>43</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>52</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>51</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2 exhibits among the cancer patients, a total of 5 out of 90 (5.55%) individuals were identified to have HBV markers (HBsAg, anti-HBc, HBV PCR). Out of these cases, 3 (3.33%) were diagnosed with ductal carcinoma (one histological grade III, and two histological grade II) while 1 (1.11%) had lobular carcinoma (histological grade II). Remarkably, 1 (1.11%) patient with ductal carcinoma (histological grade II) exclusively exhibited anti-HBc positivity.
Hepatitis B Virus Markers among the Women with Breast Cancer

Ahvaz, Iran.

Ethical consent

The project was endorsed by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, with the ethical code IR.AJUMS.MEDICINE.REC.1401.058. All patients included in the study were verbally informed of the purpose of the study and invited to participate on a voluntary basis. Written informed consent was collected from all patients.

Conflict of Interests

All authors declared that they have no conflict of interest.

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Venus Fakheris Sueini et al.

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552

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Hepatitis B Virus Markers among the Women with Breast Cancer


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