

Up-regulation of BRCA1 and Rad51 genes expression in peripheral blood mononuclear cells is associated with hepatitis B patients

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Abstract

Background: Many viruses increase their replication by affecting DNA damage response (DDR) signaling and activating and inhibiting DNA damage response factors. to date, few studies have investigated Homologous Recombination (HR) system in the field of hepatitis B patients. The current study set out to investigate HR system by examining the expression of RAD51, BRCA1, and HBx genes in peripheral blood mononuclear cells (PBMCs) of hepatitis B patients. Two groups of subjects were enrolled including healthy controls (n=61) and hepatitis B patients (n=60). The serum levels of HBV DNA, Rad5 1and BRCA1, in the PBMCs were measured using real-time quantitative polymerase chain reaction (PCR.) Moreover, serum levels of liver enzymes (ALT and AST) levels were assessed by automatic biochemical analyzer technique.

Results: In HBV-infected patients, the gene expression of Rad51 and BRCA 1 were significantly upregulated compared to healthy controls ($P < 0.05$). However, There is a statistically significant correlation between Rad51($r = 0.838$; $P \text{ value} < 0.001$) and BRCA1($r = 0.588$; $P \text{ value} < 0.05$), genes expression and viral load.

Conclusions: These results demonstrated that hepatitis B virus affected the homologous recombination system by increasing the expression of Rad51 and BRCA1 genes, suggesting these genes could be considered as valuable therapeutic targets for treating hepatitis B patients.

Keywords: BRCA1, HBV, Homologous Recombination, Rad51.

Background

Hepatitis B virus (HBV) is one of the enveloped viruses with DNA genetic structure. Infection with this virus is one of the global problems [1]. According to studies, one-third of the world's population is affected by this virus, of which 5% are carriers. 25% of carriers suffer from chronic hepatitis and cirrhosis of the liver, and eventually from hepatocarcinoma (HCC). Previous studies show that despite the vaccination against hepatitis B and available treatments, an average of 780,000 people dies each year from the disease [2, 3].

One of the main causes of HCC can be the presence of hepatitis B virus DNA in the serum of people with chronic hepatitis B (CHB) infection [4]. The severity of hepatocarcinoma is related to the degree of viral load [5]. In general, the HBV virus causes HCC through two mechanisms; first, by inserting a viral genome into the host genome structure, and removing cancer-inhibiting genes, which leading to DNA damages. As a result, instability in the host genome has occurred, resulting in cancer progression. Second, by disrupting cellular signaling pathways, which in turn can disrupt the cell cycle, eventually leading to cell proliferation and cancer development [6]. Earlier studies demonstrated that Protein x in hepatitis B (HBx) 154 amino acids and N-terminal region with negative regulatory properties and having C-terminal region with its transactivator properties has a dual play key role in the pathogenesis of hepatocarcinoma. It causes malignancies caused by viral infections in host liver cells [7]. HBx protein does not directly increase the expression of target genes, however, it effects by acting on the promoter and enhancers of the target genes [8]. HBx affects various signaling pathways, including reactive oxygen species (ROS), apoptosis and cellular growth, Ras-Raf-MAP kinase pathway, transforming growth factor- β , Tumor necrosis factor, Wnt/catenin signaling, cell, chromosomal division, and DNA repair, which can eventually lead to disease progression and hepatocarcinoma [9-12].

Many viruses increase their replication by affecting DNA damage response (DDR) signaling and activating and inhibiting DNA damage response factors [13]. HBV affects the DNA repair system and DDR in various dimensions. In a study conducted by Su Jeong Kim *et al.*, they found that γ - H2AX, a marker of double-stranded DNA breaks (DSBs), and phospho-Chk2 rates were considerably greater in HCC associated with HBV and neighboring restoring nodules [14]. Many studies have found HBx attaches to DNA binding protein 1 like X-associated protein 1 (XAP-1) and UV-damaged DNA-binding protein (UVDDDB). It has been revealed that interaction is necessary for HBV replication [15, 16]. A great deal of previous research has also shown that HBx

could impact on the nucleotide excision repair system by interacting with key proteins such as TFIIH, XPD, and XPB [17, 18]. Also, HBx protein interacts with the DNA glycosylase enzyme thymine to affect the Base Excision Repair (BER) system [19].

In studies on oncogene viruses such as HPV, KSV, HTLV1, etc. [20], they can increase the pathogenicity of viral infections by affecting another DNA repair system called the homologous repair (HR) pathway. Important members of this system include BRCA1 and Rad51. BRCA1 plays a key role in genome stability. This protein, known as an assembly protein, interacts with other cellular proteins. This protein has a role in DNA repair, cell cycle progression, and gene transcription [21]. Rad51 is an important member in repairing double-stranded DNA breaks by homologous recombination method. Throughout the recombination process, Rad51 attaches transiently to both single-strand and double-strand DNA [22].

Since studies have shown that HBV virus can replicate pbmcs causing disruption of gene expression in the host [23, 24] to date, few studies have investigated homologous recombination system in the field of HBV. Therefore, this study set to investigate the gene expression of the two main factors of this system including BRCA1 and Rad51.

Method

Study design

This study was approved by the Birjand University of Medical Sciences (BUMS) (No: 455976). Patients with hepatitis B are referred to us in the Center for Infectious Diseases and the Eastern Hepatitis Network. all methods were performed in accordance with the relevant guidelines and regulations.

Blood sample collection

After obtaining informed consent from the introduced individuals, blood samples were collected from patients and controls.

Laboratory testing

All samples were investigated for Anti-HBc, (anti-HBc, Biokit, Barcelona, Spain HBs antigen (HBsAg, Dade Behring, Germany), anti-HDV (Biokit, Barcelona, Spain), and anti-HCV (HCV Antibody ELISA kit, Pishtazteb, Iran) using ELISA kits, according to manufactures instrument.

Sample selection

Samples with HBs positive were considered as patients with hepatitis B, and those with negative HBc and negative HBs were considered as the control group. Anti-HDV and anti-HCV were negative in the study samples, and they did not take any special medicine. Finally, 60 patients and 61 control subjects were selected for analysis, and the enzymatic levels of aminotransferase and aspartate aminotransferase were evaluated in patients using an automatic biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA).

HBV Viral Load Analysis

All patients were evaluated for viral loading of HBV using cobas® 4800 System (Roch-USA).

RNA extraction and reverse transcription (RT) quantitative PCR detection

Whole-blood samples were extracted from patients and controls, then peripheral mononuclear blood cells (PBMCs) were separated by Ficoll density gradient (inno-trian, Germany) from EDTA-treated blood samples.

Complete extraction of RNA from PBMCs was carried out using the wizol reagents (wizbio Solutions-South Korea) to purify total RNA, according to the manufacturer's guidance. Before analysis, the extracted RNA was held at -70 °C. The integrity of RNA was evaluated by 1% modified electrophoresis of the agarose gel and the RNA. The detection of density and purity of total RNA was performed using NanoDrop ((NanoDrop 8000 spectrophotometer, Thermo Fisher Scientific, Wilmington, Delaware). Two micrograms of total RNA were reversed to cDNA via random hexameric primers and cDNA synthesis package RevertAid™ First-Strand (Glen Burnie, MD, Fermentas, USA). Using the PCR technique for the internal GPDH gene to assess the quality of cDNA synthesis then 1.5 % agarose gel was used to analyze post-PCR products to confirm the amplification

The BRCA1, Rad51, and HBx expression patterns were tested with real-time PCR assay by the sybr green method (Table 1). The expression level of each target gene was doubled and normalized to the level of human expression of beta-2-microglobulin, as a reference gene. PCR was utilized in real-time using an ABI Step One Plus Sequence Detection Method 2.1 (Applied Biosystem, USA). Ultimately, about the related effectiveness of different qPCR assays, the $-\Delta\Delta C_t$ method formula was used to assess the relative rates of gene expression.

Statistical analysis

Statistically, the study results are described as mean \pm SEM. SPSS program ver.13.0 (SPSS, Chicago, IL, USA) did data analysis. To check the normality of the data, we use the Kolmogorov-Smirnov test. If the data is normal, we use parametric tests and if the data is not normal, we use non-parametric tests

Results

Demographic characteristics

One hundred and twenty-one participants (64 males and 47 females) with an average age of 38.4 ± 18.7 years old were included in the study. Among them, 60 patients (39 males and 21 females) with an average age of 38.4 ± 16.8 years old were included in the study. The mean HBV-DNA was 1.449 ± 5.738 ng/ml. AST 38.30 ± 28.27 , and ALT 19.567 ± 13.2527 IU/L. In 54 cases, HBeAg was negative, and in 6 cases, HBeAg was positive (Table 2). In the control group, sixty-one samples (25 males and 26 females) with an average age of 32.9 ± 19.7 years old were included in the study. AST 26.5 ± 9.11 , and ALT 14.9 ± 6.3 IU/L.

mRNA Expression of Rad51 and BRCA1

Homologous recombination is one of the DNA repair pathways. One of the important components of this system is Rad51. This protein reacts with RPA and Rad52 to induce strand transfer of DNA. This protein also reacts with BRCA1 due to double-strand breaks [25]. BRCA1 plays an important role in maintaining the integrity of the genome and induces DNA restorative signaling through the homologous DNA repair system [26].

To determine the possible roles of Rad51 and BRCA1 in the pathogenesis of hepatitis B, the mRNA expression levels of Rad51 and BRCA1 were measured and results can be seen in Figures 1 & 2. Rad51 and BRCA1 gene expression were significantly up-regulated in hepatitis B patients compared to healthy controls ($P < 0.01$ and $P < 0.05$, respectively). There is a positive significant correlation between Rad51 and viral load ($r = 0.83$; $P = 0.0004$). Moreover, a positive significant correlation was found between BRCA1 gene expression and viral load ($r = 0.58$; $P = 0.029$) (Table 3).

Discussion

Today, it is one of the most popular novels for the treatment of cancer. It targets the signaling pathways of DNA repair. Defects or overexpression of unique pathways that recognize or repair specific types of DNA damage may serve as biological markers with a favorable or poor response to therapies that cause DNA damage of this kind [27]. One of the DNA repair pathways that has recently been considered in the treatment of cancer is the HR system. Among the important components of this system, BRCA1 and Rad51 can be mentioned[28]. Upregulation of Rad51 protein in cancer is has been proven to increase radioresistance of cancer cells [29] In certain cancers, high Rad51 expression is correlated with an adverse prognosis in tumor tissue [30, 31] According to studies, Rad51 can be considered as one of the main goals of cancer treatment [32, 33]. BRCA1 with 1800 amino acids plays a special role in DNA repair, Cell proliferation and Regulation of transcription[34]. Changes in the expression of this protein factor can lead to the progression of cancer and the cause of cancer, and can also affect the treatment options based on this factor [27, 35, 36].

Despite many advances in treating and preventing people from being infected with the hepatitis B virus, we also observe the spread of the disease and the chronicity of the disease in people and the development of hepatocarcinoma in infected people [37]. Hepatocarcinoma is the sixth most common cancer in general, but the third most prevalent cause of cancer death (24, 25). New studies show that more than 50 percent of people who get hepatocellular carcinoma are infected with the hepatitis B virus [38, 39]

Viruses cause 20% of cancers in humans [40]. Oncogenic DNA viruses cause cancer in a variety of ways, one of the most important of which is disrupting the host genome structure by inserting it into the host cell DNA or disrupting the host's genetic repair system [41]. Studies have shown that DNA damage and chromosomal abnormalities due to chronic inflammation have a special role in causing hepatocarcinoma [42]. There are many studies on the effect of hepatitis B virus on DNA repair systems such as NER and BER repair systems [17, 18], but few studies have been performed on homologs recombination system. Many studies on other viruses with the dsDNA genetic structure have shown that these viruses cause the virus to be located in the host genome and also prevent cell apoptosis by increasing the expression of the two major factors of the homologs recombination, Rad51 and BRCA1. Our study also confirms this evidence for the hepatitis B virus

[43-45]. By increasing the expression of these two factors, the virus appears to inhibit apoptosis due to genetic instability of the host and cause it to spread in the host [46].

In a study by Yan Liao et al. on hepatocarcinoma, a complex called BRCA1-associated RING Domain 1 (BARD1) was found to be highly expressed in this cancer, which causes cancer cells to invade, proliferate, and migrate. The high expression of this complex has been introduced as a predictive biomarker for the progression of the disease. They also found examining the HBs antigen of the participants, a significant relationship between people infected with the hepatitis B virus and the expression of this complex, which is also consistent with our data [47].

According to the data obtained from this study and other available studies, two factors, BRCA1 and Rad51, can be considered as biomarkers to study the disease process. Therefore, BRCA1 plays a major role in HBV-related HCC tumorigenesis and may serve as a new molecular targeted therapy for further clinical practice. Tumor DNA repair failure has been a treatment option in oncology for over a century, as demonstrated by the broad use of chemotherapy drugs that destroy DNA and ionize radiation [48].

In the current study, we tried to prevent interfering factors such as viral disease, the effect of drugs, and underlying diseases that affect the study process and to get more reliable results; this led to a small number of samples to be studied

Conclusion

Rad51 and BRCA1 were found to have significantly higher gene expression in people with hepatitis B than healthy controls. However, the current study requires to be further investigation with a larger sample size to confirm the findings. HBx expression was seen in only three samples, assessing the relationship between the effect of this oncoprotein HBx and the expression of Rad51 and BRCA1 is required.

List of abbreviations

HBV: Hepatitis B virus

HCC: hepatocarcinoma

CHB: chronic hepatitis B

HBx: Protein x in hepatitis B

BRCA1: breast cancer gene 1

XAP-1: X-associated protein 1

UVDDDB: UV-damaged DNA-binding protein

BER: Base Excision Repair

DDR: DNA damage response

DSBs: double-stranded DNA breaks

HR: homologous repair

Declaration section

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Birjand University of Medical Sciences, Center for Infectious Diseases and the Eastern Hepatitis Network(Ir.bums.REC.1398.236). All methods were performed in accordance with the relevant guidelines and regulations. Written informed consent to take part was obtained from all participants (or their parents or legal guardians in those under 18 years of age) for clinical and genetic investigation.

Consent for publication

Written informed consent for publication of clinical details was obtained from all of the all participants. (or their parents or legal guardians in those under 18 years of age). A copy is available to the journal if needed.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, Mohsen Naseri, upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

M.N was responsible for overall supervision. **A. A, N.V** and **D.J** Participated in study design, conducted molecular experiments and PCR analysis, data collection and evaluation, drafting and statistical analysis. **M.Z and S.A.R**: They have been involved in collecting samples and introducing hepatitis B patients. **A.A** and **D.J**: they were responsible for editing the article. **A. A**: Participated in study design. All authors read and approved the final manuscript.

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Table 1. The list of primers employed in specific qPCRs

| Gene | Primers |
|--------------|--|
| BRCA1 | Forward: 5' - GCTCGTGGAAGATTCGGTGT -3' Reverse: 5' - TCATCAATCACGGACGTATCATC -3' |
| Rad51 | Forward: 5' - TCTCTGGCAGTGATGTCCTGGA -3' Reverse: 5' - TAAAGGGCGGTGGCACTGTCTA -3' |
| HBx | Forward: 5' - CTTGGACTCTCAGCAATG -3' Reverse: 5' - CCTACAGCCTCCTACTAC -3' |
| B2MG | Forward: 5' -CTTGTCTTTTCAGCAAGGACTGG-3' Reverse: 5'-CCTTAACTATCTTGGGCTGG-3' |

Table 2. Clinical Characteristics of healthy controls and patients

| Variables | Hepatitis B patients | Healthy controls |
|-----------------------------|-----------------------------|-------------------------|
| N | 60 | 61 |
| Gender | | |
| Male | 39 | 25 |
| Female | 21 | 26 |
| Age ,y | 48.8 ± 10.8 | 32.9 ± 19.7 |
| ALT,U/L | 15.17 ±13.7 | 14.9 ± 6.3 |
| AST,U/L | 35.34 ±28.25 | 26.5 ± 9.11 |
| HBV DNA, log10 IU/ML | 4.99±1.8 | Undetectable |
| HBs Ag | | |
| Positive | 60 | 0 |
| Negative | 0 | 61 |
| HBe Ag | | |
| Positive | 6 | 0 |
| Negative | 54 | 61 |
| Anti HBc total | | |
| Positive | 60 | 33 |
| Negative | 0 | 28 |
| HBx Ag | | |
| Positive | 3 | 0 |
| Negative | 57 | 61 |

Table 3. Correlation between Rad51 and BRCA1 gene expression and viral load

| Spearman test | Viral load vs. Rad51 | Viral load vs. BRCA1 |
|--------------------------------|-------------------------------------|-------------------------------------|
| r | 0.8384 | 0.5889 |
| 95% confidence interval | 0.5423 to 0.9493 | 0.06749 to 0.8577 |
| P value | 0.0004 | 0.0295 |

Figure Legends

Figure2. Comparison of BRCA1 gene expression between study groups. Statistical analysis using Mann-Whitney U. test. Results are reported median.($P^* < 0.05$)

Figure1. Comparison of Rad51 gene expression between study groups. Statistical analysis using Mann-Whitney U. test. Results are reported median.($P^{**} < 0.01$)

Figure2

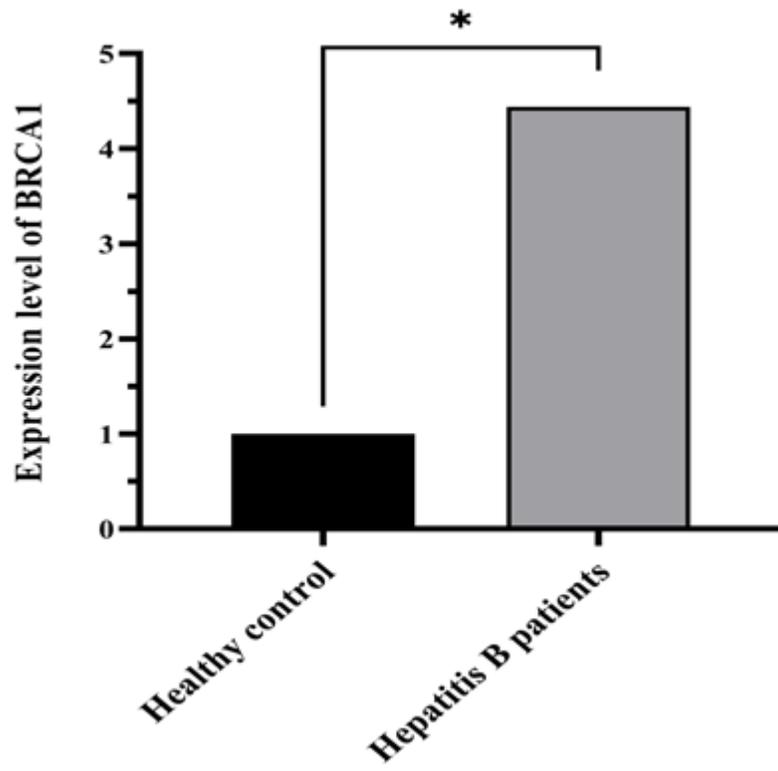


Figure1

