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SERUM LEVELS OF BCL-2 AND CELLULAR OXIDATIVE STRESS IN PATIENTS WITH VIRAL HEPATITIS

HG Osman, OM Gabr, S Lotfy, *S Gabr

Abstract

Purpose: This study was conducted to investigate the presence of bcl-2 protein in the serum of patients with viral hepatitis and to find out if there is any correlation between bcl-2 protein levels and cellular oxidative stress in the pathogenesis of viral hepatitis.

Methods: This study was carried out on 130 patients with viral hepatitis, 70 with chronic hepatitis, 30 with liver cirrhosis and 30 with hepatocellular carcinoma (HCC) in addition to 20 healthy persons as the control. Serum bcl-2 protein was estimated by enzyme-linked immunosorbent assay, serum malondialdehyde (MDA), nitric oxide (NO) and antioxidant enzymes (GSH, GSH-px, GR and SOD) were measured using spectrophotometric analysis.

Results: bcl-2 protein level was significantly elevated in the serum of HCC, cirrhosis and chronic hepatitis groups as compared to control group. There were significant positive correlations between higher bcl-2 protein level and viral hepatitis markers (HBsAg, anti-HCV antibodies) in HCC and cirrhotic patients as compared to chronic hepatitis group. An increase in oxidative stress markers (MDA, NO) and a decrease in antioxidant enzyme activities (SOD, GSH and GSH-px) were observed. However, there was a negative correlation between bcl-2 levels and GR in all studied patient groups.

Conclusions: The release of oxidative free radicals, deficiency in antioxidant enzymes and the expression of bcl-2 protein might play a role in the pathogenesis of viral hepatitis. The ability to measure bcl-2 protein in the serum could be useful as a prognostic marker of cancer patients.

Key words: Antioxidant enzymes, apoptosis, malondialdehyde, nitric oxide, oxidative stress, viral hepatitis

Viral hepatitis has emerged as a major public health problem throughout the world, affecting several hundred millions of people. Hepatitis is an inflammation of the liver characterized by a diffuse or focal necrosis that affects all hepatocytes. Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Several viral oncogenes, such as the X protein of hepatitis B (HBV) and the core protein of C viruses (HCV), are related to hepatocarcinogenesis.1

Chronic infection with HBV and HCV often results in cirrhosis and enhances the probability of developing HCC. There was a close association between chronic hepatitis C infection, liver cirrhosis and the development of HCC.2

Hepatic tissue homeostasis depends on maintaining the balance between cell proliferation and apoptosis. Disruption of this balance may lead to hepatic carcinogenesis. Hepatitis C virus core protein shows to deregulate the control of apoptosis that implicates in liver carcinogenesis. Hepatitis B virus-X (HBx) protein is known as a multifunctional protein that not only co-activates transcription of viral and cellular genes but also coordinates the balance between proliferation and programmed cell death (PCD) by inducing or blocking apoptosis.3

Reactive oxygen species (ROS) mediated liver injury may trigger by three main mechanisms: lipid peroxidation, cytokine induction and Fas ligand induction. Cytotoxic products of lipid peroxidation, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), may impair cellular functions including nucleotide and protein synthesis, which may play a role in hepatic fibrogenesis.4

Bcl-2 is a proto-oncogene originally identified at the breakpoint of a chromosomal translocation (t14:18) in human follicular B-cell lymphomas. Physiologically bcl-2 protein blocks the apoptotic process by inhibiting the release of cytochrome C from mitochondria whereas it locates at the cytoplasmic surface of the mitochondrial membrane, nuclear membrane and endoplasmic reticulum.5

Bcl-2 expression in cancer tissue was assessed indirectly by immunohistochemistry and its determination was found to correlate with apoptotic activity in cancer tissue. However, its detection necessitates the availability of cancer tissue from a surgical specimen or biopsy, which is subject to the methodology used for the preparation of specimens and interpretation of findings. In addition, the preparation of specimens cannot easily perform serially as the disease progresses with formation of metastases. However, bcl-2 immunohistochemistry has limitations in the preparation of specimens; it represents a useful prognostic factor in selected categories of patients with colorectal cancer (CRC).6

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The aim of this study was to investigate the presence of bcl-2 protein in the serum of patients with viral hepatitis and to find out if there is any correlation between bcl-2 protein levels and cellular oxidative stress in the pathogenesis of viral hepatitis.

Materials and Methods

This study was carried out on 70 patients with chronic hepatitis (CH), 30 with liver cirrhosis, 30 with HCC and 20 healthy individuals as controls. Both patients and controls were age- and sex-matched (Table 1). Patients were recruited from the Gastroenterology Surgical Center, Mansoura University, Egypt. Those who were negative for HBs antigen (HBsAg) and anti-HCV antibodies were excluded from this study. Those with negative HBsAg and positive anti-HCV antibodies were 90 cases (50 CH, 20 cirrhosis and 20 HCC). In addition, those with positive HBsAg and negative anti-HCV antibodies were 40 cases (20 CH, 10 cirrhosis and 10 HCC). Patients and control groups were subjected to clinical examination. Abdominal ultrasound and computed tomography were done for all patients and controls. Staging of liver pathology was based on Iskak’s scoring system.

Peripheral venous blood samples were obtained from patients and controls in the morning following an overnight fast. The samples were collected in sterile tubes and allowed to coagulate for 1 hour at room temperature. Then, serum samples were aliquoted in smaller containers that were marked with the patients’ name, date and stored at −80 °C until assaying.

Serum albumin, bilirubin and aminotransferases (ALT and AST) were determined using bioMérieux kits (France).

HBsAg and anti-HCV antibodies were detected by enzyme-linked immunosorbent assay (ELISA) technique (Biopkit, S.A, Barcelona, Spain).

Serum bcl-2 concentrations were determined using a commercially available, non-isotropic, enzyme-linked immunosorbent assay (ELISA, Biopkit, S.A, Barcelona, Spain).

Malondialdehyde was determined by the thiobarbituric acid method. Aliquots of 0.2 mL of serum mixed thoroughly with 0.8 mL of phosphate-buffered saline (pH 7.4) 25 μl of butylated hydroxytoluene solution. The samples were placed on ice for 2 hourly after addition of 0.5 mL of 30% trichloroacetic acid. Then, samples were centrifuged at 2000 xg at 25 °C for 15 minutes. After that, 1 mL of each supernatant was mixed with 0.075 mL of 0.1 mol/L ethylene diamine tetra acetic acid (EDTA) and 0.25 mL of 1% thiobarbituric acid in 0.05 N sodium hydroxide (NaOH). Supernatant of each sample was kept in boiling water for 15 minutes and then cooled to room temperature. Finally, the absorbance of thiobarbituric acid reactive substances (TBARS) was measured at 532 nm. The data of TBARS were expressed in MDA, using a molar extinction coefficient for MDA of 1.56 × 105 cm−1 M−1 and the results were expressed in nmol/mL.

Serum NO levels were measured with Griess reagent as previously described. The first step is the conversion of nitrate using nitrate reductase. The second step is the addition of Griess reagent, which converts nitrite to a purple azo-compound. Protein interference was avoided by treatment of the reacted samples with zinc sulphate and centrifugation for 5 minutes at 10,000g; the azochromophor spectrophotometry was performed at 450 nm; sodium nitrate was used as the standard and results were expressed in nmol/L.

Superoxide dismutase (SOD) activity was measured at 560 nm by detecting the inhibition of the NBTH 2 reduction rate. One SOD unit is defined as the enzyme activity that caused 50% of inhibition of nitroblue tetrazolium (NBTH2) reduction rate. SOD activity is expressed as U/mL. GSH-px activity is measured when oxidized glutathione is converted to the reduced form in the presence of glutathione reductase (GR) and NADPH that oxidizes to NADP. The diminished absorbance of NADPH was measured at 340 nm. The ΔA/min and the molar extinction coefficient of NADPH were used to calculate GSH-px activity. GSH-px activity was expressed in IU/mL.

GR activity was assayed using oxidized glutathione as a substrate. The GR assay depended on the absorbance change at 340 nm owing to oxidation/reduction of the NADPH/ NADP system. Absorbance of the reduced chromogen was measured at 412 nm, which is directly proportional to the GSH concentration.

Statistical analysis

Statistical analysis was carried out with spss (Statistical Package for Social Science) program version 10 for Windows (spss Inc, Chicago, IL, USA). The quantitative data were presented in the form of mean and standard deviation. Student t-test was used to compare between quantitative data of two groups. Correlations were analysed by Pearson’s correlation coefficient (r) and P values <0.05 were deemed statistically significant.

Table 1: Age and gender distribution of the studied groups

<table>
<thead>
<tr>
<th>Item</th>
<th>CH</th>
<th>LC</th>
<th>HCC</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>70</td>
<td>30</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Range of age (year)</td>
<td>17-50</td>
<td>21-60</td>
<td>33-68</td>
<td>35-50</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>4:1</td>
<td>3:1</td>
<td>4:1</td>
<td>3:2</td>
</tr>
</tbody>
</table>

CH - Chronic hepatitis, LC - Liver cirrhosis, HCC - Hepatocellular carcinoma
Results

Serum total bilirubin, ALT and AST were significantly increased while serum albumin was significantly decreased in all studied patient groups in comparison with control group (Table 2). HBsAg and anti-HCV antibodies were reported in all patient groups as compared to control group (Table 2). There were highly significant increases (P < 0.001) in bcl-2 protein levels in all studied patient groups as compared to control group. In addition, there were highly significant increases (P < 0.001) in bcl-2 protein levels in HCC and cirrhosis versus chronic groups. However, in chronic hepatitis patients, bcl-2 protein levels showed low significance increases (P < 0.05) as compared to cirrhotic group (Table 3).

Serum MDA and NO were significantly increased (P < 0.001, P < 0.01) in all studied patient groups as compared to control group (Table 2). HBsAg and anti-HCV antibodies were reported in all studied patient groups in comparison with control group (Table 5). In addition, serum bcl-2 levels showed a significant positive correlation with viral hepatitis markers HBsAg and anti-HCV antibodies in all studied patient groups (Table 5).

The presence of excess oxygen radicals and/or lack of decreases in antioxidant enzyme activities (SOD, P < 0.002; GSH, P < 0.001; GSH-px, P < 0.001; GR, P < 0.03) in all studied patient groups as compared to control group (Table 4).

Serum bcl-2 levels showed significant positive correlation with total serum bilirubin, AST and ALT, but negative significant correlation with albumin in HCC group. However, in patients with cirrhosis, serum bcl-2 showed significant positive correlation with total bilirubin and negative correlation with AST, ALT and albumin. On the other hand, there was a significant positive correlation between bcl-2 levels and total bilirubin but negative significant correlation with AST, ALT and albumin in chronic hepatitis patients (Table 5). In addition, serum bcl-2 levels showed a significant positive correlation with viral hepatitis markers HBsAg and anti-HCV antibodies in all studied patient groups (Table 5).

### Table 2: Liver function test results and viral hepatitis markers (HCV-Ab and HBsAg) in chronic, cirrhosis and hepatocellular carcinoma versus control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CH (n = 70) (M ± SD)</th>
<th>Cirrhosis (n = 30) (M ± SD)</th>
<th>HCC (n = 30) (M ± SD)</th>
<th>Control (n = 20) (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/mL)</td>
<td>160 ± 16.72 &lt;0.001</td>
<td>145 ± 13.76 &lt;0.001</td>
<td>130 ± 19.86 &lt;0.001</td>
<td>32 ± 7.64 -</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>196 ± 16.74 &lt;0.001</td>
<td>156 ± 14.65 &lt;0.001</td>
<td>112 ± 17.65 &lt;0.001</td>
<td>28.54 ± 6.4 -</td>
</tr>
<tr>
<td>T. bilirubin (mg/dL)</td>
<td>4.96 ± 0.64 &lt;0.001</td>
<td>6.96 ± 0.56 &lt;0.001</td>
<td>7.34 ± 1.44 &lt;0.001</td>
<td>0.67 ± 0.16 -</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.66 ± 0.54 &lt;0.001</td>
<td>2.67 ± 0.46 &lt;0.001</td>
<td>2.34 ± 0.97 &lt;0.001</td>
<td>4.54 ± 0.67 -</td>
</tr>
<tr>
<td>HCV-ELISA</td>
<td>1.96 ± 0.35 &lt;0.001</td>
<td>1.5 ± 0.18 &lt;0.001</td>
<td>0.96 ± 0.25 &lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>HBsAg-ELISA</td>
<td>1.6 ± 0.19 &lt;0.01</td>
<td>1.2 ± 0.14 &lt;0.01</td>
<td>0.9 ± 0.17 &lt;0.01</td>
<td>-</td>
</tr>
</tbody>
</table>

CH - Chronic hepatitis, HCC - Hepatocellular carcinoma.

### Table 3: Bcl-2 levels in chronic, cirrhosis, hepatocellular carcinoma groups versus control group

<table>
<thead>
<tr>
<th>Bcl-2 (U/mL)</th>
<th>CH (n = 70) Mean ± SD</th>
<th>Cirrhosis (n = 30) Mean ± SD</th>
<th>HCC (n = 30) Mean ± SD</th>
<th>Control (n = 20) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.34 ± 0.11</td>
<td>0.65 ± 0.13</td>
<td>1.25 ± 0.15</td>
<td>0.28 ± 0.12</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P1</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bcl-2: B-cell lymphoma -2 protein, P - comparison between the studied groups and control group, P1 - comparison between chronic hepatitis versus cirrhosis and HCC, P2 - comparison between chronic hepatitis versus cirrhosis.

### Table 4: Levels of cellular oxidative stress markers (MDA, NO, GSH, SOD, GSH-px, GR) in chronic, cirrhosis, HCC groups versus control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CH (n = 70) (M ± SD)</th>
<th>Cirrhosis (n = 30) (M ± SD)</th>
<th>HCC (n = 30) (M ± SD)</th>
<th>Control (n = 20) (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mL)</td>
<td>6.28 ± 1.42 &lt;0.001</td>
<td>4.95 ± 0.54 &lt;0.001</td>
<td>3.50 ± 0.25 &lt;0.001</td>
<td>2.59 ± 0.27 -</td>
</tr>
<tr>
<td>NO (mmol/L)</td>
<td>155 ± 0.29 &lt;0.01</td>
<td>145 ± 0.31 &lt;0.01</td>
<td>135 ± 0.26 &lt;0.01</td>
<td>113 ± 0.35 -</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td>18.35 ± 8.15 &lt;0.002</td>
<td>12.97 ± 10.66 &lt;0.002</td>
<td>10.84 ± 9.96 &lt;0.002</td>
<td>45.59 ± 4.2 -</td>
</tr>
<tr>
<td>GSH (mmol/L)</td>
<td>640 ± 128 &lt;0.001</td>
<td>580 ± 112 &lt;0.001</td>
<td>440 ± 115 &lt;0.001</td>
<td>770 ± 128 -</td>
</tr>
<tr>
<td>GSH-px (U/L)</td>
<td>45.82 ± 1.65 &lt;0.001</td>
<td>28.35 ± 2.79 &lt;0.001</td>
<td>15.89 ± 4.96 &lt;0.001</td>
<td>72.85 ± 1.88 -</td>
</tr>
<tr>
<td>GR (U/L)</td>
<td>45.3 ± 1.65 &lt;0.03</td>
<td>33.5 ± 5.79 &lt;0.03</td>
<td>35.50 ± 5.27 &lt;0.03</td>
<td>86.7 ± 3.88 -</td>
</tr>
</tbody>
</table>

MDA - Malondialdehyde, NO - Nitric oxide, GSH - Glutathione, GSH-px - Glutathione peroxidase, SOD - Superoxide dismutase, CH - Chronic hepatitis, HCC - Hepatocellular carcinoma, (M ± SD) - mean ± standard deviation, P-value <0.05 is significant.

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sufficient antioxidants to scavenge these radicals can either directly contribute to hepatocyte necrosis and participate in PCD regulation based on excessive ROS or inhibitions of antioxidant pathways, which induce apoptosis. MDA is lipid peroxide, whose content usually reflects the level of lipid peroxidation and indirectly reflects the extent of injury in vivo. NO may potentiate cytotoxicity by reaction with superoxide anion to form peroxinitrite, a strong oxidant that promotes nitration of tyrosine to form nitro tyrosine. Intrahepatic accumulation of nitro tyrosine is associated with the histological severity of liver cells. In elevated oxidative stress, NO production greatly increased, which might induce hepatocyte to progress to irreversible channel of necrosis and cell death.

Table 5: Correlations coefficients between bcl-2 levels and studied parameters in different groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CH (n = 70)</th>
<th>Cirrhosis (n = 30)</th>
<th>HCC (n = 30)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>AST (IU/mL)</td>
<td>-0.664 &lt;0.05</td>
<td>-0.49 &lt;0.01</td>
<td>0.45 &lt;0.05</td>
<td>-0.14 &gt;0.05</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>-0.554 &lt;0.05</td>
<td>-0.16 &lt;0.01</td>
<td>0.50 &lt;0.05</td>
<td>0.09 &gt;0.05</td>
</tr>
<tr>
<td>T. bilirubin</td>
<td>0.665 &lt;0.05</td>
<td>0.12 &lt;0.01</td>
<td>0.49 &lt;0.05</td>
<td>-0.27 &gt;0.05</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>-0.459 &lt;0.01</td>
<td>-0.36 &lt;0.05</td>
<td>-0.45 &lt;0.01</td>
<td>-0.36 &gt;0.05</td>
</tr>
<tr>
<td>HCV-ELISA</td>
<td>0.616 &lt;0.001</td>
<td>0.61 &lt;0.001</td>
<td>0.60 &lt;0.001</td>
<td>-0.17 &gt;0.05</td>
</tr>
<tr>
<td>HBsAg-ELISA</td>
<td>0.334 &lt;0.01</td>
<td>0.32 &lt;0.01</td>
<td>0.33 &lt;0.01</td>
<td>-0.16 &gt;0.05</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>0.664 &lt;0.001</td>
<td>0.47 &lt;0.001</td>
<td>0.66 &lt;0.001</td>
<td>-0.34 &gt;0.05</td>
</tr>
<tr>
<td>NO (mmol/L)</td>
<td>0.334 &lt;0.001</td>
<td>0.39 &lt;0.001</td>
<td>0.33 &lt;0.001</td>
<td>-0.64 &gt;0.05</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td>0.664 &lt;0.001</td>
<td>0.22 &lt;0.001</td>
<td>0.54 &lt;0.001</td>
<td>-0.23 &gt;0.05</td>
</tr>
<tr>
<td>GSH (mmol/L)</td>
<td>0.664 &lt;0.001</td>
<td>0.65 &lt;0.001</td>
<td>0.64 &lt;0.001</td>
<td>0.49 &gt;0.05</td>
</tr>
<tr>
<td>GSH-px (U/L)</td>
<td>0.664 &lt;0.001</td>
<td>0.49 &lt;0.001</td>
<td>0.45 &lt;0.001</td>
<td>0.41 &gt;0.05</td>
</tr>
<tr>
<td>GR (U/L)</td>
<td>-0.554 &lt;0.01</td>
<td>-0.16 &lt;0.01</td>
<td>-0.50 &lt;0.05</td>
<td>0.18 &gt;0.05</td>
</tr>
</tbody>
</table>

MDA - Malondialdehyde, NO - Nitric oxide, GSH - Glutathione, GSH-px - Glutathione peroxidase, SOD - Superoxide dismutase, CH - Chronic hepatitis, HCC - Hepatocellular carcinoma, (M ± SD) - mean ± standard deviation, P-value <0.05 is significant

Serum bcl-2 levels showed a significant positive correlation with the increase in free radical oxidative stress markers (MDA, NO) in all studied patient groups (Table 5). On the other hand, there was a significant positive correlation between bcl-2 levels and decrease in antioxidant enzyme activities (SOD, GSH and GSH-px). However, there was a negative correlation between bcl-2 levels and GR.

Discussion

Chronic infection with hepatitis B and C viruses often results in cirrhosis and enhances the probability of developing HCC. In 60% of HCC cases, no etiologic factors could be identified. This might be due to the difference in environment and incidence of HCC and other chronic liver diseases. Eighty to 85% of persons infected by HCV cannot resolve their infection and suffer from persistent chronic hepatitis and persistent HCV and HBV infections are strongly associated with the development of hepatocellular carcinoma.9

In the present study, there were highly significant (P < 0.001) increases in total serum bilirubin, AST and ALT in all studied patient groups as compared to control group, whilst there were highly significant (P < 0.001) decreases in albumin in all studied patient groups as compared to control group. These results were in agreement with the presence of viral hepatitis markers (HBsAg and anti-HCV antibodies), which were significantly detected in all studied patient groups. The significant increase in liver enzymes was in concomitant with Vincent,10 who reported that viral hepatitis was encountered with hepatocellular injury, massive necrosis, dramatic increase of liver enzymes and different degrees of hepatic inflammation as well as fibrosis.

As reported, bcl-2 antigen is a proto-oncogene responsible for the inhibition of the death of cells in the mechanism of apoptosis. Bcl-2 protein was shown to prevent apoptosis of normal liver and enhance the cellular anti-oxidant capacity. Therefore, it prolongs the survival of liver cells.11 In patients with chronic hepatitis C, the expression of bcl-2 antigen was directly proportional to the degree of inflammatory activity and it was inversely proportional to the degree of fibrosis. In addition, in hepatic tissues, a positive colour reaction with bcl-2 antigen might be of prognostic value.12

The present study revealed that the mean serum level of bcl-2 protein in the healthy control group was (0.28 ± 0.12). The data were in agreement with Hamazaki et al.,13 who studied the expression of bcl-2 protein in normal liver. The expression of bcl-2 protein in normal liver was 8.5%. Moreover, they stated that bcl-2 protein is an oncogene product located at the mitochondrial inner surface and could prolong cell survival by blocking apoptosis.

In this study, the mean serum level of bcl-2 protein in chronic hepatitis patients was 0.34 ± 0.11. There was significant increase in serum bcl-2 level in chronic
hepatitis patients as compared to control group. These data accompanied the estimation of viral hepatitis markers (HBsAg and anti-HCV antibodies) in the serum of chronic hepatitis patients, whereas the mean serum levels were HBsAg, $1.6 \pm 0.19$; anti-HCV, $1.96 \pm 0.35$. The data obtained are in harmony with Toubi et al.,$^{14}$ who reported that the expression of bcl-2 did not increase in the livers and peripheral T-cells of patients with chronic HCV infection, signifying that bcl-2 does not exert an anti-apoptotic effect in HCV-infected patients. In addition, the expression of bcl-2 protein was reported more often among controls than in chronic hepatitis patients and that positive colour reaction with bcl-2 antigen in lobules might be of prognostic value and that the expression of bcl-2 protein in portal spaces have a limited diagnostic value for the prediction of the scope of fibrosis in patients with chronic hepatitis C.$^{12}$

In our HCC cases, serum bcl-2 levels were highly significantly increased when compared to cirrhotic and chronic hepatitis patients ($1.25 \pm 0.15$, $0.65 \pm 0.13$ and $0.34 \pm 0.11$, respectively, $P < 0.001$). The significantly higher bcl-2 values were in concomitant with Frommel et al.,$^{15}$ who stated that bcl-2 expression was elevated in the liver of cirrhotic patients and this might correlate with the development of hepatocellular carcinoma given the anti-apoptotic/oncogenic potential of bcl-2. These observations put the hypothesis forward that the higher expression of bcl-2 protein in the serum of cancer cases with viral hepatitis (HBsAg, $0.9 \pm 0.17$; anti-HCV, $0.96 \pm 0.25$) might be of prognostic value.

The data suggested a decrease in the anti-apoptotic bcl-2 activity in chronic hepatitis patients, which may be related to the regulatory function of viral hepatitis proteins like HBx protein of HBV upon bcl-2. These data were in agreement with Terradillos et al.,$^{16}$ who reported interference of the pro-apoptotic function of HBx with anti-apoptotic bcl-2 during hepatic apoptosis in transgenic mice. However, the pro-apoptotic activity of HBx overcomes or bypasses the inhibitory effect of bcl-2 against Fas cytotoxicity. This dominant function of HBx upon bcl-2 might play an important role in the pathogenesis of chronic hepatitis B.

In this study, the mean serum level of bcl-2 protein in patients with liver cirrhosis was $0.65 \pm 0.13$, whereas in chronic group, the mean value of serum bcl-2 was $0.34 \pm 0.11$. A significantly higher bcl-2 concentration was observed in cirrhotic patients ($P < 0.001$, $P < 0.05$) when compared to the corresponding mean values in control and chronic patient groups, respectively. These data matched with Kim et al.,$^{17}$ who reported that HBx sensitizes liver cells to apoptosis upon hepatitis B virus infection, which contributes to the development of hepatitis and the subsequent generation of hepatocellular carcinoma.

Serum bcl-2 levels showed significant positive correlations ($P < 0.001$, $P < 0.01$) with viral hepatitis markers (HBsAg, anti-HCV antibodies) in all studied patient groups. These results significantly correlated with a recent report that HBx could potentiate c-myc, which induces liver oncogenesis and elicits apoptotic responses in transgenic livers. These data strengthen the notion that HBx may contribute to HBV pathogenesis by enhancing apoptotic death in the chronically infected liver.$^{18}$

Serum bcl-2 levels showed significant positive correlations ($P < 0.05$) with total serum bilirubin, AST, ALT and negative significant correlation ($P < 0.01$) with albumin in HCC group. In patients with cirrhosis, serum bcl-2 showed significant positive correlation ($P < 0.01$) with total bilirubin and negative correlations ($P < 0.01$, $P < 0.05$) with AST, ALT and albumin. On the other hand, in chronic hepatitis patients, there was a significant positive correlation between bcl-2 levels and total bilirubin ($P < 0.05$) and negative significant correlations ($P < 0.05$, $P < 0.01$) with AST, ALT and albumin.

Lipid peroxidation products are one of the main causes of hepatocyte injury. In patients with viral infection, serum MDA levels were found to be higher as compared to the control. Evidence of lipid peroxidation in the form of increased MDA production has been reported in previous studies and that serum MDA levels were correlated with the severity of chronic hepatitis. In addition, it was reported that serum LPO products significantly increased in patients with chronic hepatitis C before interferon (IF)-2 treatment.$^{19}$

In the present study, there were highly significant ($P < 0.001$, $P < 0.01$) increases in the levels of MDA and NO as cellular oxidative markers in all studied patient groups with viral hepatitis as compared to control group. The results of this study were matched with Mansurova et al.,$^{20}$ who reported that serum MDA concentration increased in CHC patients and this fits well with GSH depletion observed in hepatic tissue, plasma and peripheral blood mononuclear cells of CHC patients. The obtained results may confirm the involvement of oxidative stress as part of the pathophysiology of CHC patients.

In the current study, although there was an increase in NO levels in the serum of hepatitis patients with different pathological stages as compared to control group, we did not find correlations between histologic severity and NO concentration. This may reflect the protective role of NO in the liver through vasodilatory, antioxidative and antiapoptotic effects. However, in the presence of massive injury (e.g. high level of inducers and elevated oxidative stress), greatly increased NO production might induce the hepatocyte to progress to irreversible channel of necrosis and cell death.$^{21}$

In addition, these results matched with reports concerning the contradictory role of NO in liver damage during inflammatory conditions. It was found that NO protects against liver injury by scavenging lipid radicals and inhibiting the lipidperoxidation chain.$^{22}$
Serum bcl-2 levels showed significant positive correlations ($P < 0.001$) with the increase in free radical oxidative stress markers (MDA, NO) in all studied patient groups. The peculiarity of mechanisms that lipid regulates peroxidation in different grades of hepatitis is of a role in disease pathogenesis and underlies the prediction of the course of hepatitis.$^{23}$

Hepatocytes are continuously exposed to ROS and are protected from oxidative injury by a range of antioxidant pathways. The defenses against free radical-mediated injury include enzymatic deactivation and direct reaction with free radicals.

In our study, we observed a significant decrease ($P < 0.002, P < 0.001, P < 0.001, 0.03$) in the level of antioxidant enzyme activities (SOD, GSHp-x, GSH and GR) in all studied patient groups as compared to control group. The depletion of antioxidant enzyme defense system was significantly observed among chronic hepatitis, cirrhosis and cancer patients when compared with controls. The data were in agreement with Anderson,$^{24}$ who found a low level of GSH and a decrease in antioxidant enzyme activities with the release of serum malondialdehyde level in patients chronically infected with hepatitis C and/or B virus. These findings may indicate that oxidative free radicals play a role in liver cell damage and apoptosis through impairment of antioxidant enzymatic defense system. This matched with Carmela and Alessandro,$^{12}$ who reported that in patients with viral or alcoholic liver disease, the consequent alteration of cellular redox state is potentiated by a correlated decrease in antioxidant enzymes and increase of free radical-mediated damage and apoptosis of liver cells.

In our study, there were significant positive correlations ($P < 0.001$) between bcl-2 level and decrease in antioxidant enzyme activities (SOD, GSH and GSH-px). However, there was a negative correlation ($P < 0.01$) between bcl-2 levels and GR in all studied patient groups.

Conclusions

The release of oxidative free radicals, deficiency in antioxidant enzymes and increase in the expression of bcl-2 protein might play a role in the pathogenesis of viral hepatitis. In addition, the ability to measure bcl-2 protein in the serum could be useful as a prognostic marker of cancer patients.

References

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