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INDICES OF LIVER AND KIDNEY FUNCTION AND THE LEVEL OF CYTOKINES AND TUMOR BIOMARKERS IN THE SERUM OF PATIENTS WITH HEPATITIS C AND HEPATOCELLULAR CARCINOMA

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Hepatitis C virus (HCV) selectively targets the liver and causes persistent infection, often evading the immune system, leading to chronic liver failure, kidney disease and HCV-related hepatocellular carcinoma (HCC). This study aims to estimate the indices of liver and kidney functions and the level of inflammatory cytokines and tumor markers in patients with both HCV-positive chronic hepatitis and HCV-related HCC in comparison to HCV only patients and healthy controls. The study included 156 persons divided into four groups: control group I – 27 healthy individuals; HCV group – 45 patients with HCV (proved by PCR); HCC group – 42 patients with HCC (proved by radiological investigations and laboratory tests); HCV+HCC group – 42 patients with HCV and HCC (HCV – positive chronic hepatitis with HCC). Routine clinical tests for kidney and liver function were used. The levels of IL-6, IL-1B, TNF-α and tumor markers carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and alpha-Fetoprotein (AFP) were examined by ELISA. The results showed a highly significant increase in ALT, ALP, and AST activity, as well as uric acid, urea and creatinine levels, with a significant decrease in albumin levels in HCC and HCV+HCC groups compared to HCV-only patients and healthy controls. The elevation in the serum levels of the studied tumor markers and cytokines in the HCC and HCV+HCC groups, with the highest levels in the latter, was observed. The data obtained indicate the progressive deterioration in liver and kidney functions and a significant effect of chronic inflammation in HCV-related liver carcinogenesis.

K e y w o r d s: hepatitis C virus, hepatocellular carcinoma, liver, kidney, IL-6, IL-1B, TNF-α, tumor markers.

Hepatitis C virus (HCV) infection is a major global health concern, affecting an estimated 58 million people worldwide, with approximately 1.5 million new infections occurring annually, according to the World Health Organization (WHO). The prevalence of HCV varies significantly by region, with the highest burden observed in low- and middle-income countries. Transmission primarily occurs through exposure to infected blood, including unsafe medical procedures, injection drug use, and blood transfusions without proper screening [1].

HCV is a positive-sense, single-stranded RNA virus that belongs to the genus Hepacivirus in the Flaviviridae family and infects humans and other higher primates. It selectively targets the liver by triggering cellular machinery for viral replication and causes persistent infection, often evading the immune system, leading to liver disease that can progress to cirrhosis, liver failure, hepatocellular carcinoma (HCC), and even death [2-4].

HCV infection is one of the causes of death in the general population as well as in the population represented by hemodialysis patients. However, hemodialysis patients are at a higher risk of contracting the infection due to the potential for insufficient sterilization of the dialysis equipment during the dialysis process. As a result, the mortality rates among hemodialysis patients are higher due to the increased risk of infection [5].

For HCV to spread throughout hepatocytes and cause several pathological processes in the liver, it is heavily dependent on host variables. Examining these viral host requirements is essential to understanding the pathophysiology of HCV-related illnesses and developing new treatment approaches [6].

Approximately 80% of initial liver tumors are HCCs. Approximately 70% of individuals may experience an intrahepatic recurrence within 5 years after receiving curative surgical or non-surgical treatment, making ongoing follow-up necessary even after treatment. During follow-up, magnetic resonance imaging, contrast-enhanced computed tomography, and ultrasound are used. Due to its advantages in determining the risk of recurrence, cost, examination time, and detection of extrahepatic metastases, contrast-enhanced computed tomography is the most often employed of these modalities [7-9].

HCC is the third most common cause of cancer-related mortality (about 800,000 deaths annually) and the sixth most common malignancy globally. Hepatitis B, hepatitis C, or nonalcoholic steatohepatitis are three common chronic viral infections that can produce persistent cycles of inflammation and repair in the liver, which is the cause of HCC in most cirrhosis patients [10-12].

Hepatitis and liver cirrhosis are two of the main chronic liver illnesses that increase the chance of developing HCC. Cirrhosis, an irreversible outcome of chronic liver disease marked by fibrous scarring and regenerated parenchyma, is the precursor to more than 80% of HCCs [13].

Material and Methods

Sample collection. This study comprised 156 samples, aged between 29 and 76 years, with 86 males (55.1%) and 70 females (44.9%). Ethical committee approval was obtained on Feb,2023 from the Faculty of Pharmacy at Minia University with a protocol No: MPEC230602 (MPEC refers to Minia Pharmacy Ethical Committee), and informed consent was obtained from all patients from Minia Oncology Center. Samples were collected in the period from Feb 2023 to Feb 2024 and were classified into four groups as follows.

Control group involved 27 samples who appeared to be healthy, with ages ranging between 28 and 68 years and a mean value of 55.8 ± 7.3. They had normal liver function tests and were seronegative for HCV antibodies and hepatitis B surface markers. (There is a limitation in obtaining more than 27 healthy samples due to the lack of available healthy volunteers).

Group HCV comprised 45 patients (age range was 35-76 years with a mean value of 58.7 ± 11.2) with liver hepatitis without HCC based on ultrasonography results, biochemical evidence of parenchymal damage, and liver biopsy. Hepatitis C infection was confirmed by PCR.

Group HCC without HCV included 42 patients aged between 38 and 76 years with a mean value of 59.7 ± 8.9. Radiological investigations, including abdominal ultrasonography and triphasic CT scans, as well as laboratory tests, were used to diagnose HCC in this group.

Group HCV+HCC involved 42 patients with positive chronic hepatitis with HCC aged between 39 and 74 years with a mean value of 54.5 ± 8.9. Ultrasonography findings, biochemical evidence of parenchymal damage, and a liver biopsy were used to diagnose the patients in this group. Hepatitis C infection was confirmed by PCR.

Samples from the following patients: those with any other type of tumor other than HCC; those with autoimmune, hepatitis B (acute); those with localized hepatic lesions other than HCC; those with inflammatory illnesses or bone lesions; and those who had previously had treatment for HCC were excluded.

All samples collected from the patients were subjected to the following procedures to obtain the serum: 10 ml of venous blood from each patient was taken, and the serum was separated by centrifugation for 15 min at 1,000×g. Serum was subsequently refrigerated at -80 ˚C. The following procedures were applied to all patients and controls: complete clinical assessment, taking a full history, and abdominal ultrasonography [14].

Biochemical examination. Colorimetric assay kits (catalog numbers ZL-211 001, 264 002, 216 001, 260 002, 234 001, 318 002 and 323 009, Spectrum-diagnostics, Cairo, Egypt) were used for measuring standard laboratory tests, including serum albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and kidney function tests (creatinine, urea and uric acid) respectively [15-21].

Measurement of serum pro-inflammatory cytokines and tumor markers. Commercially available ELISA kits (catalog numbers E-EL-H6156, E-EL-H0149, E-EL-H0109, E-EL-H6047, E-EL-H0637 and E-EL-H0070 Elabscience, Houston, Texas, USA,) were used to assess the serum levels of interleukin 6 (IL-6), interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and Alpha-Fetoprotein (AFP) respectively in accordance with the manufacturer's instructions [22-27].

Statistical analysis. The data were analyzed using the statistical software SPSS 18 by One-Way

ANOVA. The post-hoc test used is the Tukey test. The results were presented as M ± SD. The quantitative variables between the two groups were compared using chi-square analysis. $P < 0.05$ was considered statistically significant, while $P < 0.001$ was considered highly significant.

Results

Diagnostic value of kidney function and liver function. When measuring kidney function, a very high significant increase ($P < 0.001$) was found in creatinine (5.6 ± 0.7 mg/dl, 95% confidence interval (CI): 3.96, 4.72), urea (22.3 ± 7.3 mg/dl, 95% CI: 5.52, 12.93) and uric acid (9.6 ± 2.8 mg/dl, 95% CI: 2.84, 5.82) in HCV+HCC group compared to control group (1.1 ± 0.4, 11.7 ± 5.2 and 5.3 ± 2.1, respectively), HCV group (2.8 ± 0.5, 15.4 ± 4.7 and 6.8 ± 1.9, respectively) and HCC group (4.4 ± 0.6, 18.7 ± 5.6 and 7.8 ± 2.4, respectively) as shown in Fig. 1, *A*.

The values of the functional liver enzymes (AST, ALT, and ALP) were considerably ($P < 0.0001$) higher in the HCV group (67.5 ± 8.6, 60.8 ± 12.4 and 105.5 ± 21.5 IU/l, respectively), HCC group (91.5 ± 11.3, 89.8 ± 16.2 and 243.0 ± 18.7, respectively), and HCV+HCC group (98.8 ± 7.5 [95% CI: 59.4, 70.63], 98.4 ± 12.2 [95% CI: 64.5, 79.1] and

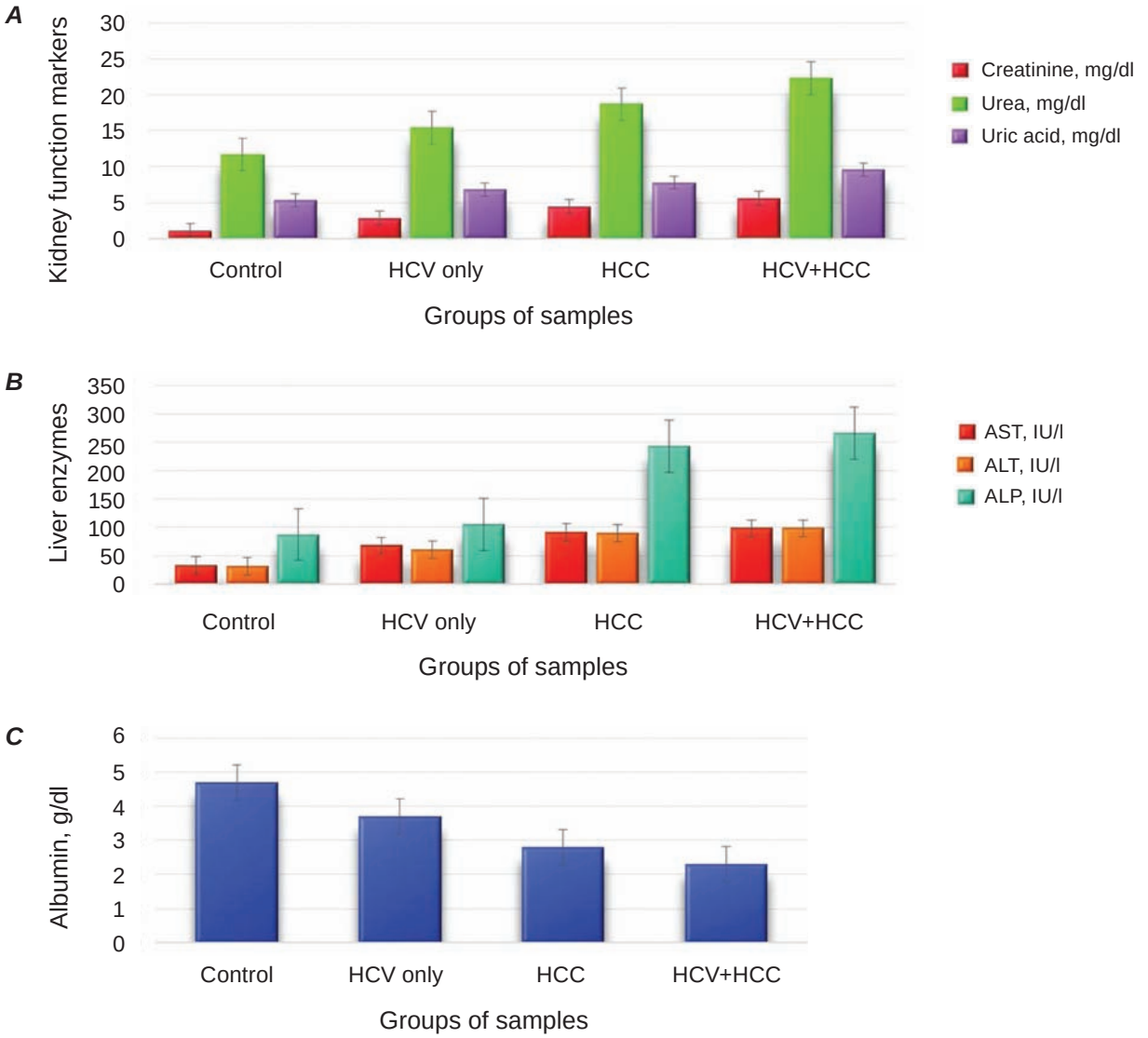

Fig. 1. ***A****. Effect of HCV, HCC, and combined HCV+HCC on kidney function markers (creatinine, urea, and uric acid) compared to control.* ***B****. Effect of HCV, HCC, and combined HCV+HCC on liver enzymes (ALT, AST, and ALP) compared to control.* ***C****. Effect of HCV, HCC, and combined HCV+HCC on serum albumin concentration compared to control*

266.1 ± 27.5 [95% CI: 167.04, 194.91], respectively) compared to control group (32.5 ± 3.7, 31.6 ± 4.7, and 87.3 ± 15.7, respectively) as represented in Fig. 1, *B*. The albumin value in the HCV+HCC group (2.3 ± 0.3 mg/dl, 95% CI: -2.71, -2.21) was considerably lower than the other groups (control: 4.7 ± 0.4, HCV: 3.7 ± 0.5 and HCC: 2.8 ± 0.30) (Fig. 1, *C*).

Diagnostic value of tumor biomarkers. Serum CEA (ng/ml) and CA19.9 (U/ml) in HCV (35.4± 5.4, 98.8 ± 14.2), HCC (298.2 ± 14.3, 211.2 ± 12.5), and HCV+ HCC (354.7 ± 15.2 [95% CI: 343.16, 357.62], 285.7 ± 15.3 [95% CI: 239.93, 257.49]) groups were high significantly ($P < 0.001$) elevated in comparison to the control group (2.8 ± 1.2, 35.5 ± 7.5). Serum levels of AFP were extremely significantly higher in the HCV+HCC (394.1 ± 16.4 ng/ml [95% CI: 383.21, 396.73]) group compared to the control group (5.3 ± 1.5 ng/ml) as represented in Fig. 2, *A*.

Concentration of cytokines biomarkers in serum. The levels of pro-inflammatory cytokines IL-6, TNF-α and IL-1β significantly increased ($P < 0.001$) in the HCV group (26.8 ± 2.7, 65.2 ± 4.5 and 47.6 ± 3.5 pg/ml, respectively), HCC group (30.4 ± 3.4, 87.3 ± 5.3 and 66.8 ± 7.3, respectively) and HCV+HCC group (41.5 ± 5.7 [95% CI: 31.66, 36.04], 95.8 ± 8.6 [95% CI: 68.5, 75.77] and 83.2 ± 6.7 [95% CI: 64.25, 70.93], respectively) compared to the control group (6.7 ± 1.4, 23.1 ± 2.7, 15.4 ± 2.1, respectively) as shown in Fig. 2, *B*.

Discussion

One of the main causes of end-stage liver disease, including HCC, is a persistent HCV infection. Persistent viral replication in the majority of HCV-infected people can advance to cirrhosis and then HCC. It is hypothesized that HCV plays crucial role in the development of HCC through mechanisms that include modifications to various cellular signaling pathways [28].

HCV replicates in liver cells, causing fibrosis and ultimately leading to cirrhosis. Moreover, HCV replicates in B cells, triggering the synthesis of cryoglobulin. Furthermore, HCV-tissue protein association promotes a chronic inflammatory response by enhancing autophagy and the release of pro-inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF). Due to unclear mechanisms, the inflammatory response is associated with the generation of reactive oxygen species and nitric oxide, which can cause damage to the kidneys and liver [29, 30]. This mechanism is supported by our

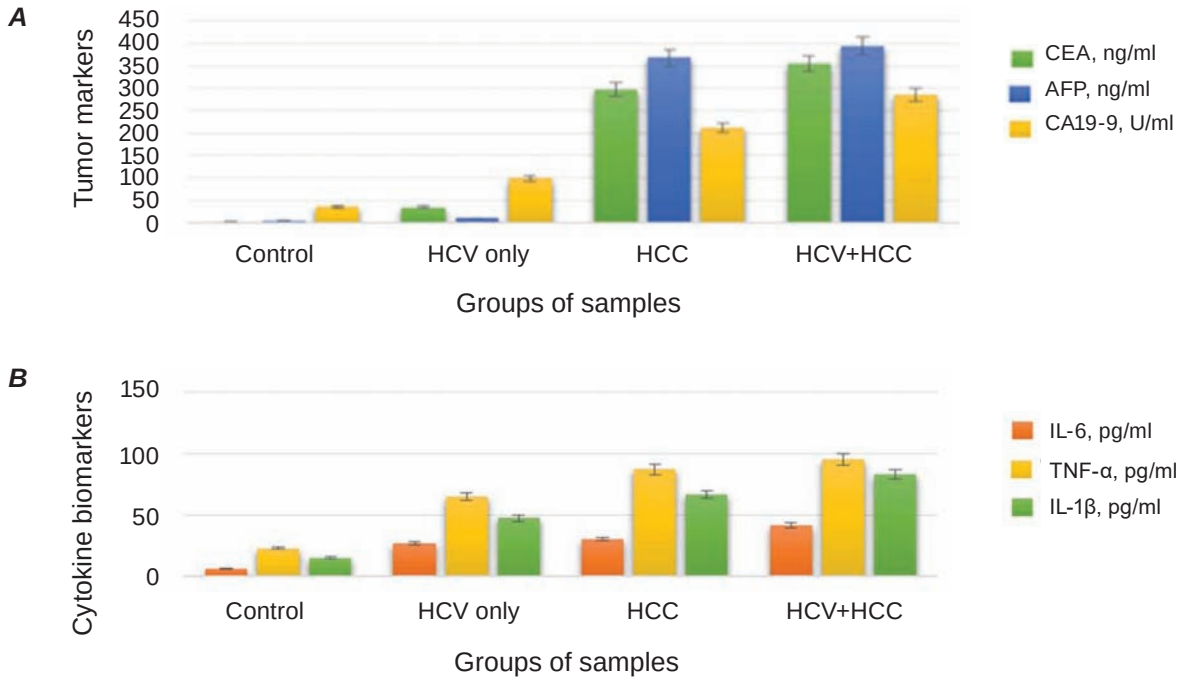

Fig. 2. ***A****. Effect of HCV, HCC, and combined HCV+HCC on tumor markers (CEA, AFP, and CA19-9) compared to control.* ***B****. Effect of HCV, HCC, and combined HCV+HCC on cytokine levels (IL-6, IL-1β, and TNF-α) compared to control*

findings, where IL-6, IL-1β, and TNF-α levels were significantly elevated in the HCC and HCV+HCC groups compared to both the control and HCV-only groups, indicating a strong inflammatory milieu associated with liver tumorigenesis. HCV infection causes dramatic changes in serum cytokine and chemokine levels. HCV clearance with antiviral drugs modifies the body's immune system, which may interfere with immune-mediated cancer surveillance and lead to altered cytokine levels. Identifying HCV patients at risk for developing HCC after eradication remains an urgent need in our country.

There is an epidemiological relationship between HCV and chronic kidney disease (CKD) because (a) CKD patients are more likely to be exposed to HCV in dialysis units and (b) HCV infection directly causes renal harm [31]. In line with this, creatinine, urea, and uric acid levels were markedly elevated in the HCV+HCC and HCC groups, while albumin levels were reduced, reflecting combined hepatic and renal dysfunction in advanced disease states.

In the previous study, individuals with HCC who also had ascites had reduced renal function as indicated by the estimated glomerular filtration rate determined by modifying the diet in the renal disease formula. Furthermore, ascitic patients also exhibited larger HCC and vascular invasion more frequently, indicating that cirrhosis and tumor factors may be involved in the production of ascites. Patients with HCC in this study had considerably higher creatinine levels and significantly lower serum albumin at study admission [32]. Our data align with this observation, as patients in the HCC and HCV+HCC groups demonstrated significantly higher creatinine and lower albumin levels compared to controls.

According to our findings, the median IL-6 levels in HCC patients were greater than those in HCV patients and the controls, suggesting that the tumor cells are responsible for the elevated cytokine production. The immunohistochemical result and the observation that Okuda's stage III, where the neoplastic mass is at its largest, had the highest IL-6 values, both corroborate this. Serum levels of IL-1B and IL-6 that are higher than control values may be the result of remnant hepatic cells responding to cytolysis and an attempt to restore liver mass linked to a definite impairment [33, 34]. This is further supported by the statistically significant elevations in IL-6 and IL-1β in the HCC and HCV+HCC groups, with the highest levels observed in the latter.

TNF-α increased statistically significantly as the tumor got bigger. One of the most sensitive cytokines for tracking the course of the disease in HCV-infected patients was TNF-α, whose serum level increased significantly as the illness progressed. This could be explained by TNF-α's critical function in the progression of HCC and its potential use as a marker of hepatocyte damage, as TNF-α secretion increases with higher stages of inflammation and fibrosis [35-37]. Indeed, in this study, TNF-α showed a clear stepwise increase across groups, peaking in HCV+HCC patients.

Well-known tumor markers for the diagnosis and prognosis of hepatobiliary pancreatic cancers, including alpha-fetoprotein, carcinoembryonic antigen, and CA19-9, have been included in a number of prognostic models. Elevated serum AFP levels are typically associated with a poor prognosis and a high risk of HCC development [38]. Our data revealed a dramatic rise in AFP levels in HCC and HCV+HCC patients, in contrast to the much lower levels in HCV-only and control groups, confirming the strong diagnostic value of AFP.

A common antigen used in the clinical diagnosis and therapy monitoring of gastrointestinal cancer is CEA, which is comparatively non-specific. In individuals with HCC, its elevated serum level is thought to be an independent indicator of recurrence. The primary application of CA19-9 is as a sensitive biomarker for pancreatic cancers; in patients with HCC, a high level of this biomarker is also linked to a bad prognosis. The CA19-9 test is typically used for individuals diagnosed with pancreatic cancer, but it can also have applications in other types of cancer, such as HCC or conditions related to the digestive system, due to its high value, as mentioned in the results obtained in our study. It was discovered that CA19-9 was connected to severe cirrhosis and liver inflammation, which resulted in a bad prognosis for HCC patients [39]. This study found that both CEA and CA19-9 were significantly elevated in HCC and HCV+HCC groups, underscoring their added value when combined with AFP for tumor monitoring and prognostication.

Conclusion. This study provides strong evidence that the serum levels of AFP, CA19-9, and CEA are significantly elevated in patients with HCC and HCV-related HCC, in comparison to both HCV-only patients and controls. These tumor markers demonstrated high specificity and positive predictive value, supporting their utility in distinguishing

HCC from other liver conditions. Moreover, the analysis of liver and kidney function markers (such as ALT, AST, ALP, creatinine, and urea) revealed a clear pattern of progressive deterioration in function as disease severity increased, with statistically significant differences, particularly in the HCC and HCV+HCC groups. In addition, inflammatory cytokines, including IL-6, TNF-α, and IL-1β, were markedly elevated in patients with HCC and HCV+HCC, reflecting the role of chronic inflammation in liver carcinogenesis. While the combination of AFP, CA19-9, and CEA improves the diagnostic accuracy for HCC, the early detection of HCC still demands further research and larger sample sizes to identify more sensitive and specific biomarkers.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПОКАЗНИКИ ФУНКЦІЇ ПЕЧІНКИ ТА НИРОК, РІВЕНЬ ЦИТОКІНІВ І ПУХЛИННИХ БІОМАРКЕРІВ У СИРОВАТЦІ КРОВІ ПАЦІЄНТІВ ІЗ ГЕПАТИТОМ С І ГЕПАТОЦЕЛЮЛЯРНОЮ КАРЦИНОМОЮ

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Вірус гепатиту С (HCV) спрямовано вражає печінку і спричиняє стійку інфекцію, яка часто уникає імунної системи, що призводить до хронічної печінкової недостатності, захворювань нирок і HCV-асоційованої гепатоцелюлярної карциноми (HCC). Метою цього дослідження було оцінити показники функції печінки і нирок, рівень запальних цитокінів і пухлинних маркерів у пацієнтів із HCV-позитивним хронічним гепатитом і HCV-асоційованою HCC порівняно з пацієнтами з HCV і контролем. У дослідженні брали участь 156 осіб, розділених на чотири групи: контрольна група – 27 здорових осіб; група HCV – 45 пацієнтів з HCV (підтверджено методом ПЛР); група HCC – 42 пацієнти з HCC (підтверджено рентгенологічними дослідженнями та лабораторними тестами); група HCV+HCC – 42 пацієнти з HCV і HCC (HCV-позитивний хронічний гепатит з HCC). Використовували звичайні клінічні тести для дослідження функції нирок і печінки. Рівень IL-6, IL-1B, TNF-α і пухлинних маркерів карциноембріонального антигену (CEA), вуглеводного антигену 19-9 (CA19-9) і альфа-фетопротеїну (AFP) досліджували методом ELISA. Результати показали значне підвищення активності ALT, ALP, AST, рівня сечової кислоти, сечовини і креатиніну за значного зниження рівня альбуміну в групах HCC і HCV+HCC порівняно з пацієнтами з груп HCV і контролем. Відзначено підвищення рівнів досліджуваних пухлинних маркерів і цитокінів у групах HCC і HCV+HCC, з найбільшими значеннями в останній. Отримані дані свідчать про прогресуюче погіршення функцій печінки та нирок і значний вплив хронічного запалення в HCV-асоційованому канцерогенезі печінки.

Ключові слова: вірус гепатиту С, гепатоцелюлярна карцинома, печінка, нирки, IL-6, IL-1B, TNF-α, пухлинні маркери.

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