Ehrlich ascites carcinoma bearing mice as model of human hepatocellular carcinoma

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ABSTRACT

Aims: Ehrlich ascites carcinoma (EAC) is a transplantable neoplasia from a malign epithelium; it is a form of ascites when inoculated into the intraperitoneal cavity. This study aimed to investigate to compare among the effects of EAC and human hepatocellular carcinoma (HCC) on liver in mice and human.

Methodology: Female Albino mice (n=10) and human liver biopsy (n=10) were used in this work; mice were divided into two equal groups, five of mice for each group: group1 served as control group; and group2 were inoculated once intraperitoneal with EAC cells. Human liver was divided into two equal groups (five liver biopsy for each group). Group1 was collected from healthy male and group 2 patients infected with HCC. Blood samples and liver tissues were collected from all groups.

Results: The obtained results showed that increase in liver enzymes activity (AST, ALT and ALP), and decrease level of albumin in serum, also changes in histopathological and proliferating cell nuclear antigen (PCNA) expressions in liver sections when compared to the control group.

Conclusion: It could be concluded that EAC bearing mice lead to a same defect on liver enzymes, histopathological and immunohistochemical which simulates and corresponds the same problems in the human HCC.

Keywords: Ehrlich ascites carcinoma, hepatocellular carcinoma, immunohistochemistry, histopathology, liver enzymes.

1. INTRODUCTION

Cancer is a major and serious problem that affects human societies because it is the second leading cause of death throughout of the world. Unfortunately, the diagnosis and treatment of cancer in the body require a great challenge because it is considered a diverse disease at the level of tissues [1-4]. The rapid formation of abnormal cells growing away from their natural forms is one of the important aspects of cancer. After that, they can attack nearby components and then spread to other organs in the body. This process is defined to as metastasis which is considered the main cause of cancer deaths (about 90 % of all mortality from cancer cases) in the world and it show an outstandingly different situation of clinical characteristics [5].

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. In the United States, HCC is the ninth

leading cause of cancer deaths. Hepatocellular carcinoma (HCC) is a common malignancy which usually grows on a background of chronic hepatic disease. Hepatocellular carcinoma is a one of main cause that leading to cancer-related morbidity and mortality. Relative to other malignancies, the incidence of (HCC) that is fifth highest in men and ninth highest in women, and it regard for the second most cancer deaths worldwide [6]. Growth and development of (HCC) is a complex multistep process that comprise sustained inflammatory damage, including hepatocyte necrosis and regeneration, associated with fibrotic deposition. Danger of (HCC) emerging when cirrhosis is decided, and it increases in parallel to progressive hepatic function impairment [7].

Ehrlich ascites carcinoma (EAC) is an undifferentiated tumor has a high capacity for transplant in transplantation, absence of regression, shorter life span, assured malignancy, originally hyper diploid, quick proliferation and described by absence of TSTA (tumor-specific transplantation antigen) [8].

Islam et al. [9] demonstrated that the effusion, which contained neoplastic cells that are proliferated after injection of tumor cells into the peritoneal cavity, is referred to as the ascites (EAC). Commonly, tumor virulence rises by repetitious passages, while the proliferating rate of such tumors increases gradually. Moreover, differentiation gradually disappears, while the cells get free growth control mechanisms, getting hetero transplantability and in the end, are converted to the ascites form. However, in the therapeutic studies of (HCC), a human hepatocellular cancer cell line (HepG2) and (EAC) line were used and found similar antitumor activities [10]. The present work is aimed to compare among the effects of EAC and HCC on liver in the mice and human, in order to know whether the (EAC) liver damage is similar to human (HCC) via assessing liver enzymes, histopathological and immunohistochemical (PCNA) examination

2. MATERIAL AND METHODS

2.1. Patients and animals sample collection

Liver biopsy from 10 patients have been included in this study; patients were divided into two equal categories according to the specific criteria, five of patients for each group: Group 1: five samples (blood) and liver biopsy were collected from healthy male; Group 2: five samples (blood) and liver biopsy were collected from patients infected with liver cancer. The patients sample was taken from the people referred for treatment at Tumor Institute, Tanta, Egypt. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the Faculty of Medicine, Kafer Elsheek University for liver biopsy and Faculty of Science, Tanta University guide for animal, as approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-0046).

Ten female Swiss albino mice were used in the present study. Mice were divided into two equal groups, five of mice for each group: Group1 include healthy animal; and Group2 include animals inoculated once intraperitoneal I.P with Ehrlich cells. After two weeks tumor inoculation, fluid cells of (EAC) were isolated from the peritoneal cavity of mice from infected group through withdrawing peritoneal fluid containing the tumor cells. In addition, all animals of each group were anaesthetized with diethyl ether and sacrificed. Blood samples and liver tissues were immediately removed for biochemical, histological and immunohistochemical studies. Animals were obtained from animal house colony Egypt Vaccine Company, Giza, Egypt.

2.2. Serum preparation

Blood samples have been collected aseptically by venepuncture into a dry clean and sterile tube without anticoagulant substances and allow it to clot. Blood samples allowed to stand for 30 min at 4^{o}C for clot formation and centrifuged for 10 minutes at 3000 rpm. The

supernatant serums were stored in Eppendorf tube at -20^{o}C for subsequent analysis or use, Clinical samples (blood) were collected according to Tuck et al. [11].

2.3. Biochemical parameters

Serum aspartate transaminase (AST) and alanine transaminase (ALT) were estimated in the rat sera according to Moustafa et al. [12] and Al-Rasheed et al. [13] respectively while alkaline phosphatase (ALP) was estimated in the rat serum according to El-Moghazy et al. [14]. Serum albumin was estimated according to Basuony et al. [15] while serum total proteins level was estimated according to Tousson et al. [16].

2.4. Histopathological examination

Liver tissues were taken and immediately fixed with 10% buffer neutral formalin solution for 24 to 48 h, and then processed for paraffin sectioning. Sections were stained with haematoxylin and eosin (H&E) for histopathological examination according to Tousson [17].

2.5. Immunohistochemical detection of PCNA expressions

Expression of proliferating cell nuclear antigen (PCNA) in the liver sections were detected using avidin Biotin Complex (ABC) method according to Tousson et al. [18,19].

2.6. Statistical analysis

Results are reported as means ± SE. Statistical analysis for all studied parameters were performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute [20]. Duncan's New Multiple Range Test was used to test the significance of the differences between means. Values of $p<0.05$ were considered statistically significant.

3. RESULTS

3.1. Liver function parameters of EAC bearing mice and human HCC

Figures 1 represented the data of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and albumin in serum of Ehrlich ascites carcinoma (EAC) bearing female mice and human hepatocellular carcinoma (HCC). The results showed significantly ($P<0.05$) increased in the activity of liver enzymes (AST, ALT and ALP) and a significant ($P<0.05$) decrease in the serum albumin in both (EAC) bearing mice and patients (HCC) groups compared with normal control group.

3.2. Histopathological changes of EAC bearing mice and human HCC

In the current study, we evaluated the effects of Ehrlich ascites carcinoma (EAC) bearing female mice and human hepatocellular carcinoma (HCC) on liver histopathology. Microscopic examination of liver control group reveals normal hepatic architecture showing no sinusoidal congestion, no fibrosis, no inflammation, and no piecemeal necrosis (Figure 2A&B). Mice infected with (EAC) revealed hydropic changes petal inflammation, piecemeal necrosis, mild to moderate fibrosis, lytic necrosis, congestion of sinusoidal blood vessels (Figure 2C). Patients infected with (HCC) revealed increase in hydropic changes, inflammation and lytic necrosis (Figure 2D).

3.3. PCNA immunohistochemical changes of EAC bearing mice and human HCC

Figures (3) represented proliferating cell nuclear antigen (PCNA) expression in the liver sections for human and mice groups. Negative or faint positive reaction for PCNA expressions were observed in the liver section for control mice and human group (Figures 3A&3B), while, strong positive reaction for PCNA was observed in nuclei of hepatocytes in liver sections of EAC and HCC group when compared with the normal control group (Figures 3C&3D).

4. DISCUSSIONS

Most patients with hepatocellular carcinoma (HCC) have liver cirrhosis, which develops following long periods of chronic liver disease. Cirrhosis is characterized by a decrease in hepatocyte proliferation, indicating an exhaustion of the regenerative capacity of the liver, and results in an increase in fibrous tissue and a destruction of liver cells, which may ultimately lead to the development of cancerous nodules. Hepatocellular carcinoma (HCC) is the dominant form of primary liver cancer and is histologically and etiologically distinct from other forms of primary liver cancer.

These results agree with Chakraborty and Bhattacharya [21] who find that albumin levels were decrease and ALT and AST levels were increase in (EAC) bearing mice. This indicates that the (EAC) bearing mice lead to a defect in liver function simulates the same problems in the human hepatocellular carcinoma (HCC). Liver function parameters include three enzymes that are often measured, which are (ALT), (AST) and (ALP), as well the albumin also measured in both animal and human. If the liver is injured, the enzymes level will be elevating due to liver cells spill the enzymes into blood and signaling hepatic damage such as liver necrosis and inflammation.

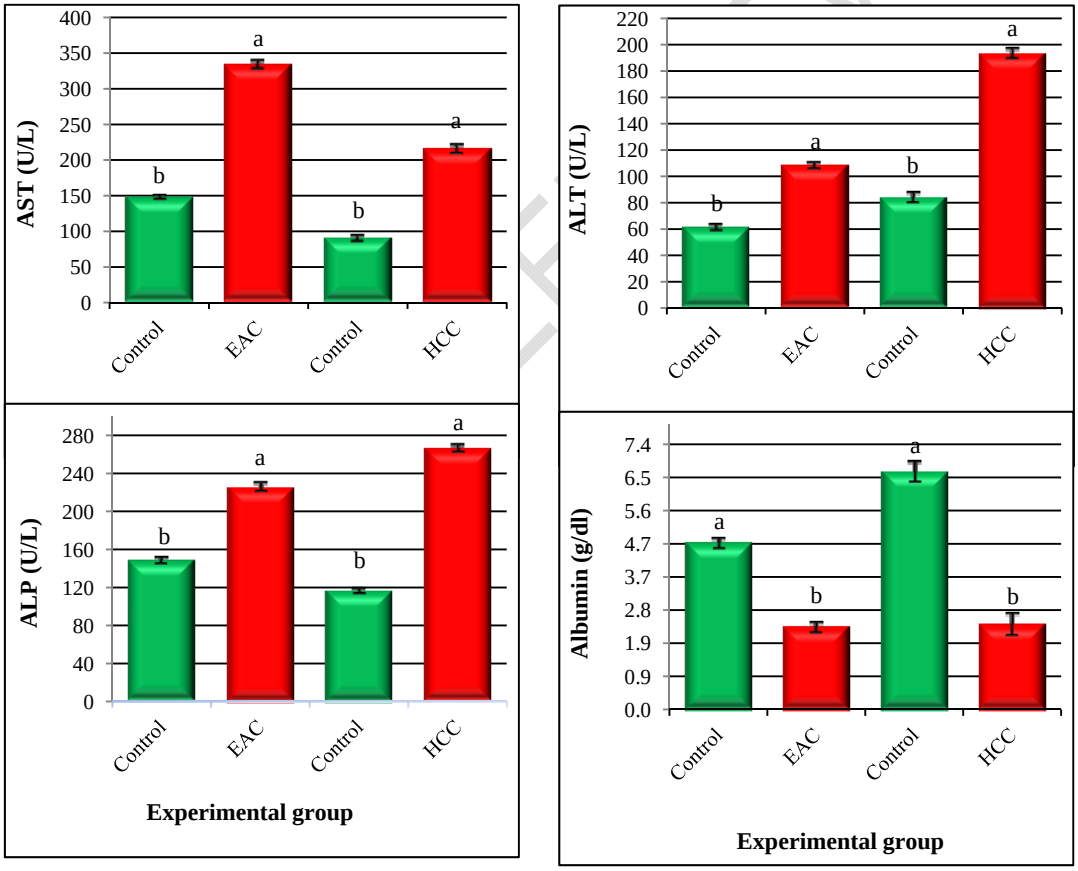
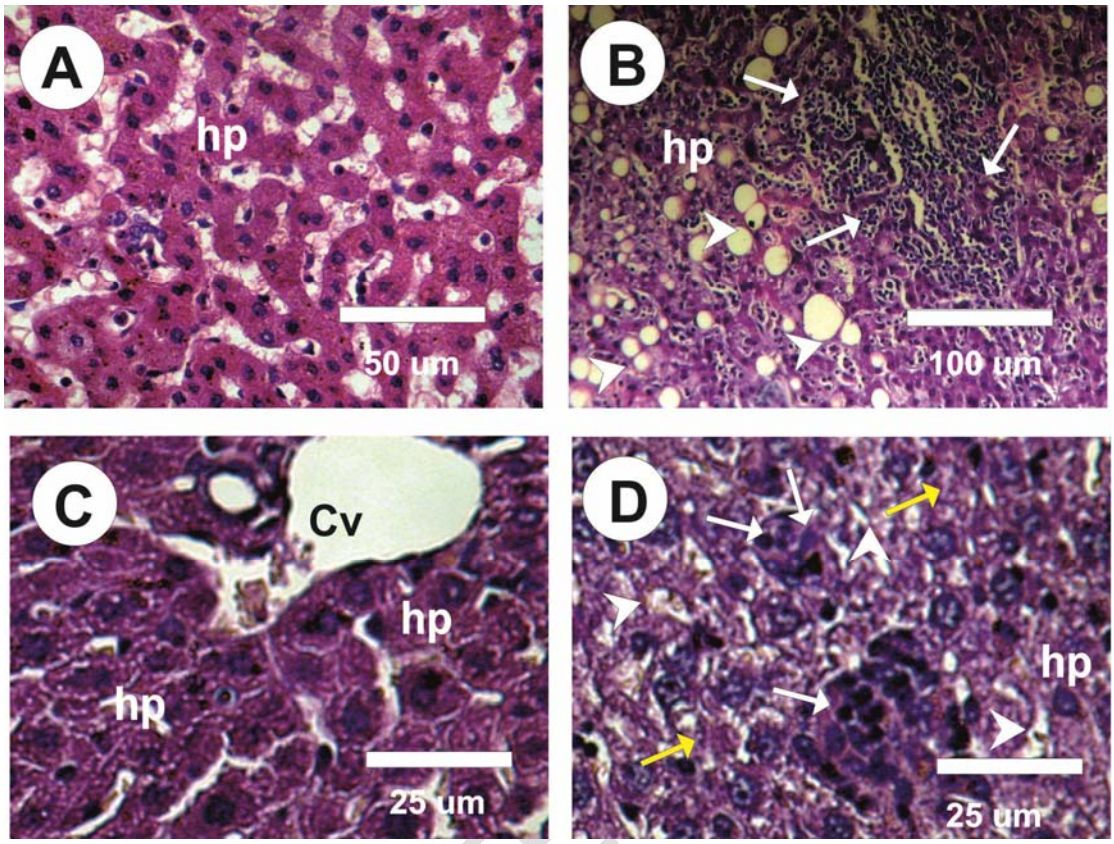


Figure 1: Mean values ± SE of (AST), (ALT), (ALP) and albumin infected with (EAC) mice and human (HCC).

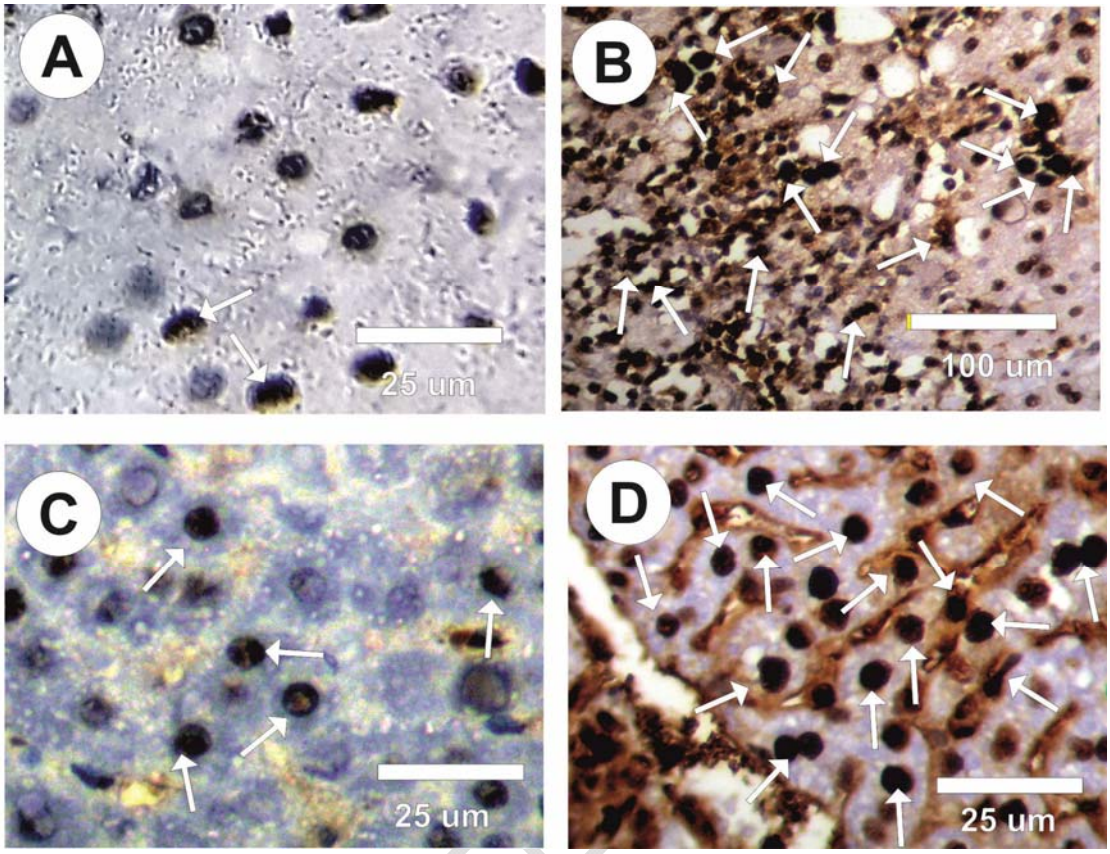


Figures 2: Photomicrographs of liver histological section in the mice and human groups. A&B: Normal structure of hepatocytes (hp) in liver sections in control mice (A) and healthy human (B). C&D: Marked degeneration and inflammatory cells and diffuse necrosis of hepatic tissue in EAC (C) and HCC (D) group.

Khalifa et al. [22]; Berk and Korenblat [23] reported that the increase in (AST) and (ALT) levels may indicate to liver tumor, liver necrosis, cirrhosis, lack of blood flow to the liver (liver ischemia), hepatitis, acute haemolytic anaemia, skeletal muscle trauma, acute renal failure, acute pancreatitis, primary muscle disease and progressive muscular dystrophy.

In the same contrast, Shim and Kwon [24]; Tayeb et al. [25] found that very high levels of (ALP) can be caused by hepatic problems such as liver cancer, hepatitis, cirrhosis, gallstones and blockage of the bile ducts (obstructive jaundice). On the other hand, the reduction in the albumin level (hypoalbuminemia) may be referred to cases of liver diseases, increased mitotic division of tumor cells and certain cases of massive ascites and also associated with hepatic cancer. The infected mice with EAC and human (HCC) leads to an increase in the level of liver enzymes and decrease of albumin. This may be indicative of a simulation between EAC and HCC because they have the same effects on the liver, which were in accordance to Chakraborty and Bhattacharya [21]; Badr et al. [26].

Liver sections of human and mice in the control group revealed the hepatocytes contain radially arranged cords which extend from a central vein to the periphery of the hepatic lobules, as well the shape of hepatocytes is polygonal with eosinophilic granular cytoplasm and vesicular basophilic nuclei [27-29].



Figures 3: Photomicrographs of liver sections in the different experimental groups stained with PCNA immunoreactivity. A&B: Negative or faint positive expression for PCNA in liver sections in control mice (A) and human (B). C&D: Strong positive reactions (arrows) for PCNA in the nuclei of hepatocytes in EAC (C) and HCC (D) group.

Liver histopathological examination presented that (EAC) bearing mice lead to a defect in liver sections simulates the same problems in the human (HCC), showed marked degeneration, necrosis and infiltration also, numerous newly formed blood capillaries (neovascularization) were seen in the surrounding tissue with mild or no inflammatory response. Such tumor showed tissue architectural disarray, as well as marked degree of cellular anaplasia, pleomorphism, and anisocytosis, with nuclear vascularity, a typicality, hyperchromasia and mitoses [30,31].

The pathological changes that seen in biopsy specimen from ruptured (HCC) and vascular injuries specially was seen in the small arteries included increased elastin proliferation and collagenase expression as well degradation of type IV collagen fibril. Also, makes the small arteries supplying the tumor stiff and brittle, which rupture easily when subjected to raise in vascular load secondary to minor trauma or portal hypertension. Moreover, these alterations more selectively in ruptured (HCC) and small arteries, on the contrary from non-rupturing (HCC) [27,32].

Immunohistochemical markers is necessary to diagnosis of hepatocellular carcinoma (HCC), because the morphologic features of (HCC) often overlap on the one hand with intrahepatic cholangiocarcinoma and metastatic tumors, and on the other hand with those of benign or

borderline entities like hepatocellular adenoma (HCA), focal nodular hyperplasia (FNH) and high-grade dysplastic nodule (HGDN) [33]. Therefore, in this study we investigated on the effects of p53 and proliferating cell nuclear antigen (PCNA) in liver sections which were examination by immunohistochemistry. As well, to find the similarity effects among of (EAC) model and (HCC).

Proliferating cell nuclear antigen (PCNA) protein in the liver sections is very highly observed in various types of cancers. Moreover, liver immunohistochemistry examination in eac bearing mice and human (HCC) groups showed a significant decrease in reactivity for the expression of (PCNA) in the liver section compared with the control group. This indicates that the (EAC) bearing mice lead to a defect in liver immunohistochemical for (pcna) simulates the same problems in the human (HCC), these results according to previous studies [34-37]. In order to understand the similarity effects among of human hcc and eac bearing mice on liver, we detected changes in the expressions of p53 protein and (PCNA) in (HCC) and (EAC) liver tissues by immunohistochemistry. The immunohistochemical markers were observed that overexpression of p53 protein and (PCNA) in the liver tissues of patients or mice. This may be indicative of found a simulation between (EAC) and (HCC) because they have same effects on immunohistochemical markers.

4. CONCLUSION

It could be concluded that EAC bearing mice lead to a similarity of the defects in liver enzymes, histopathological and immunohistochemical examinations which simulate and correspond the same problems in the human HCC.

ETHICAL APPROVAL

“All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.”

The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the Faculty of Medicine, Kafer Elsheek University for liver biopsy and Faculty of Science, Tanta University guide for animal, as approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-0046).

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