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Serum serotonin as unexpected potential marker for staging of experimental hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the primary cancer of the liver. The present study aimed to assess the potential role of the endogenous regulators of angiogenesis like neurotransmitters, as possible HCC biomarkers. Five groups of rats were used in this study (8 rats per each): control healthy group (I), four intoxicated groups (II, III, IV, and V) used for induction of HCC with a single IP dose of diethylnitrosamine (DENA), 200 mg/kg. Groups II, III, IV, and V were sacrificed after 8, 16, 24, and 32 weeks of DENA injection respectively. Serum levels of epinephrine, nor-epinephrine, serotonin, and dopamine of all animals were estimated using high performance liquid chromatography technique coupled with fluorescence detector (HPLC-FLD). Development of HCC was confirmed histopathologically. Our results showed a significant increase in 3 neurotransmitters (epinephrine, nor-epinephrine, and serotonin) in DENA intoxicated HCC rat model. Only serotonin exhibited a significant increase in early histological stage HCC development (16 weeks post DENA injection) in comparison to alpha-fetoprotein (AFP), (24 weeks post DENA injection). These results suggest that neurotransmitters (Epinephrine and Norepinephrine) may have a role as a biomarker for late histological stage HCC. Like AFP, while serotonin may be used for early stage HCC.

1. Introduction

Liver cancer in adult men is the fifth most frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. In adult women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death [1]. The silent growth nature of HCC may delay its diagnosis for as long as three years from the time of its onset [2,3]. Therefore, early detection of HCC is the most crucial step in the management process. The main diagnostic tools for HCC include serum markers, different imaging and histological examinations. Best diagnosis of HCC is done by combination of liver imaging and measuring serum AFP which has been used as a serum marker for HCC for a long time. However the sensitivity of AFP test ranges between 39 and 65% and its specificity ranges between 76 and 94% in the presence of HCC [4,5]. This is referred to its probable rising in the sera of other cases such as pregnancy and tumors of gonadal origin [6]. Tumor expansion depends mainly on angiogenesis (development of new capillaries from preexisting capillaries), in order to provide the tumor with a suitable supply of oxygen and nutrients [7,8]. Among the various endogenous regulators of angiogenesis, are catecholamines (CAs), which are crucial because it opposes the effects on tumor angiogenesis [9–13].

Dopamine (DA) acts through its D2 receptors, suppressing the action of vascular permeability factor/vascular endothelial growth factor-A (VPF/VEGF) on adult endothelial cells as well as endothelial progenitor cells, therefore inhibits angiogenesis [9,10]. In contrast, norepinephrine (NE) and epinephrine (E) by acting through the β adrenoceptors, increase the synthesis of many proangiogenic factors in the cancer cells and induce angiogenesis [11–13].

Serotonin, known as 5-hydroxytryptamine (5-HT), acts as a ligand for a big family of 5-HT receptors [14]. About 90% of serotonin in the body is synthesized in the gastrointestinal (GI) tract by enterochromaffin cells, which regulates intestinal motility [15]. Recently, both *in vitro* and *in vivo* studies have shown that serotonin is involved in the tumor growth of various cancers including HCC [16], colon cancer [17], and cholangiocarcinoma [18,19]. Within the liver, 5-HT has been shown to be involved in the

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pathogenesis of viral hepatitis [20], nonalcoholic steatohepatitis [17], and liver fibrosis [21,22]; all these conditions are implicated in the tumorigenesis of HCC. On the other hand, serotonin was included as a diagnostic/or therapeutic approach in many depressive and brain diseases [23].

In present study, we used rat model of HCC to evaluate the benefit of serum levels of CAs and 5-HT as biomarkers. We compared serum AFP values against CAs and 5-HT levels.

2. Material and methods

2.1. Chemicals

Diethylnitrosoamine (cat. no. N0258-1G), was purchased from Sigma Chemical Company, St Louis, MO, USA. Other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Experimental groups and animal sampling

Fifty male Sprague-Dawley albino rats about 250 g were used in the present experiment. They were purchased from the animal house of Asyut University, Asyut, Egypt. The animals were housed under standardized environmental conditions, fed with standard diet and left to acclimatize for one week prior to inclusion in the experiment at 22 °C ± 2 °C under a 12/12 h. light/dark cycle. All animal experiments were conducted in accordance with the guide for the care and use of laboratory animals of the National Institutes of Health (NIH publication No. 85-23, revised 1985).

Animals were divided randomly into five groups as follows, 8 rats per each: (I): control healthy group (injected with saline), (II, III, IV, and V): HCC induced groups (injected with DENA, 200 mg/kg single intra peritoneal dose). Groups II, III, IV, and V were sacrificed following 8, 16, 24, and 32 weeks of the DENA injection respectively. Rats were initially anesthetized with 3% halothane before they were sacrificed to collect blood and livers for experimental analyses. Blood samples were collected on the final day of the experiment after a 12 h. Fasting blood samples were left for 15 min for *in-vitro* coagulation and then centrifuged at 3000*g* for 15 min for serum collection. Blood samples were collected for catecholamine, serotonin, and AFP measurements.

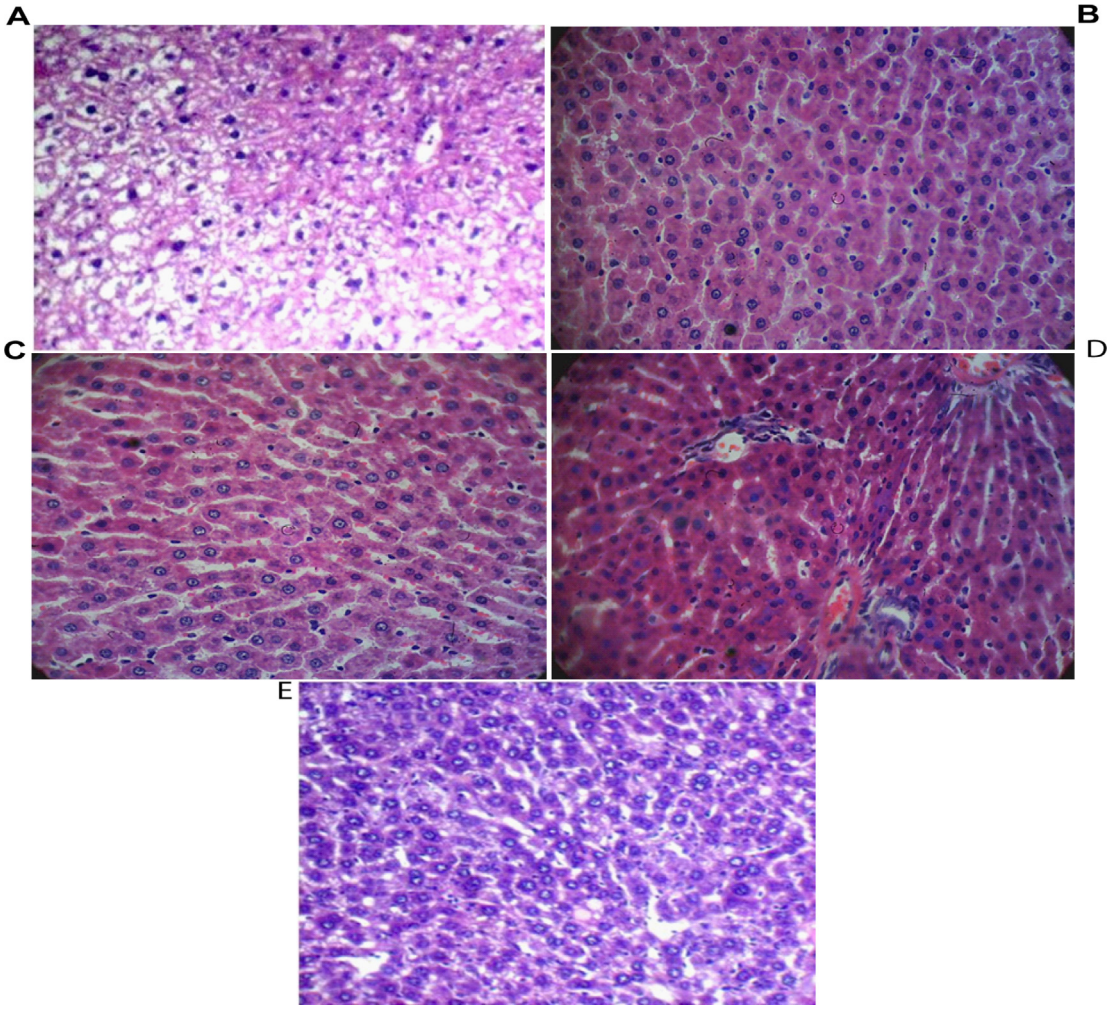

Fig. 1. Histopathological photographs of HCC during the different time points of the experiment: A. Control hepatic tissue sections showing normal cellular architecture (H&E × 100). B. Hepatic tissue section after 8 weeks from DENA injection, showing increased nuclear/cytoplasm (N/C) ratio with high grade sinusoidal pattern (H&E × 100). C. Hepatic tissue section after 16 weeks from DENA injection, showing multinuclear giant cell formation with increased mitotic figures (H&E × 100). D. Hepatic tissue section after 24 weeks from DENA injection, showing multinuclear giant cell formation and increased width of cord cells more than two cells and poorly differentiated HCC (H & E × 100). E. Hepatic tissue section after 32 weeks from DENA injection, showing cellular and nuclear poleomorphism, increased width of cord cells more than two cells, with microacinar formation (H&E × 100).