lymphocytes and other lymphoid tissue components [6].

The most common cause of chronic hepatitis is infection with HBV and HCV [7]. Persistent infections with these viruses are frequently associated with the development of hepatocellular carcinoma [8]. The role of these infections in the induction of neoplastic changes in liver tissues is still to be elucidated. The core protein of hepatitis C virus is known to have multifunctional features, including binding to the death domain of the tumor necrosis factor receptor type 1 (TNFR 1). It also known to bind to the cytoplasmic tail lymphotoxin β receptor, reflecting a possible involvement in the signaling pathways of apoptosis [9].

The over expression of certain cytotoxic cytokines has been implicated as a possible inducing factor for the progression towards hepatocellular carcinoma (HCC) [10].

This study aims to determine the extent of expression of lymphotoxin α, β and their receptor (TLRs) using immunohistochemistry techniques in patients with chronic HCV infection and hepatocellular carcinoma. The study also aims to elucidate the correlation between these cytokines expression and different clinicopathological variables such as age, gender, histopathological activity index (HAI), stage and grade.

Methods

Study population. Thirty five formalin-fixed, paraffin embedded liver tissue blocks were obtained from patients with confirmed cases of chronic HCV infection and hepatocellular carcinoma. The age of patients was ranged from 17 to 65 years. The histopathological types of hepatocellular carcinoma included in this study were moderately differentiated adenocarcinoma (4 cases) and poorly differentiated adeno-carcinoma (6 cases). All patients had positive test for anti-HCV antibodies (third-generation enzyme linked immunosorbent assay (ELISA). The patients' samples were collected during the period from January 2010 till December 2011 from the archives of histopathology laboratories of liver and digestive system technical hospital and private laboratories in Baghdad, but this research perform during 23, March 2012 till 20, September 2012.

Normal liver specimens were obtained from thirteen persons were collected from the Forensic Medicine Institute Archives.

Formalin-fixed, paraffin embedded tissue blocks were sectioned (4 μm) thickness, one section was stained with Haematoxylin and Eosin, and four sections were mounted on positively charged slides to be used for immunohistochemistry technique for the detection of lymphotoxin α, β and their receptor TLRs.

The histopathological diagnosis of the tissue blocks used in this study was primarily based on that obtained from histopathological records of liver biopsy samples and hospital laboratory records. Confirmatory histopathological re-evaluation of each obtained tissue blocks was done.

Immunohistochemical staining. Was carried out using mouse anti-human lymphotoxin alpha (US Biological- USA Cat. Number L2610-03B), mouse anti-human lymphotoxin beta (abcom-UK Cat. Number ab89568), mouse anti-human lymphotoxin beta receptor (US Biological- USA Cat. Number L8015-03L) and immune-histochemistry detection kit (US Biological/USA Cat. Number 17506).

The slides were deparaffinized by immersion two times in xylene for 5 minutes each time, and they were then rehydrated in serial alcohols in the following order: 100%, 95%, 70% and water for 5 minute each. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide for 30 minutes. Slides were then washed with distilled water followed by two times in phosphate-buffered saline for 5 minutes.

All of the slides were treated with 1% normal serum and incubated for 30 minutes at room temperature. Excess normal serum was tipped off slides before adding the primary antibody, dilution 1:250 for each lymphotoxin α and TLβRs, dilution 1:500 for lymphotoxin β, as recommended by manufacturer's instructions.