

Immunological Assessment for Detection of Carcinogens in Liver

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Abstract

The aim has been to assess the performance and efficacy of immunological techniques for the detection of carcinogen in acute liver cancer. The following techniques were used to examine 86 sera samples of the different patient who has been hospitalized for the different liver damages; direct enzyme linked immunosorbent assay (ELISA), antibody capture ELISA (CHORUS ELISA), and antibody capture agglutination. We collect the sera of these patients and keep them in fridge and after that we tested these sera by ELISA.

Our results demonstrated the improved positive detection rates and diagnostic value for cancer screening, showing potential application in clinic diagnosis. The immune methods has specificity and sensibility which vary from 80% to 100%, and for this reason they are used so much in clinical tests. Detection of carcinogens by sera antibodies is a useful diagnostic marker for acute liver tumor.

KEYWORDS: ELISA, sera samples, liver, carcinogen

Introduction

Liver cancer or hepatic cancer (from the Greek *hēpar*, meaning liver) is a cancer that originates in the liver. Liver tumors are discovered on medical imaging equipment (often by accident) or present themselves symptomatically as an abdominal mass, abdominal pain, yellow skin, nausea or liver dysfunction.

The leading causes of liver cancer are cirrhosis due to hepatitis B, hepatitis C, and alcohol. Hepatitis B caused 300,000 deaths, hepatitis C because 343,000 deaths and alcohol caused 92,000 deaths due to liver cancer (1,3,7,10). Liver cancers are different than liver metastases, which originate from elsewhere in the body and spread to the liver. Liver cancers are formed from either the liver itself or from structures within the liver, including blood vessels or the

The most frequent liver cancer, accounting for approximately 75% of all primary liver cancers, is hepatocellular carcinoma (HCC) (also named *hepatoma*, which is a misnomer because adenomas are usually benign). HCC is a cancer formed by liver cells, known as hepatocytes, that become malignant (4,5,18,21). Another type of cancer formed by liver cells is hepatoblastoma, which is specifically formed by immature liver cells. It is a rare malignant tumor that primarily develops in children, and accounts for approximately

1% of all cancers in children and 79% of all primary liver cancers under the age of 15. Most hepatoblastomas form in the right lobe (6,13,17).

Liver cancer can also form from other structures within the liver such as the bile duct, blood vessels and immune cells. Cancer of the bile duct (cholangiocarcinoma and cholangiocellular cystadenocarcinoma) account for approximately 6% of primary liver cancers. There is also a variant type of HCC that consists of both HCC and cholangiocarcinoma. Tumors of the blood vessels (angiosarcoma and hemangioendothelioma, embryonal sarcoma and fibrosarcoma are produced from a type of connective tissue known as mesenchyme (8,11,15,20). Cancers produced from muscle in the liver are leiomyosarcoma and rhabdomyosarcoma. Other less common liver cancers include carcinosarcomas, teratomas, yolk sac tumours, carcinoid tumours and lymphomas. Lymphomas usually have diffuse infiltration to liver, but It may also form a liver mass in rare occasions.

Because liver cancer is an umbrella term for many types of cancer, the signs and symptoms depend on what type of cancer is present. Cholangiocarcinoma is associated with sweating, jaundice, abdominal pain, weight loss and liver enlargement (9,12).

Hepatocellular carcinoma is associated with abdominal mass, abdominal pain, emesis, anemia, back pain, jaundice, itching, weight loss and fever.

Viral infection with either hepatitis C virus (HCV) or Hepatitis B virus (HBV) is the chief cause of liver cancer in the world today, accounting for 80% of hepatocellular carcinoma (HCC). The viruses cause HCC because massive inflammation, fibrosis and eventual cirrhosis occurs within the liver. HCC usually arises after cirrhosis, with an annual incidence of 1.7% in cirrhotic HCV-infected individuals (14, 16). Around 5-10% of individuals that become infected with HBV become chronic carriers, and around 30% of these acquire chronic liver disease, which can lead to HCC. HBV infection is also linked to cholangiocarcinoma. The role of viruses other than HCV or HBV in liver cancer is much less clear, although there is some evidence that co-infection of HBV and hepatitis D virus may increase the risk of HCC.

Many genetic and epigenetic changes are formed in liver cells during HCV and HBV infection, which is a major factor in the production of the liver tumours (19). The viruses induce malignant changes in cells by altering gene methylation, affecting gene expression and promoting or repressing cellular signal transduction pathways. By doing this the viruses can prevent cells from undergoing a programmed form of cell death (apoptosis) and promote viral replication and persistence

METHODS

After antibodies have been developed and characterized, they can be used in a wide variety of assays, as outlined below. However, before use on biological samples, it is important to carry out experiments to validate the particular assay to be used.

Competitive ELISAs.

Competitive ELISA is the most commonly used immunological method for quantification of liver damage. There are numerous variations of the ELISA using alkaline phosphatase or peroxidase-conjugated secondary antisera for primary antibody detection and colored,

fluorescent, or radioactive substrates for end point detection. Antigen, either *in vitro* modified mono-adduct coupled to carrier protein, is coated onto 96-microwell plates, and nonspecific binding to the plate is blocked by incubation of the wells with a dilute protein solution. Antibody binding to the antigen on the plate is competed by antigen in solution. After incubation and washing off non-bound material, bound primary antibody is quantitated with enzyme-conjugated secondary antisera, followed by the appropriate substrate. With the highest affinity antibodies, 50% inhibitions can be obtained with adduct levels in the femtomol range.

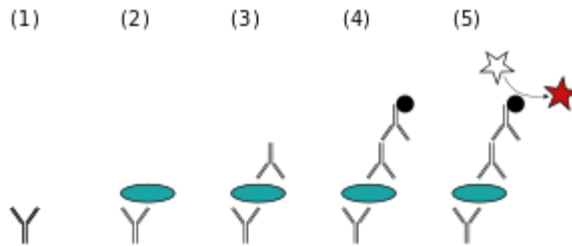


Fig.1. A sandwich ELISA.

(1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

Results and discussion

We have analyzed 86 sera of the different patient who has been hospitalized for the different liver damages.

We collect the sera of these patients and keep them in fridge and after that we tested these sera by ELISA.

Our results demonstrated the improved positive detection rates and diagnostic value for cancer screening, showing potential application in clinic diagnosis.

We have presented our positive and negative cases basing to ELISA results (Chart 1). The results which were from CHORUS ELISA are considerate:

- Positive cases – if index is $> 1,1$
- Negative cases – if index is $< 0,9$
- Doubt cases - if index is from 9,0 to 1,1

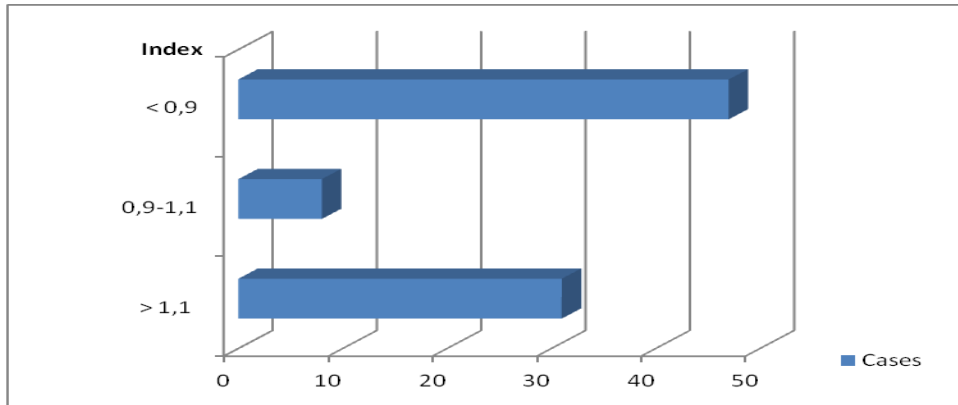


Fig. 2. The cases basing in the index

From Fig.2 we can say that the negative cases were higher than positive cases, while the doubt cases were only 8 cases. After the immunological test the patient were diagnosed and with other methods, but we have almost the same rate as immunological test.

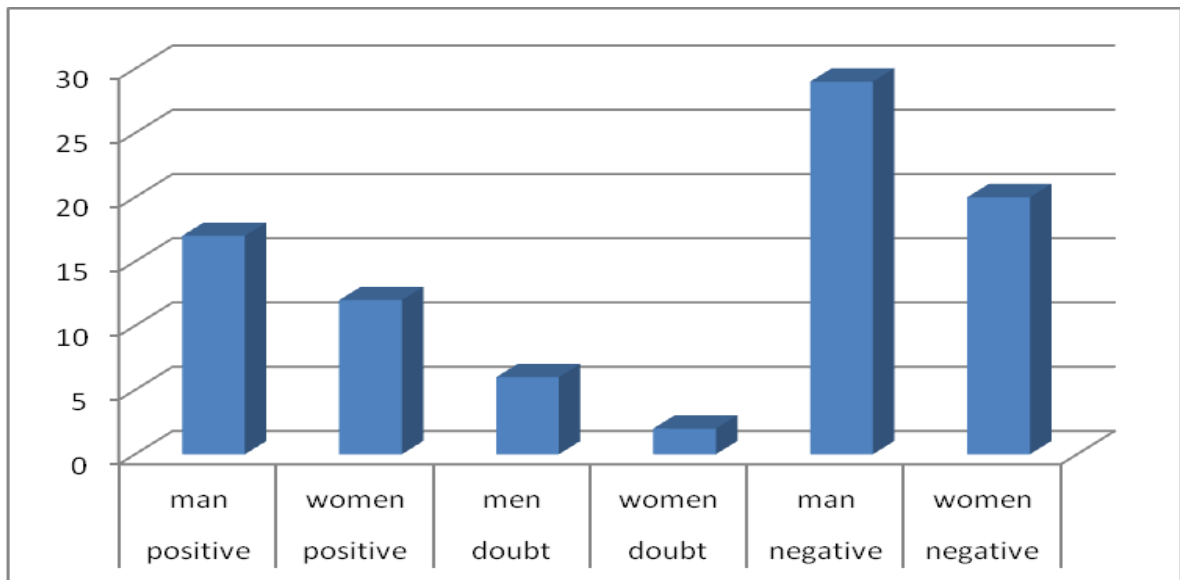


Fig.3. The genus in according to cases

Hepatocellular carcinoma is much more common in males than in females. Much of this is probably because of behaviors affecting some of the risk factors. Most cases in the Albania occur in people who abuse alcohol or have chronic HBV or HCV infections.

Non-alcoholic fatty liver disease, a condition in which people who consume little or no alcohol develop a fatty liver, is common in obese people. People with a type of this disease known as *non-alcoholic steatohepatitis* might go on to develop cirrhosis.

The immune method has high specificity and sensibility and for this reason they are used so much in clinical tests.

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