

LP detection System HRP Polymer & DAB Plus Chromogen (Lab Vision corporation).

The first assays of biomarker of HCV infection detection were performed by using the human monoclonal Ab anti-Core B12.F8 (gift from Dr. Mario Mondelli, University of Pavia, Italy). However the technique is under standardization and another antibody is being considered.

The evidence of exposition to AFB1 are being assessed by the detection of DNA-AFB1 adducts in HCC paraffin embedded hepatic tissues using the monoclonal antibody 6A10 (kindly donated by Dr. Regina Santella, Columbia University, USA), which recognizes the imidazole ring in the open guanine adduct DNA. The DNA adducts-albumin complexes will also be detected in the serum sample obtained from the patients diagnosed in the period 2005-2007. This detection method corresponds to a competitive ELISA using policlonal antiserum.

Additionally, the mutation in the third base of p53 gene codon 249, associated with exposure to AFB, is being evaluated by PCR of exon 7 p53 and its flanking introns, and by restriction analysis with the enzyme HaeIII.

This study will contribute to the understanding the importance of these three risk factors in hepatic cirrhosis and HCC in a Latin-American country. The results of the study are essential for the instauration of public health policies aimed to reduce the incidence of hepatic cirrhosis and HCC. It will also facilitate the transfer of technology for the implementation of techniques on HCC's risk factors.

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## Epidemiology of hepatocellular carcinoma

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**INTRODUCTION.** The incidence of hepatocellular carcinoma (HCC) has increased both due to the world-

wide increase on the virus C infection and to the increase on the survival rate of patients with chronic liver disease.

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It usually is an aggressive tumor with survival rate directly associated with the hepatic residual liver function as well as the size of the tumor at the diagnosis.

Despite occupying the 7th position among malignant neoplasias worldwide, it is currently considered as the 3rd cause of death due to cancer occurring most frequently among men than among women (3:1) and between the 6th and 7th decades of life. In hyperendemic regions for the hepatitis B virus (HBV), it occurs in younger individuals, between the 3rd and 5th decades of life 1.

About 60 - 80% of patients with HCC present liver cirrhosis 2. Because of this association the treatment of this tumor is a challenge for the modern hepatology, since it presents poor response to chemotherapeutic agents. The surgical and non-surgical ablation methods depend on factors inherent not only to the tumor itself but also to the etiology and stage of liver disease.

**HCC ETIOLOGY AND EPIDEMIOLOGY.** Usually, the liver structural alterations occur along the years, before HCC develops in the hepatic tissue. The HCC diagnosis may be verified in individuals with no known previous history of liver disease; however, this diagnosis will be coincident with biochemical and laboratorial evidences of hepatocellular dysfunction or viral infection, besides metabolic disorders of associated diseases, making HCC the starting point for the hepatic disease diagnosis. However, in patients under screening, HCC may be detected in early phases, with better prognosis. The HCC development in normal livers is unusual; thus, the HCC epidemiology will be established based on its etiological factors.

**HEPATITIS B VIRUS (HBV).** Approximately 400 million people worldwide are chronic hepatitis carriers. Over than 40% of these patients will develop serious hepatic complications such as the HCC.

The hepatitis B is present in all continents. There is a variability of infection rates in function of the different regions of the globe (from 0.1% to 20%). Therefore, the different regions of the world are divided into high endemicity, intermediate endemicity and low endemicity areas.

In low endemicity areas, the prevalence of chronic HBV carriers is lower than 2%. In intermediate endemicity areas,

the prevalence of chronic HBV carriers ranges from 2% to 7% and from 20% to 50% of the population have serological evidence of past infection. The highest infection rates are among older children, adolescents and young adults. Intermediate prevalence regions include Eastern Europe countries such as Russia, countries from the Mediterranean basin, Southeastern Asia, Japan and Northern Latin America.

In high endemicity areas, the risk of HBV infection is higher than 60% and most infections occur at birth or early in the childhood. All children from this population present high risk of acquiring chronic infection before the age of 5 years. In these areas, the rate of chronic HBV carriers ranges from 8% to 25% and the antiHBs prevalence is around 60 to 85%.

Since the implementation of HBV vaccination in counties from North America and Europe from 1985 on, a significant reduction on the number of new cases in those regions was documented. However, the continuous immigration of from endemic regions (Asia and Africa) reveals that viral reservoirs persist in these areas, what makes crucial the continuous disease surveillance 4.

Approximately 5 - 10% of infected adult individuals become chronic, and may evaluate toward advanced liver disease and hepatocellular carcinoma. HBV carriers present a risk 100 times higher than normal individuals of developing hepatocellular carcinoma (HCC). About 60 - 80% of HCC cases detected worldwide are associated with HBV 5.

**HEPATITIS C VIRUS (HCV).** It is estimated that about 175 million people are infected with the HCV worldwide. The mortality rate associated to the HCV will increase two or three times in the next decades in infected patients who develop cirrhosis and will become the greatest indication of liver transplantation.

Two zones of HCV prevalence may be defined based on data obtained from world blood banks: the zone of strong prevalence, with 0.5 to 1.5% of blood donors includes Japan, Spain, Hungary, Southeastern Italy, Africa, Asia and South America. Egypt presents the highest prevalence, between 10% and 30% 6. The zone of low prevalence, with 0.001 to 0.05% of blood donors includes European countries, USA and Canada.

It is also estimated the 10 - 20% of patients with chronic hepatitis due to HCV at cirrhosis stage may develop HCC within ten years of disease evolution. The carcinogenesis mechanisms are not yet fully elucidated. Since it deals about a RNA-type virus, it cannot integrate to the hepatocyte genome. It has been postulated that the HCV core protein presents oncogenic properties 7. It is believed that the presence of cirrhosis is a preponderant factor for HCV emergence in HCV carriers 8. An asiatic study demonstrated that 25% of patients at cirrhosis stage developed HCC within five years 9.

**ALCOHOL AND AFLATOXIN.** Alcohol is associated with digestive tract cancer such as esophagus and pancreas (alcohol-induced chronic pancreatitis). The alcohol intake in HBV or HCV carriers can induce the fibrosis progression and hence to hepatic carcinogenesis.

The Aflatoxin, a toxin produced by the *Aspergillus* fungus, may contaminate stored food such as peanuts, soybeans and rice, and is, at least, partially responsible for the development of HCC among some populations from Africa and China 10.

**METABOLIC AND HEREDITARY DISEASES.** Other hepatic diseases, among which, autoimmune hepatitis, hemochromatosis, Wilson disease, alpha-1 antitrypsin deficiency and primary biliary cirrhosis may be associated to the HCC development, when in cirrhosis stage. The HCC incidence in the hemochromatosis seems to be higher than in other metabolic diseases 11.

**STUDIES ASSOCIATED TO THE HCC EPIDEMIOLOGY.** The geographic distribution of HCC coincides with the epidemiology of hepatotropic viruses such as HBV and HCV, as mentioned before. The most recent epidemiological studies corroborate this characteristic. In Canada, ElSaadany & Giulivi (2006) 12 evaluated 403 cases of HCC and found that 15% of cases were associated hepatitis (B and C) and 22% to alcohol. Dissimilarly, Abdel-Whab et. al (2007) 13 in Egypt, found HCV as etiologic factor for HCC in 76.6% of cases

and B virus in 3.3% of cases. Southeastern Asia, Japan and South Africa present high HCC incidence, what is coincident with the high HBV prevalence.

In Brazil, according to data from the National Cancer Institute (Inca), the HCC rate per each 100 thousand inhabitants is different from state to state, being of 1.07 (males only) in Belém (Eastern Amazonia) in the year of 1988 and 9.34 in Porto Alegre (South) in 1991. However, there is still a lack of data for the HCC epidemiological characterization, factor associated to the difficulty of access to image and laboratorial exams that could clear the diagnosis.

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## Hepatitis B virus DNA integration and transactivation of cellular genes

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Chronic hepatitis B virus (HBV) infection is etiologically related to human hepatocellular carcinoma (HCC). Most HCCs contain integrated HBV DNA in hepatocyte, suggesting that the integration may be involved in carcinogenesis. Available data on the integrants from human hepatocellular carcinomas seem to represent primary integrants as well as the products of secondary rearrangements. By means of structural analyses of the possible primary integrants, it has been observed that the replication intermediates of the viral genome are the preferred substrates for integration. The integrated HBV DNA and the target cellular DNA are invariably associated with deletions, possibly reflecting the substrate for, and the mechanism of, the integration reaction. The host DNA sequences as well as the target site of integration in chromosomes are selected randomly suggesting that HBV DNA integration should bring about random mutagenic effects. Analysis of the samples recovered from hepatocellular carcinomas show that the integrated HBV DNA can mediate secondary rearrangements of chromosomes, such as translocations, inversions, deletions and (possibly) amplifications. The integration of HBV DNA into the host genome occurs at early steps of clonal tumor expansion. The integration has been shown in a number of cases to affect a variety of cancer-related genes and to exert in-

sertional mutagenesis. However, in contrast to the wood-chuck model, in which specific HBV-DNA integration is detectable in most cases, insertional activation or inactivation of cellular genes appears to be a rare event in man. The discovery of transactivating functions exerted by HBx and truncated HBs(urface) proteins supports the notion that these could be relevant to hepatocarcinogenesis as these transactivator sequences have been found in a large number of HCC tumors or hepatoma-derived cell lines. The HBx transactivator can stimulate a wide range of cellular genes and displays oncogenic potential in cell culture as well as in a transgenic environment. The HBs transactivators are encoded by the preS/S region of S gene and may involve carboxy terminal truncation to gain transactivation function. Expression of host genes by viral transactivators is mediated by regulatory elements of the cellular transcription factors like c-fos, c-myc, NF-kappa B, SRE and Sp1. Thus, during hepatitis B infection, the tendency of rearrangement of hepatocyte chromosomes is combined with the forcible turnover of cells. This is a constantly operating system for the selection of cells that grow better than normal cells, possibly involving important steps in multi-staged hepatocarcinogeneses. Gene expression profiling and proteomic techniques may help to characterize the mo-

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