DETECTION OF HEPATITIS B VIRAL DNA IN PATIENTS OF CHRONIC LIVER DISEASES BY NON RADIOACTIVE LABELLED TECHNIQUE

Thesis
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بسم الله الرحمن الرحيم

(... قال رب اشرح لى صدرى ويسر لى امرى واحلل عقدة من لسانى يفقهوا قولى ...)

صدق الله العظيم

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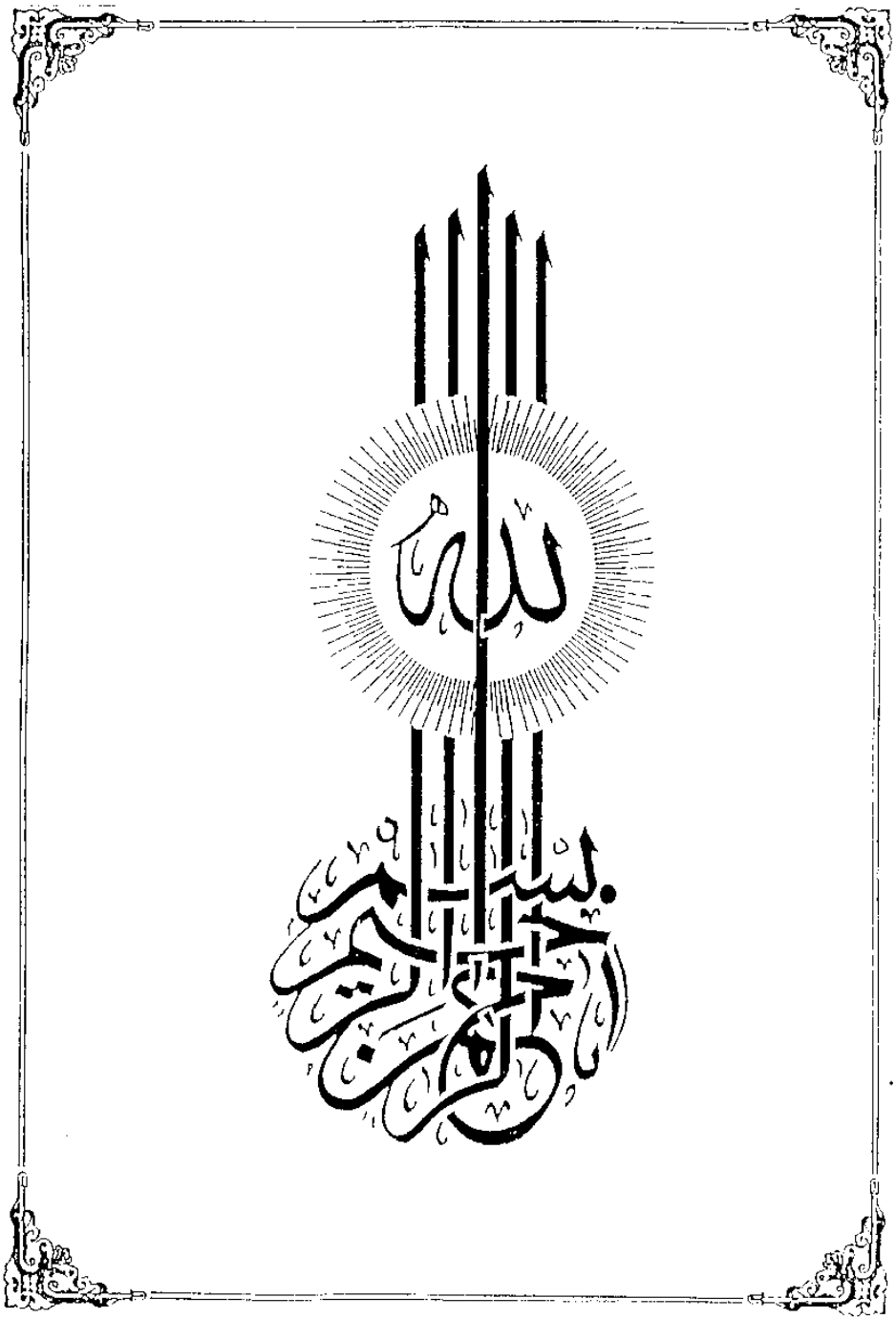

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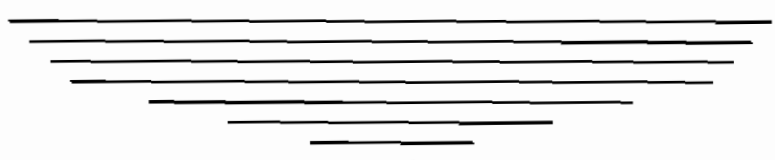

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Hepatitis B virus (HBV) infection represents a major public health problem because of the ability of HBV to cause a chronic infection. While a chronic infection may remain asymptomatic, a significant number of infected individuals subsequently develop liver disease, including cirrhosis and primary hepatocellular carcinoma (Locarnini and Gust, 1988).

To determine whether active virus replication is taking place, one can assay a number of serum markers, such as hepatitis Be antigen (HBe Ag) and HBV-specific DNA polymerase, or the presence of virions detected in serum by electron microscopy (Hoofnagle and Schafer, 1986).

Unfortunately, these assays suffer either from a lack of sensitivity or from being technically involved.

Nucleic acid hybridization assays have become useful tools for the diagnosis of many infectious diseases (Tenover, 1988). The detection of HBV DNA in serum by hybridization techniques is a direct measure of the quantity of virus present and correlates closely with infectivity (Lieberman et al., 1983 and Scotto et al., 1983).

One of the major obstacles to the wider application of such assays has been the requirement for radiolabelled probes, usually (P32), to obtain maximum sensitivity. The use of radioisotopes is not desirable in most laboratories because of safety considerations, and the short half-life means frequent probe preparation. To overcome such limitations, researchers investigated a number of non-radioactive labelling procedures, the biotin-avidin system being the most commonly used. However,when biotinylated probes were used to assay HBV DNA in serum, the limit of detection was found to be significantly poorer than that for radiolabelled probes and, furthermore, a significant proportion of samples gave false-positive signals (Saldanha et al., 1987).

More recently, an alternative non radioactive labelling and detection system has been developed by random primer labelling of DNA with digoxigenin-dutp (Casacuberta et al., 1988).

The aim of the work:

The aim of this work is to evaluate the importance of Hepatitis B viral (HBV) DNA detection in patients of chronic liver diseases with HBs antigenemia with or without HBe antigen or antibody positive by non radioactive labelled technique.

MORPHOLOGY OF HBV

MORPHOLOGY OF HBV

Infection with HBV is endemic throughout much of the world, with an estimated 200 million people carrying persistent HBV infection. HBV infects only humans and certain non-human primates; it belongs to a group of hepatotropic DNA viruses (the hepadnaviruses) that includes HBV and the hepatitis viruses of the woodchuck, ground squirrel, tree squirrel, pekin duck and heron (Feitelson et al., 1986; and Sprengel et al., 1988). In humans, HBV infection is associated with a wide spectrum of clinical states, ranging from the healthy carrier state to acute or chronic hepatitis and liver cirrhosis. Further, chronic HBV infection is involved in the development of hepatocellular carcinomas (HCCs), a major cause of death from cancer throughout the world (Hubert et al., 1989).

Structure of hepatitis B virus:

In infected individuals, HBV is found in three morphologically distinct forms: as spheres of about 22 nm diameter, as filamentous particles of the same diameter and up to several hundred nm in length and as more complex structures known as Dane particles. The spheres and filaments both represent incomplete viral particles, while the Dane particles are considered to be the hepatitis B

virion (Hubert et al., 1989).

The hepatitis B virion consists of an electron-dense internal core structure (the nucleocapsid) and an envelope. The envelope is made up of the hepatitis B surface antigens (HBsAgs) which share antigenic determinants with the incomplete viral particles mentioned above. Three main serotypes of HBsAg (adw, adr, ayw) are commonly observed and each has a distinct geographical distribution. Biochemical analysis of the viral envelope revealed three polypeptides, termed the major, middle and large proteins (Heermann et al., 1984). These proteins occur in both glycosylated and nonglycosylated forms and are encoded by the S gene, the pre-S2/S genes and the pre-S1/ pre-S2/ S genes, respectively. The nucleocapsid contains the hepatitis B core antigen (HBcAg), a DNA polymerase/ reverse transcriptase and the viral DNA, which has a protein covalently attached to the 5′ end of the minus strand (Gerlich and Robinson, 1980) and an oligoribonucleotide attached to the 5′ end of the plus strand (Will et al., 1987). HBcAg has been proposed to be related to HBe antigen (HBeAg), a second HBV-induced antigen, on the basis of the fact that HBcAg can be converted into HBeAg upon denaturation and/or limited proteolysis (Takahashi et al., 1980; and McKay et al., 1981). In nature, however, HBeAg is always

present in a nonparticulate state in the serum of HBV-infected individuals, whereas the polymeric HBcAg occurs only in the enveloped virus or inside HBV-infected hepatocytes (Salfeld et al., 1989).

Structure of hepatitis B virus genome:

Recent studies have shown that the genome of hepatitis B virus is highly variable and it is likely that no two viral isolates are genetically identical. Because hepatitis B virus gives rise to a number of distinct diseases it is tempting to suppose that different viral genotypes cause different diseases. Recipients of contaminated blood from patients with hepatitis B virus, however, do not necessarily develop the same disease, indicating that different hepatitis B virus infections are caused by the interaction between a particular viral genotype and their host (Foster and Thomas., 1993).

The HBV genome is a circular DNA molecule of about 3.2 kbp (Tiollais et al., 1985; and Ganem and Varmus, 1987). The DNA is only partially double-stranded, having a single-stranded region that varies in length, and the 5′ end of the incomplete plus strand is 200-300 nucleotides downstream from the 5′ end of the minus strand, thus creating a cohesive overlap that maintains the

circular structure of the DNA. The complete minus strand is terminally redundant, having a short sequence of 5-10 bases that is present at both the 5′ and 3′ ends; a protein is covalently attached to the 5′ end. The 5′ end of the plus strand incorporates a covalently attached oligoribonucleotide, 11 nucleotides of which are complementary to the adjacent minus strand. This 11 nucleotide sequence is directly repeated near the other end of the cohesive overlap (Will et al., 1987), and partially repeated again in the minus strand's terminal redundancy. These direct repeats (DR2 and DR1) are important for viral DNA replication and are frequently used as sites of integration of HBV into the host genome.

DNAs of similar size and structure to HBV (including the single -stranded region, short direct repeats and a DNA polymerase that functions to fill in the gap by elongation from the 3′ end of the incomplete plus strand) have been found so far only in the other hepadnaviruses of the woodchuck, ground squirrel, tree squirrel, pekin duck and heron (Hubert et al., 1989).

Genetic organization of hepatitis B virus genome:

The DNAs of hepadnaviruses have been cloned and the complete nucleotide sequences of HBV, WHV, DHBV and HHBV DNAs have been established (Tiollais et al., 1985; Ganem and Varmus, 1987; and

Sprengel et al., 1988). Analysis based on the three reading frames of the nucleotide sequence and the position of ATG start and TAA, TGA or TAG stop codons indicated a compact coding organization with four major open reading frames, all encoded by the DNA minus strand. These coding regions are :

(1) A region coding for the three HBsAg proteins (S: major protein; pre-S2/S: middle protein; pre-S1/pre-S2/S: large protein);

(2) A region coding for HBcAg (C) and a short upstream in-phase reading frame (pre-C) specifying a larger HBcAg (pre-C/C); the C region specifies a cytoplasmic HBcAg which assembles to give viral core particles; the pre-C region encodes a signal sequence that is essential for the synthesis and secretion of processed core proteins, one of which is the hepatitis B e Ag (HBeAg);

(3) A region denoted P coding for DNA polymerase/ reverse transcriptase and the protein covalently attached to the 5' end of the DNA minus strand;

(4) A region denoted X encoding a protein, HBxAg, that may have a trans-activating function.

Because region P overlaps extensively with the other genes, the small HBV genome can code for all the known constituent proteins of the virus (Hubert et al., 1989).