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STUDY ON HEPATITIS B VIRUS INFECTION USING ELECTRON MICROSCOPY AND GEL DIFFUSION TECHNIQUES

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Hepatitis B Virus is causative agent of an acute and some times subclinical disease which is transmitted through direct inoculation of contaminated blood or through skin and mucous memberane by close contact with the contaminated material (Mc Collum 1977, Laver et al 1979). The virus is spherical containing DNA and is almost a unique type of animal virus (Robinson, 1977). The viral DNA is a circular molecule and for 15 to 45 % is single stranded (Hruska et al 1977, Landers et al 1977). The virus contains three antigens.

(I) Hepatitis B. Surface antigen (HBsAg) designated before as Australia antigen located on the outer layer of the virion (Dane et al 1970).

(II) Hepatitis B core antigen (HBcAg) which is located at the interior central part of the virion (Almedia et al 1971).

(III) Hepatitis B antigen which is also in the interior part of the virion (Takahashi, et al 1979).

The complete virion refered to as Dane particles contains the DNA molecule plus the three above mentioned antigens and a DNA polymerase activity used in diagnostic tests (Kaplan et al 1973). On the basis of antigenic subdeterminants a number of related subtypes have been identified. The virus contains a group specific antigen, a, and two independent sets of antigens d,y and W, r which make in total combination four genotypes of hepatitis virus such as adw, adr, ayw and ayr.

Viral hepatitis is world wide distributed and is common in many countries of the world. Among the total cases about 50% or more are caused by HBV

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(Demihardt et 1980). In this communication we report the results of detection and isolation of HBV from the blood of several patients suffering from hepatitis.

Samples to be examined were obtained from different parts of the country. From Mashad area, were collected and delivered by Dr. Tabarestani and Dr. Bigdelli, Razi Institute, Mashad. Sera were obtained in frozen state and processed immediately after arrival. Some blood samples were also taken from local patients around Karadi and Hessarak. Few samples were also obtained from Dr. M. Shariaty cancer Research Institute, Tehran. All the sera were centrifuged at 4000 rpm. for 20 min. to sediment , the large particles. The supernatant from each sample was takei and centrifuged again at 30,000 rpm. for 1 hr. in a sorval OTD-65 ultracentriguge, using SW.50 swinging bocket . The pellet was disolved in 0.3 ml. of phosphate buffer saline and in ammunium acetate Ph. 7. Samples were negatively stained using 3% phospho tungstic acid and examined in a Philips EM 400 electron microscope. Some samples were slso tested by the micro - techniqiue of agar gel diffusion test using sera containing HBsAg as the antigen and convaliscent sera with titer of HBs antibody as the antisera. From the samples examined about 50% of them were positive for hepatitis B virus infection (Table 1). The positive samples contained HBs Ag in the form of 22 nm. Spherical particles (Fig 1). in addition filementous form HBsAg were also present but less frequent than the spherical particles. In most samples large aggregates of mixed spherical and filementous form were present (Fig 2). In several cases Dane particles which were about 44 nm. in diameter were also bserved (Fig. 3). but their frequency was low. In few cases particles of 22 nm. in size with hollow center were seen (Fig 4). These particles were the core anti gen (HBcAg) which appered in the blood. From the results obatined it seemes that hepatitis B virus infection is common in some region of the country. Most of the patients tested did not have detectable anti HBsAg in their serum as examined by the immunodiffusion test. This means that patients were probably in the early convalescent phase (Deinjard 1980). We did not attempt to determine Anti HBc in the area. The scarcity of Dane particles in the samples was also another evidence for the non acute phase of the disease. Although there are several techniques for the diagnosis of hepatitis B virus infection, we used the technique of electron microscopy because the results are conclusive and the state of the disease can be determined particularly where the tests for detection of HBcAg are not available. Presence of core antigen (HBcAg) in the blood is not common and it is usually found in the infected liver cells. However. we have no satisfactory explanation for the appearance of HBcAg in the few serum samples examined byt its presence is an indication of continuing viral replication and infectivity.

Table 1. Positive samples for HBV infection in different area

No. of samples tested	Arca	Type of test.	No. of positive asmples for		
			HBsAg.	HBcAg.	Danc particle
16	Mashad	EM GD	7	2	4
8	Karadj	EM	3	—	1
4	Hessarak	EM GD	2	—	—
7	Tehran	EM	4	1	1

All the samples were processed as described some samples were examined by the agar gel diffusion technique (GD) but all of them examined by electron microscope (EM).

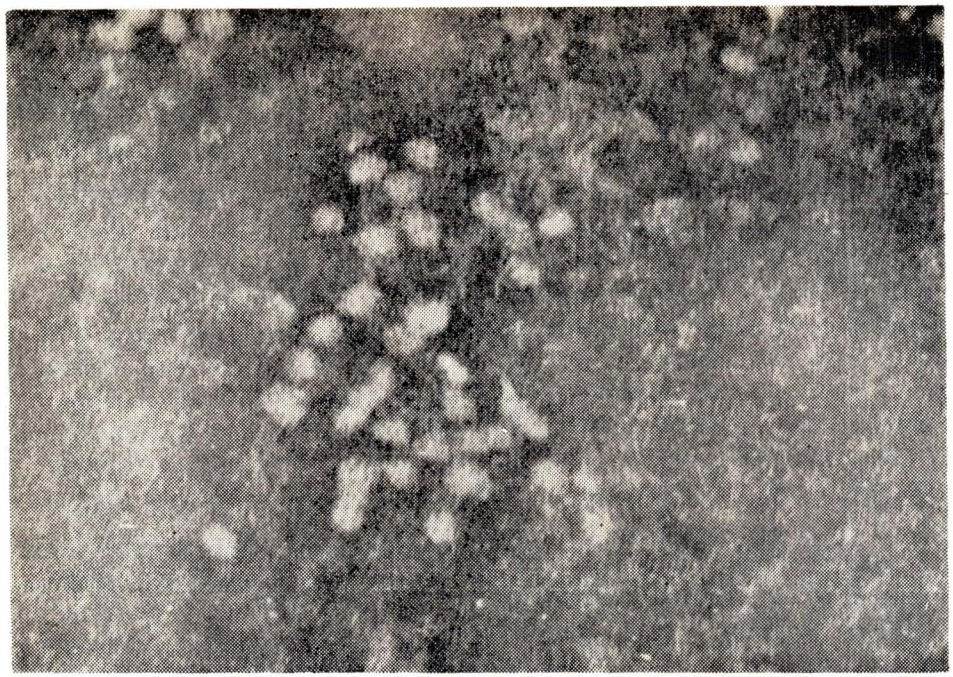

Fig. 1 Electron micrograph of negatively stained sample showing hepatitis B surface antigen. X 220000

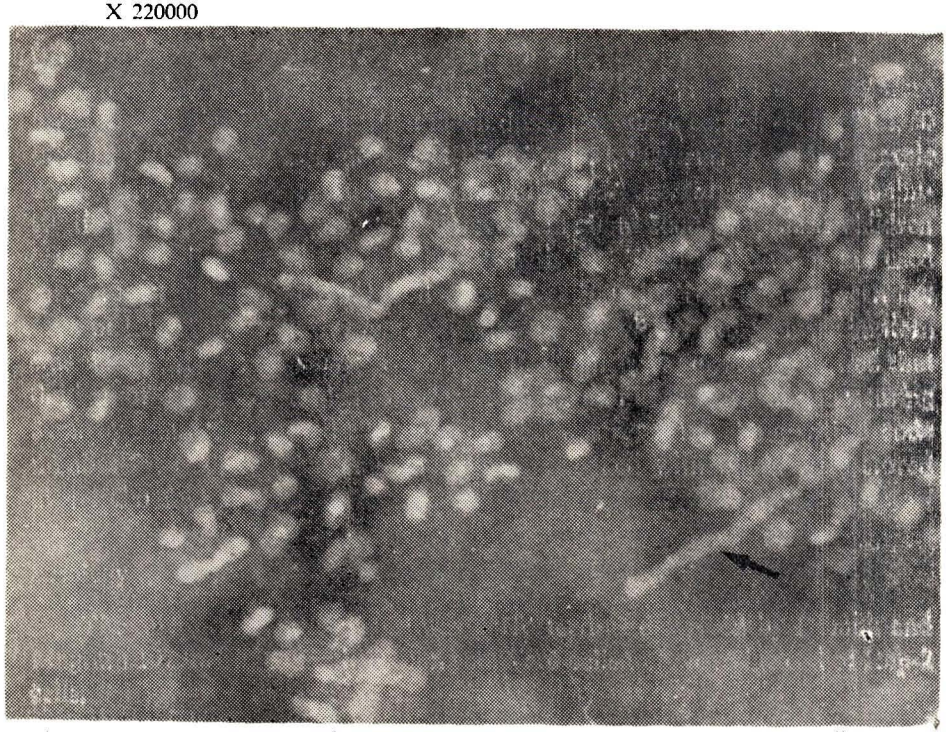

Fig. 2 Electron micrograph of an aggregate of HBsAg. Both the spherical and filementous form are present (Arrow). X 220000

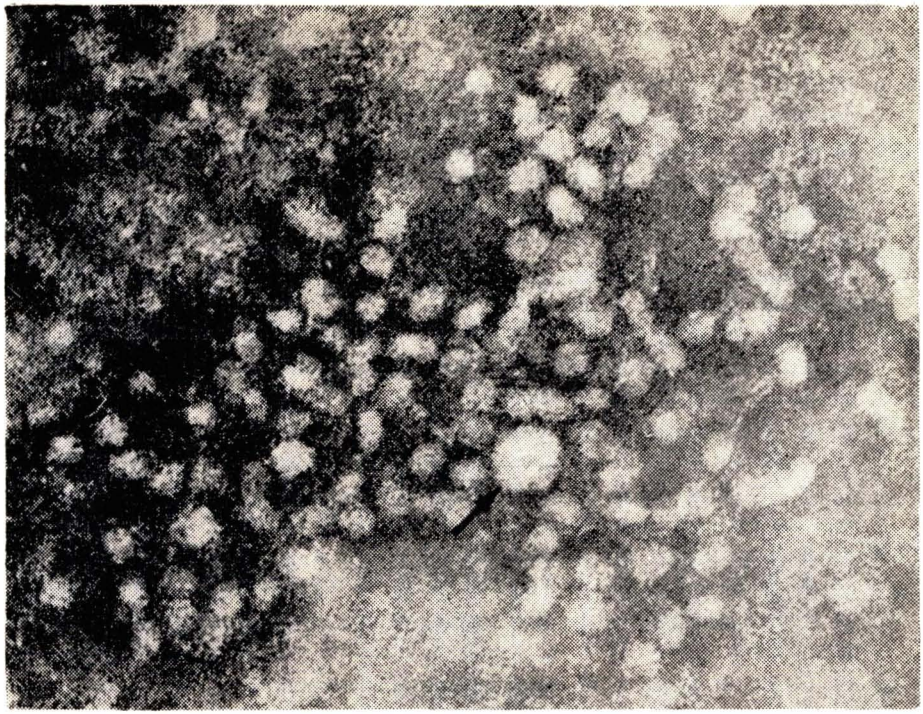

Fig. 3 Electron micrograph of HBsAg showing presence of Dane particle (Arrow) X 200000

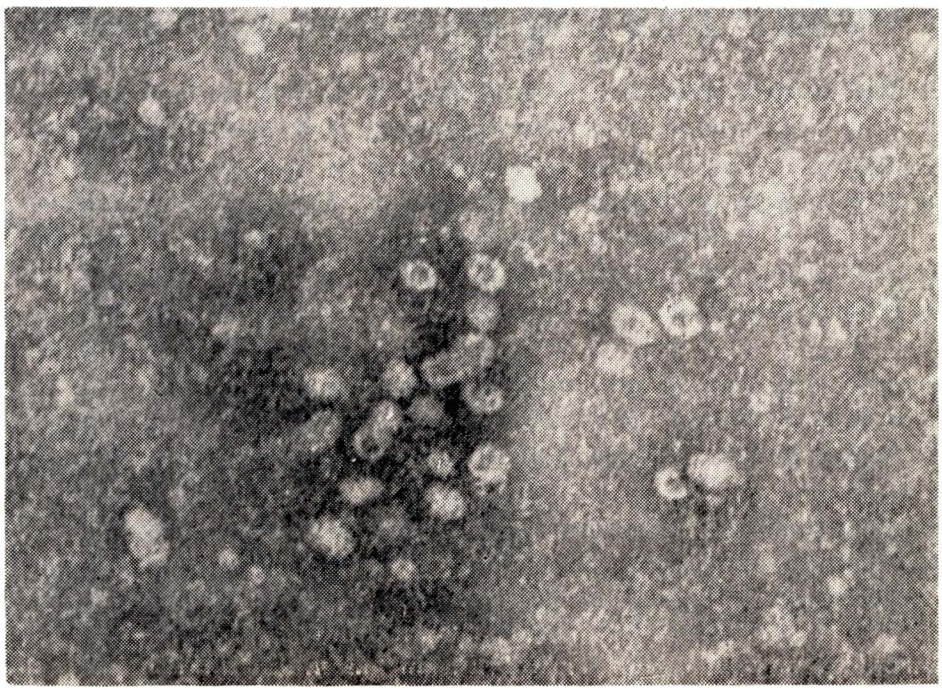

Fig. 4 Electron micrograph of HBcAg. The particles are about 22 nm. in diameter with dark center. X 220000

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