

Hepatitis—associated Antigen (HAA) and Hepatitis in Thailand

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OBJECTIVE: The purposes of this laboratory's initial 6 months of research in hepatitis were to:

- 1) establish laboratory methods for the detection of Hepatitis—associated antigen (HAA).
- 2) determine the prevalence of HAA in the serum of patients with hepatitis and matched controls.
- 3) determine the prevalence of HAA in Thai blood donors.

DESCRIPTION: These studies are based on the following premises:

- 1) HAA can be detected in the serum of patients with serum hepatitis. The most specific diagnostic distinction between serum and infectious hepatitis is the presence or absence of HAA in patient serum. The detection of HAA provides an epidemiologic marker for the study of serum hepatitis.
- 2) Serum hepatitis is infective orally, as well as parenterally. The transmission of serum hepatitis is not limited to parenteral inoculation of the virus.
- 3) Serum hepatitis accounts for a major proportion of sporadically occurring hepatitis cases in adults.
- 4) Chronic carriers of HAA exist. In general, they are asymptomatic with little or no evidence of liver disease. In the United States, at least 0.1% of volunteer blood donors are carriers of HAA. Estimates in Southeast Asia indicate that the background prevalence of HAA may be 50 times higher than in the United States.
- 5) Preliminary studies have indicated that predisposition to the HAA carrier state may be genetically determined by an autosomal—recessive mode of inheritance.
- 6) In the United States, over 50% of recipients of HAA positive blood develop serum hepatitis.

PROGRESS: 1) Laboratory detection of HAA

A microtiter complement fixation (CF) test using five 50% hemolytic units of complement and 2—4 units of antibody has been established. The test system is identical to that used by Department of Virus Diseases, WRAIR. The antiserum used is from a Thai adult (Korn) who developed antibody following transfusion with HAA positive blood. The sensitivity and specificity of this human antiserum compares favorably with guinea pig reference antiserum prepared by the Department of Virus Diseases, WRAIR. An experiment has been completed which demonstrated acceptable reproducibility in detecting HAA by the CF test between replicates in this laboratory and between this laboratory and Department of Virus Diseases, WRAIR. Over 2,400 sera were tested for HAA as of 31 March 1971.

In order to provide a complementary method of detecting HAA suitable for the testing of larger numbers of serum of small volume, an immunoelectrophoresis (IEOP) test for HAA was established. IEOP is a technique which accelerates the formation of immunologic precipitin reactions in an agarose gel by forcing

antigen to migrate toward antibody in an electric field. In an electrophoretic field, HAA (with a negative charge) moves toward the anode and specific antibody diffuses toward the cathode. Where they meet and reach equivalence, a visible light scattering band is formed. The IEOP procedure used is similar to that employed in the Department of Virus Diseases, WRAIR. The power supply is a simple transformer-rectifier supply (Model 19, Arthur H. Thomas Co., Philadelphia) with 300 volt, 50 ma output; the electrophoresis cabinet (4937-V20, Thomas Co.) has been modified so that voltage can be measured across the wicks. Ten ml. of a 1% agarose solution (Seakem, Marine colloids, Springfield, N.J.) in 0.05M Barbitol buffer, pH 8.6 is pipetted onto a clean 3 1/4 by 4 inch glass slide. After solidification of the agarose, 3 mm diameter antigen wells and 2 mm diameter antiserum wells (5 mm apart center to center) are punched and aspirated. Wells are loaded brim full with serum to be tested for antigen and with antiserum. Hyperimmune rabbit antiserum R#244, prepared in Dept. of Virus Diseases, WRAIR, has been used at a 1:4 dilution. A known HAA positive and HAA negative control serum is tested on each slide. The slides are laid in the electrophoresis chamber with antigen wells closest to the cathode, and the power is adjusted to give 12 volts across the agar which yields approximately 10m amp/slide. Slides are read after 2 hours of electrophoresis.

In order to determine if HAA determined by CF or IEOP are identical to standard HAA, an agar gel diffusion (AGD) test was established. The procedure used is identical to that used in the Department of Virus Diseases, WRAIR. Three ml. of hot 1% Agarose in 0.01M tris-EDTA buffer, pH 7.6 are pipetted onto clean 1x3 inch glass microscope slides. After cooling, 7 hole well patterns with 2 mm. wells and 5 mm. center to center spacing between wells are punched and the wells are aspirated. The center well is filled with undiluted hyperimmune HAA antiserum (Rabbit #230, WRAIR) and the top and bottom wells in the pattern filled with known HAA positive serum. The side wells are filled with human serum to be tested for HAA. Slides are incubated in a closed, moist chamber at room temperature and read at 24, 48, and 72 hours

A recent experiment using serum from hepatitis cases and controls in Thailand compared the sensitivity of the three techniques in detecting HAA. Antiserum used in CF tests included Korn antiserum and Rabbit #244 antiserum. Rabbit #244 antiserum, diluted 1:4, was used in IEOP tests, and undiluted rabbit #230 antiserum in AGD tests. Of the 46 sera from hepatitis cases tested, 18 (39%) were positive by IEOP and 15 (33%) were positive in CF tests using both antisera. All sera positive by CF were positive by IEOP; the three sera positive by IEOP but negative by CF gave partial fixation with R244 antiserum. Only 5 sera were positive by AGD, and these sera were positive by both IEOP and CF. When tested in tandem with the standard WRAIR HAA (JF) with R230 antiserum, the 3 hepatitis sera which gave distinct precipitin lines formed a spur with JF antigen, suggesting partial identity with JF antigen.

In the same experiment, 72 sera from matched controls of hepatitis cases were run by all 3 techniques. Seven sera were positive by IEOP, 5 of which were positive by CF using both antisera. An additional serum was positive by CF using R#244 antiserum but negative by CF with Korn antiserum and IEOP. Five of the 7 sera with HAA detected by IEOP were positive by AGD; three showed spur formation in tandem with JF antigen and 2 gave lines of identity with JF antigen.

Collaborative studies with the Department of Virus Diseases, WRAIR, are planned to investigate the significance of suspected antigenic HAA variants in Thailand and to explore reasons for the differences in detection of HAA by the IEOP and CF techniques.

2) HAA in patients with hepatitis

112 hospitalized patients from Bangkok Women's and Children's Hospital and the Royal Thai Army Hospital with an admission diagnosis of hepatitis have been studied. Of the 112 patients, 69 were considered to have hepatitis on the basis of an SGOT or SGPT level exceeding 100 Sigma Units. Of these 69 hepatitis patients, 23 (33%) had sera positive for HAA by the CF test. Of 21 cases of hepatitis in the 20-29 year age range, 14 (67%) were positive for HAA.

Of the 43 patients who did not meet the criteria for a final diagnosis of hepatitis, only 1 (2%) was positive. Of 85 control cases, only 6 (7%) were HAA positive.

Thirty-one hospitalized patients from the U.S. Army Hospital, Bangkok, with an admission diagnosis of hepatitis have been studied. Of the 31 patients, 30 were considered to have hepatitis by the criteria stated above. Of the 30 patients with a final diagnosis of hepatitis, 12 (40%) had sera positive for HAA by the CF test. Of 15 control cases, only 1 (7%) was positive for HAA.

Twenty-two children with a preliminary diagnosis of hepatitis were identified in the out-patient clinic of Children's Hospital over a 10 week period ending 19 March 1971. Eighteen of these 22 had abnormal liver function tests compatible with acute hepatitis. There were 18 matched controls for 12 of these 18 cases of hepatitis. Of the 18 hepatitis cases, 2 (11%) were positive for HAA. Of the 18 controls, none were positive for HAA.

3) HAA among Thai blood donors.

Of 515 professional donors of the Royal Thai Institute of Pathology Blood Bank, 24 (4.7%) were positive for HAA by the CF test. Of 689 volunteer donors of the Thai Red Cross Blood Bank, 42 (6%) were positive for HAA by CF test. Of the total 1204 donors from both blood banks, 66 (5.5%) were positive for HAA. Preliminary results using both CF and IEOP tests indicate that about 8% of blood donors may carry HAA.