The Turkish Journal of Pediatrics
1974 / Vol. 16 / No. 1 / pp. 8 - 14

Role of Australia (Au) Antigen in Chronic Liver Disease in Infancy and Childhood

Meliha Ertuğrul, M.D.*

Introduction

During the years of the 20th century, the etiology of hepatitis was suggested to be due to a virus; this etiology was not confirmed, however, because the responsible agent could not be isolated. The discovery of the Australia (AU) antigen by Blumberg[1] in 1964 (also called hepatitisan tigen, Australia/SH antigen (AU/SH), hepatitis associated-antigen (HAA and hepatitis B-antigen (HBAg) by others) enlightened researchers in this field.

More recent studies (Krugman et. al.[2]) provided evidence for the existence of two types of viral hepatitis with distinctive clinical, epidemiologic, and immunologic features. One type, MS-1, resembled viral hepatitis type A. It was characterized by a short incubation period (30-38 days). The other type, MS-2, resembled viral hepatitis type B, and it had a longer incubation period (41-108 days). MS-2 strain was infectious by mouth as well as parenterally.

Prince et. a.[3] Giles et. al.[4] demonstrated that the Au-Antigen was detectable only in cases of MS-2 hepatitis (B type hepatitis). Electron microscopic studies indicate that the Au-Antigen might be a particle of the hepatitis virus which is called Dane particles and are 42nm. in diameter. Dane particles separate into an outer coat hepatitis B surface antigen (HBs Ag) and an inner component, hepatitis B core antigen (HBc Ag).[5]

---

* Associate Professor, Infectious Disease Unit, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey.

Later studies indicated that patients with viral hepatitis seemed to have the Australia antigen during the early phases of the illness. In most cases of Au-antigen positive viral hepatitis, the Australia antigen disappeared concominant with clinical recovery. Some of the patients, however, were observed to be persistant Au-antigen carriers.[6]

The purpose of this study is to investigate the role of the Au-antigen in childhood chronic liver disease. To realize this study, an attempt was made to compare the percentages of Au-antigen present in patients with chronic liver disease, with that of those with other liver disorders and control cases.

Materials and Methods

This study was carried out on a total of 289 subjects ranging in age from 1 month to 16 years, between January, 1971, and February, 1972. Fifty-eight of these had acute viral hepatitis, 9 neonatal hepatitis (1 to 3 months of age), 10 chronic hepatitis, 29 cirrhosis, 48 hepatomegaly, (Kala-azar, leukemia, anemia, cardiac failure, cyctic disease of the liver and Wilm's tumor), with 99 cases being healthy controls. Of the last group, 25 were taken from an elementary school and 74 from an orphanage.

The blood specimens were obtained from the patients with viral hepatitis during the first two weeks of the illness in all cases, and also sera were taken repeatedly from Au-antigen (+) patients several times in one year. For the other patients with chronic liver diseases sera were obtained when they were admitted to the hospital, and later some of the patients came to the hospital for check-ups at two or three month intervals, and we have taken repeated sera from then.

Standard procedures were used to prepare the specimens for evaluation. Ouchterlony immunodiffusion and micro complement-fixation tests were used. Detection of Au-antigen in all serum specimens was done by the double-immunodiffusion in agarose gel technique, as modified by Prince.[7]

For the complement-fixation (CF) test:[8] Serial dilutions of serums were prepared in 0.025 ml. of onefifth barbital buffered saline solution (pH 7.2) with standard loops, and 2U of both Au-antibody and guinea-pig complement were added. All reagents were in volumes of 0.025 ml. The plates were covered with tape and placed at 4°C for 18 hours. Then 0.050 ml. of 2% sensitized sheep cells was added, and the plates were recovered with tape and incubated at 37°C. Readings were made after incubation for 30 to 60 minutes. Serum specimens were inactivated at 56°C for 30 minutes before use.

Results

Table I shows the frequencies and percentages of patients with Au-antigen positive in various groups, determined both by techiniques of immunodiffusion and complement-fixation. In comparing the frequencies and percentages of patients of different groups, however, we refered only to those obtained from the CF, since this technique is considerably more sensitive than the other.

TABLE I

FREQUENCY OF DETECTION OF AU-ANTIGEN IN LIVER DISEASES

		Immunodiffusion		Complement fixation	
Type of liver disease	No. cases	No. Au positive	% positive	No. Au positive	% positive
Acute viral Hepatitis	58	13	22	21	36.2
Neonatal Hepatitis	9	2	22.2	3	33.3
Chronic Hepatitis	10	3	30	6	60
Cirrhosis	29	7	24	12	41.3
Hepatomegaly	84	3	3.5	NT	NT
Total	190	28	–	–	–

NT: none done.

Twenty-one of the 58 patients (36.2%) with acute viral hepatitis had a positive test for Australia antigen. We had also taken repeated serum from these 21 patients. Excepting for five of those patients, the Au-antigen disappeared within 2 or 3 months. Five patients showed antigen persistance over a one-year period.

Three of the nine patients (33.3%) with neonatal hepatitis were positive for Au-antigen. All neonatal hepatitis was confirmed by biopsy, 5 of which had Giant-cell hepatitis. Three of the mothers (33.3%) of these nine neonatal hepatitis cases were found to have the positive Au-antigen. Of 10 patients with chronic hepatitis, six (60%) were found to have Au-antigen positive.

Three of the 84 cases (3.5%) of hepatomegaly were found positive in antigen by using only the immunodiffusion technique.

Fifty-eight acute viral hepatitis patients gave a typical short history preceeding the onset of the jaundice. (Table II.) We can divide them into three groups for clinical diagnosis, according to their exposure

TABLE II

INCIDENCE OF AU-ANTIGEN IN 58 PATIENTS WITH A CLINICAL DIAGNOSIS OF VIRAL HEPATITIS BY CF METHOD

Clinical diagnosis	Total patients	No. Au-positive	% positive
Infectious hepatitis	11	1	9
Serum hepatitis	13	7	53
Infectious or serum hepatitis	34	13	38
Total	58	21	36.2

to hepatitis. Eleven patients had viral hepatitis contacted from neighbors or school frinends who had recently had hepatitis. Thirteen were classified in the second group on the grounds of a history of exposure to a contaminated needle or operation and blood transfusion. Thirty-four patients had an unknown exposure.

Control groups (Table III): In one control group consisting of 25 children from an elementary school, Au-antigen was shown to be present in 4% by the Ouchterlony immunodiffusion technique and in 8% by the CF technique. 74 orphanage children who lived together in the same building were tested. None of these children had had jaundice nor transfusions of blood or blood products; however, previously only one child, who was not included in this study, had had hepatitis a year earlier at this orphanage. The incidence of positive Au-antigen was 14.8% by immunodiffusion and 24.3% by the CF technique in these healthy children. Some of these (Au) positive controls' sera were examined for transaminase level and were found normal.

TABLE III

FERQUENCY OF DETECTION OF AU-ANTIGEN IN HEALTHY CONTROL CHILDREN

		Immunodiffusion		Complement fixation	
Type of control	No. tested	No. Au positive	% positive	No. Au positive	% positive
Elementary School	25	1	4	2	8
Orphanage children	74	11	14.8	18	24.3
Total	99	12	–	20	–

Statistical comparisons in this study were done by the chi square (x2) test and since some of the frequencies were below 5, Fisher's exact method was also employed. The number of cases of acute viral hepatitis was compared with those of chronic hepatitis ($p < 0.05$), cases of acute viral hepatitis with those of cirrhosis ($p < 0.05$), and patients with chronic hepatitis with cases of cirrhosis ($p < 0.05$). Statistical comparisons between elementary school children and orphanage children were found to be $p < 0.05$.

Discussion

The current concepts of infectious and serum hepatitis have been the subject of much discussion recently. Our findings showed (Table II) only one (9%) positive Au-antigen in 11 patients with infectious hepatitis. But, in the cases of hepatitis with a history of parenteral exposure, 7 of the 13 patients (53%) were found to have the Au-antigen. High incidence of Au-antigen (38%) in the patients with unknown exposure was also found. It may be postulated that the third group, too, represents primarily infection by serum hepatitis virus. The difficulties in clinical differentiation of the two types of hepatitis must be mentioned. It has been clearly demonstrated that the long incubation MS-2 strain of serum hepatitis virus is infectious orally as well as parenterally.[2] Giles et. al.[4] show that all patients with MS-1 infection have been antigen negative, whereas, those with MS-2 infection are almost always positive. In this study, with the exception of five of the 21 Au-antigen positive patients with viral hepatitis, all realized clinical and laboratory recovery in two or three months and the Au-antigen disappeared. Five patients had persistent detectable antigen for one year, but only one of these Au-antigen persistent patients developed chronic liver disease.

Of 6 chronic hepatitis patients with Au-antigen (+), only one showed persistent detectible antigen for more than ten months. Clinical and biochemical findings of this patient were similar to those associated with cirrhosis.

Four of 12 Au-antigen positive patients with cirrhosis showed a continuance of detectible antigen for one year. Thirteen of 29 patients (44.8%) with cirrhosis had a past history of hepatitis, ranging from 2 to 48 months prior to the development of cirrhosis. The occurance of antecedent acute hepatitis in cirrhosis gave further support to this opinion. In most cases of Au-antigen positive viral hepatitis, the antigen was only present transiently. Persistent antigenicity is associated with a high incidence of chronic liver disease such as chronic persistent hepatitis. It is suggested that two factors may influence the outcome in individual

patients: (1) the dose of virulence, or strain of the virus and its mode of transmission, and (2) the immunological response of the patients. The antigen is unlikely to be a non-specific product of the injured liver cells. It could be explained as an antigen antibody complex. Immunofluorescence studies.[9] showed deposits of IgG and complement in the paranchyma, suggesting the presence of immune complexes, the immune complexes being a pathogenetic factor of liver necrosis.

We used only the immunodiffusion technique in the other liver disesases with hepatomeglay, and Au-antigen positive was found to be 3.5%. These results run a parallel course to the prevalence of Au-antigen in Turkey, which is 3.2%.[10]

The frequency of antigen encountered in patients with chronic liver disease was found to be significantly greater than that in patients with hepatomegaly and that in control groups. Statistical tests carried out on the elementary school control group and on patients with cirrhosis, chronic hepatitis and acute viral hepatitis, revealed the results to be significantly different ($p > 0.05$).

In the 25 elementary school children and the 74 healthy orphanage children, percentages of antigen differed. Some studies indicate a high-rate of transmission of the virus of serum hepatitis in institutionalized children, such as patients with Down's syndrome, under care in large insitiutions.[11] Transmissions may also be seen among family members and as a result of other close contacts (such as kissing). In tropical climates transmission of the virus is by blood-sucking insects.[12]

Our findings suggest that the hepatitis virus is easily transmitted within the orphanage group; however, the statistical test results of groups of elementary school and orphanange children were found to be insignificant.

Summary

The Australia antigen was sought in patients during childhood who had acute viral hepatitis, neonatal hepatitis, chronic hepatitis and cirrhosis of the liver. The incidence of positive tests for the Au-antigen in viral hapatitis was 36.2%; in neonatal hepatitis, 33.3%; in chronic hepatitis, 60%; and in cirrhosis, 41.3%, as determined by the complemen t-fixation technique. Some of these positive patients were found to be Au-antigen negative by the immunodiffusion test. Different Au-antigen percentages were encountered in these diseases and statistical test results seemed to be insignificant. Au-antigen is much higher in acute viral hepatitis and in chronic liver disease than in healthy control groups and

patients with other kinds of liver pathology. A much higher proportion of patients (44.8%) with cirrhosis have had a past history of hepatitis or jaundice. It would seem that the Au-antigen carrier state plays an important role in the etiology and pathogenesis of most forms of chronic liver diseases.

Our study also confirmed that complement-fixation is more sensitive than immunodiffusion for detection of the Au-antigien.

REFERENCES

1. Blumberg, B. S.: Polymorphisms of Serum Protein and the Development of Isoprecipitins Intransfused Patients. Bull. N.Y. Acad. Med. 40: 377, 1964.
2. Krugman, S., Giles, J. P., Hammond, J.: Evidence For Two Distinctive Clinical, Epidemiological and Immunological Types of Infection. JAMA. 200: 365, 1967.
3. Prince, A. M., Hargrove, R. L., Szmuness, N., Cherubin, C. E., Fontana, V. J., Jeffries, G. H.: Immunological Distinction Between Infectious and Serum Hepatitis. New. Eng. J. Med. 282: 987, 1970.
4. Giles, J. P., Mc Collum, R. W., Berndtson, L. W., Krugman, S.: Viral Hepatitis Relation of Australia/SH Antigen to the Willowbrook MS - 2 Strain. New. Eng. J. Med. 281: 119, 1969.
5. Dane, D. S., Cameron, C. H., Briggs, M.: Virus - Like Particles in Serum of Patients with Australia Antigen Associated Lancet, Hepatiti. 1: 695, 1970.
6. Nielsen, J. O., Dietrichson, O., Elling, P., et all: Incidence and Meaning of Persistence of Australia Antigen in Patients with Acute Viral Hepatitis Development of Chronic Hepatitis. New. Eng. J. Med. 285: 1157, 1971.
7. Prince, A. M.: An Antigen Detected in the Blood During the Incubation Period of Serum Hepatitis. Proc. Nat. Acad. Sci. 60: 814, 1968.
8. Sever, J. L.: Application of a Microtechnique to Viral Serological Investigations. J. Immun. 88: 320, 1962.
9. Nielsen, J. O., Elling, P.: Investigations of Liver Biopsies for Australia Antigen by Immunofluorescent Technique. Clin. Exp. Immun. 9: 699, 1971.
10. Ertuğrul, M., Say, B.: Australia Antigen in Turkey. Lancet. 1: 1302, 1971.
11. Blumberg, B. S., Gerstley, B. J. S., Hungerford, D. A., et all.: A Serum Antigen (Au Antigen) in Down's Syndrome, Leukemia and Hepatitis, Ann. Intern. Med. 66: 924, 1967.
12. Prince, A. M.: Prevelence of Serum Hepatitis Related Antigen in Different Geographic Regions. Amer. J. Trop. Med. Hyg. 19: 872, 1970.