

## STUDY REPORTS

2. Title The Frequency and Classification of  
Types of Liver Disease in Thailand  
(LEPTOSPIROSIS)

Principal Investigator: Natth Bhamarapravati, M.D.

Associate investigators: Vikit Veranuvatti, M.D.  
Uthai Tuchinda, M.D.  
Captain Sylvanus W. Nye, USAF (MC)  
Praphasri Nimsomburana, M.D.

OBJECTIVE: In an area like Thailand where both viral hepatitis and leptospirosis occur endemically, the differential diagnosis on patients with acute hepatocellular damage of infective origin may at times be difficult. Since a final diagnosis of viral hepatitis rests in most instances on the histologic findings in the liver, owing to a lack of etiologic diagnostic procedures, it is essential to have more knowledge on the pathological changes which may be encountered in liver biopsies obtained from patients with leptospirosis.

DESCRIPTION: Biopsies of the liver were obtained from twenty-two patients with leptospirosis admitted to Siriraj Hospital, Vajira Hospital, and the hospital of the School of Tropical Medicine, Bangkok, Thailand during 1963 and 1964. Seventeen cases were male and five were female. All cases had this diagnosis proven by one or a combination of the following procedures: culture of blood or urine on Fletcher's media, inoculation of blood or urine into hamster, agglutination lysis. The biopsies were fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin eosin, Periodic Acid Schiff after digestion with diastase and Perl's iron stain. Clinical data were collected from the records of the cases.

DISCUSSION: A summary of the clinical and laboratory findings of these cases are presented in Table I. Cases are divided into two groups, the jaundiced (total bilirubin over 1.2 mg%) and the non-jaundiced (total bilirubin under 1.2 mg%), Table II shows the types and frequency of leptospirosis encountered in this series.

The symptoms and signs are in order of frequency; fever, injection of conjunctiva, myalgia, nausea and vomiting, abdominal pain and tenderness, chest pain and cough. No significant difference in the frequency of these observations is noted between the jaundiced and non-jaundiced group, except that seventeen per cent of the jaundiced group showed impaired consciousness.

Pathological findings. Table II shows a summary of the frequency of various lesions seen in the biopsies and graded in a semiquantitative manner. After grading was done, the findings are grouped together according to the time of illness. In the non-jaundiced group there is very little difference in the findings regardless of the time that the biopsy was done. Slight cholestasis in the form of bile droplets is noted in one case of this group.

Jaundice group. Liver cell change. Liver cell changes consist of a mild to moderate degree of unrest of liver cells manifested by an irregularity in size with an increase in number of binucleated liver cells. The unrest of liver cells may persist through the 4th week of illness. Spotty necrosis of the liver cells is present, more marked after onset. There is no concomitant swelling or ballooning of the liver

cells. A small number of acidophilic cells of the type seen in classical icteric viral hepatitis is also present in the sinusoids or in the process of being dislodged from the liver plate. Some of these acidophilic cells still retain bits of nuclear debris and some may actually be derived from the Kupffer cells. Some PAS positive pigment which resists diastase digestion and has pericanalicular arrangement is noted in the livers of this group.

Kupffer cells. The alteration of Kupffer cells consists of an increase in number of the Kupffer cells and sinusoidal lining cells. The cytoplasm of the Kupffer cells is hypertrophic, bulging into the lumen of the sinusoids and occasionally shows phagocytosis of red blood cells. Hemosiderin in the form of granules is seen in the cytoplasm of the Kupffer cells in increasing amount in some of the cases while in others, faint, homogenous bluish shade is observed in sections stained for iron.

Cholestasis. Stasis of bile is manifested by small greenish droplets in the cytoplasm of the liver cells, or occasionally by thrombi in the canaliculi but bile lake is not seen. The bile droplets and bile thrombi are evident mostly in the centrilobular areas of the lobules but occasionally can be observed focally. In one case, stasis of bile is apparent eight weeks after the onset of disease. There is no associated glandular transformation of liver cells around the bile pigment.

Cellular Reaction. An increase in number of cells in the sinusoids is present in three cases. The cells are mostly neutrophils. In four cases, infiltration of portal area is present, the cells are mostly mononuclear and lymphoid cells.

Fibrosis and ductular proliferation. In two cases biopsied within four weeks, there is some enlargement of portal area with short, thin fibrous septa not associated with an increase in number of perilobular ductules. These findings are considered as probably pre-existing changes and may not be the actual consequence of leptospirosis. There is no evidence of focal scar formation nor proliferation of intralobular ductules in any cases.

#### Severity of Morphological Changes and Time Relationship.

In the sections where biopsies were performed between two to four weeks after the onset, the morphological changes appear to be active. Some of the changes are still present in the one biopsy obtained eight weeks after the onset. Since there is only one case biopsied late, it cannot be said whether this would represent a usual type of host response in leptospirosis.

#### Types of Leptospirosis and Severity of Histologic Changes.

Table III shows the relationship between types of leptospira and the severity of histologic changes which are graded arbitrarily as minimal, mild and moderate changes. The group which reveals minimal lesions in the liver corresponds with the non-jaundice group. Most of the patients infected with L. bataviae show mild changes while the other cases infected with L. canicola, L. javanicum and L. pyrogenes show from mild to moderate alterations. Whether this would indicate that as far as the liver is concerned, one could expect relatively milder host responses in infection caused by L. bataviae than in infections caused by other leptospirae can not be definitely ascertained. The biopsies were not random depending more on voluntary consent of patients as well as judgement of physician on the clinical safety.

SUMMARY AND CONCLUSION: The findings in the liver biopsies obtained from patients suffered leptospirosis in this series are, in general, in conformity with those described by Areans, in his study of a fatal human leptospirosis autopsy. Disorganization of liver cell plates is, however, not a prominent feature in our material and this is certainly not of pathognomonic significance. In the biopsy material, a variation of the pattern of the pathological findings is present. This may be due to different host susceptibility, variations in time when the biopsy was done as well as the strains of leptospira.

The pathogenesis of leptospirosis, as far as the liver is concerned, is of interest. The general effect is not unlike that of a viral hepatitis. There is some degree of unrest of the liver cells indicating an increase in the turn over rate of the liver cells even though this is probably less than in viral hepatitis. Necrosis of the liver cells is present but rather spotty and may lead to the formation of acidophilic cells. The portal infiltration is, however, scanty but bile stasis is prominent. This has led us to make erroneous diagnosis of cholestatic hepatitis on liver biopsies in the past. There is usually little if any, ductular proliferation contrary to what is usually present in viral hepatitis. It is interesting that despite the rather severe cholestasis in some of these cases, the level of alkaline phosphatase did not rise much this is somewhat similar to observations made on leptospirosis in guinea pigs in which there was a depressed level of both serum alkaline phosphatase and depressed activity in the homogenates of liver and kidney.

The morphologic observations and liver function studies suggest that leptospira exert a general effect on the liver. Whether this could be due to the mechanical presence of leptospira or its toxin is not known. Conjugation of bilirubin in the liver cells can probably continue since there is a rise in the direct fraction of bilirubin in the blood, and bile thrombi are seen in the canaliculi. Thus, the excretory function of the liver cells which would facilitate flow of bile may be impaired. Some increase in peribiliary lipochrome is noted suggesting a disturbance of lysosomal system of the cells. Further studies utilizing advanced methodology are needed for clarifying the pathogenetic mechanism operating in leptospirosis.

PUBLICATION: In Press. American Journal of Gastroenterology, "Liver Diseases in Thailand".

TABLE I  
SUMMARY OF CLINICAL AND LABORATORY FINDINGS

|   | JAUNDICE<br>15 cases        | NON JAUNDICE<br>7 cases |
|---|-----------------------------|-------------------------|
| 1. Sex  | Male 11    Female 4         | Male 5    Female 2      |
| 2. Age  | 34.6 years (18-52)          | 25 years (15-32)        |
| 3. Duration of hospitalization                    | 24.7 days (8-56)            | 18 days (5-44)          |
| 4. Hepatomegaly                                   | 46.6%                       | 14.2%                   |
| 5. Splenomegaly                                   | None                        | None                    |
| 6. Hemoglobin level                               | 9.4 gm%                     | 13 gm%                  |
| 7. Leukocyte count                                | 17,837/cu mm (6,650-32,300) | 10,857 (6000-17,800)    |
| 8. Polymorphonuclear leukocyte percentage over 80 | 67%                         | 71%                     |
| 9. NPN  | 64.6 mg%                    | 50.3 mg%                |
| 10. Creatine                                      | 3.82 mg%                    | 1.82 mg%                |
| 11. Direct bilirubin                              | 12.88 mg%                   | 0.19 mg%                |
| 12. Indirect bilirubin                            | 8.73 mg%                    | 0.61 mg%                |
| 13. Alkaline Phosphatase                          | 4.28 Sigma Units            | 1.77 Sigma Units        |
| 14. Thymol turbidity                              | 4.7 Unit                    | 2.9 Unit                |
| 15. Serum albumin                                 | 2.91 gm%                    | 2.67 gm%                |
| 16. Serum globulin                                | 4.82 gm%                    | 3.63 gm%                |
| 17. Proteinuria                                   | 40%                         | 33.3%                   |
| 18. Bilirubinuria                                 | 46.6%                       | None                    |

Laboratory findings recorded here are the ones obtained close to or on the days that liver biopsies were performed.

TABLE II

HISTOLOGIC FINDINGS IN LIVER BIOPSIES

|              |   | NON JAUNDICE                    |      |                                      |      | JAUNDICE                             |      |       |      |
|--------------|---|---------------------------------|------|--------------------------------------|------|--------------------------------------|------|-------|------|
|              |   | Biopsy upto 2 weeks after onset |      | Biopsy between 2-4 weeks after onset |      | Biopsy between 4-8 weeks after onset |      |       |      |
|              |   | Freq.                           | Sev. | Freq.                                | Sev. | Freq.                                | Sev. | Freq. | Sev. |
| LIVER CELL   | Unrest                                  | 6/7                             | 1.0  | 5/5                                  | 1.6  | 9/10                                 | 1.7  | 1/1   | 2.0  |
|              | Necrosis                                | 0/7                             | 0.0  | 2/5                                  | 1.5  | 5/10                                 | 1.7  | 1/1   | 1.0  |
|              | Acidophilic cell                        | 0/7                             | 0.0  | 0/5                                  | 0.0  | 5/10                                 | 1.0  | 1/1   | 1.0  |
|              | Pigment                                 | 0/7                             | 0.0  | 5/5                                  | 1.4  | 6/10                                 | 1.3  | 1/1   | 2.0  |
| KUPFFER CELL | Proliferation                           | 6/7                             | 1.1  | 4/5                                  | 1.25 | 10/10                                | 1.3  | 1/1   | 2.0  |
|              | Phagocytosis                            | 0/7                             | 0.0  | 1/5                                  | 1.0  | 3/10                                 | 1.0  | 0/1   | 0.0  |
|              | Iron                                    | 1/7                             | 1.0  | 1/5                                  | 1.0  | 6/10                                 | 1.0  | 0/1   | 0.0  |
|              | Cholestasis                             | 1/7                             | 1.0  | 2/5                                  | 1.0  | 10/10                                | 1.9  | 1/1   | 2.0  |
|              | Increased number of cell in portal area | 1/7                             | 2.0  | 1/5                                  | 2.0  | 2/10                                 | 1.0  | 0/1   | 0.0  |
|              | Increased number of cell in sinusoids   | 0/7                             | 0.0  | 1/5                                  | 1.0  | 2/10                                 | 1.0  | 0/1   | 0.0  |
|              | Fibrosis                                | 0/7                             | 0.0  | 1/5                                  | 2.0  | 1/10                                 | 1.0  | 0/1   | 0.0  |
|              | Ductular proliferation                  | 0/7                             | 0.0  | 1/5                                  | 2.0  | 1/10                                 | 1.0  | 0/1   | 0.0  |

Freq. = frequency

Sev. = severity (An average of the grading from + to 3+)

TABLE III

TYPES OF LEPTOSPIROSIS AND SEVERITY OF LIVER LESIONS

|              | Minimal | Mild | Moderate | Severe |
|--------------|---------|------|----------|--------|
| L. bataviae  | 7/15    | 7/15 | 1/15     | —      |
| L. canicola  | 0/3     | 1/3  | 2/3      | —      |
| L. jananicum | 0/2     | 1/1  | 1/1      | —      |
| L. pyrogenes | 0/2     | 2/2  | 0/2      | —      |