Original Article

Histopathological Grading and Staging of Chronic Hepatitis C Patients in Rural Sindh

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ABSTRACT

OBJECTIVE: To determine the grading and staging of chronic hepatitis induced by hepatitis C virus in rural Sindh.

DESIGN: A prospective, observational study.

SETTING: Medical Research Center Liaquat University of Medical and Health Sciences Jamshoro, Department of Pathology, Nawabshah Medical College for Girls Nawabshah and Biotechnology Department of University of Karachi, from August 2006 to June 2008.

PATIENTS: A total of 344 HCV-PCR positive patients were selected by non-probability convenient sampling technique. (Overall 239 men and 105 women with age 18–55 years included in the study.)

METHODOLOGY: All the patients went for ELISA test for the presence of HCV antibodies by ELISA kit of Biokit Spain and HCV-RNA by RT-PCR on Cephid smart cycler, and then a hepatic biopsy was performed in clinically indicated patients and the biopsy fragments were submitted to conventional histopathological procedures.

RESULTS: According to histopathological analysis of 344 biopsy samples of chronic hepatitis C patients 38 (11.04%) patients presented with necro-inflammatory grade 1, 151 (43.89%) with grade 2, 117 (34.01%) with grade 3 and 38 (11.04%) cases presented with grade 4; while no biopsy specimen revealed necro-inflammatory grade 0. Majority of patients presented with fibrosis grade 2 and fibrosis grade 1 in 121 (35.17%) and 102 (29.65%) biopsy samples respectively followed by fibrosis grade 3 in 77 (22.38%), fibrosis grade 4 in 30 (8.72%) and fibrosis grade 0 in 14 (40.6%) cases.

CONCLUSION: In current study we observed majority of cases in A2, A3 grade of inflammation and F1, F2, F3 stages of fibrosis, which indicates that patients came late for medical assessment which may cause difficulties in management. This warrants the necessity of HCV awareness programs in rural areas of Sindh.

KEY WORDS: HCV RNA, Histological Grading and staging, Chronic Hepatitis, Rural Sindh.

INTRODUCTION

During the 1990s, there was a revolution in the way that pathologists and hepatologists thought about chronic viral hepatitis. These revolutions were stimulated by the realization that the traditional categorization of pathologic changes as chronic persistent hepatitis, chronic lobular hepatitis, and chronic active hepatitis were inadequate for assessing histologic changes during clinical trials and were sometimes misunderstood to be separate pathologic processes rather than part of a continuum of pathologic changes in chronic hepatitis C[1-4].

In response to this, pathologists introduced the idea of staging and grading to the pathological evaluation of chronic hepatitis C. Staging, in the broadest sense, is the determination of the position of the patient on the continuum of disease progression between its initiation and its end stage. Grading is the assessment of the activity of a disease, which is the rate at which the disease stage is changing. In the natural history of most chronic diseases, the stage of the disease generally increases with time, although relapsing or remitting diseases may be examples of disease as a disease flares and subsides, or may remain static throughout the course. Therapeutic intervention typically has most of its effect on disease activity[5]. The hepatic biopsy is the gold-standard diagnostic procedure to estimate severity of tissue damage in chronic hepatitis and to determine histological activity. The decision to treat the patient is based on these data, and tissue damage severity seems to be predictive for the future development of fibrosis[6]. However, it has been noted that fibrosis staging and inflammatory activity grading are not always related. The progression of fibrosis culminates in the disarrangement of the hepatic architecture is a variable parameter[7]. The new concepts of rate of progression of fibrosis have emerged with current knowledge on chronic HCV hepatitis[8]. The estimation of fibrosis progression and knowledge concerning associated factors in chronic hepatitis C is extremely important for understanding

its natural history[3]. Pathologic staging has focused on the assessment of fibrosis as the best surrogate marker of true disease stage. Staging divides the fibrotic continuum into discrete categories and all of the existing staging systems have cirrhosis as their highest stage. Once the pathologic stage of cirrhosis has been reached, clinical scales take over for the staging of the liver disease. During this stage of clinical progression, cirrhosis may continue to progress pathologically in which fibrotic bands widen, more vascular shunts form, and the remaining parenchyma loses any resemblance of normal architecture. However, only the Ishak modification of the Histological Activity Index (HAI) tries to subdivide cirrhosis with its categories of incomplete cirrhosis and definite cirrhosis[5].

Grading is a more nebulous concept than staging because determination of factors important in disease progression presumes knowledge of how fast the stage is changing. At the very least, this would seem to require absolute knowledge of the stage at two separate points in the course of the disease. In the absence of such longitudinal data, pathologists have used a global assessment of the inflammation as the most rational choice for pathologic surrogate marker of grade. Several systems exist for grading and staging of chronic hepatitis and all have been used effectively to assess changes in pathology following therapeutic intervention. These systems include the methods of Scheuer[1], Desmet[3], Batt and Ludwig[4] and the METAVIR system[6,9]. All of these systems provide a single global assessment of grade and stage, although the final grade may be dependent on a combined assessment of several inflammatory features. Evaluation of grade and stage is a standard part of the pathologic assessment of liver biopsies in chronic hepatitis, and pathology reports should routinely provide this information. Although grading and staging can be used in assessing histological outcome in clinical trials or as variables in statistical analyses of progression or therapeutic response, scoring of liver biopsies for particular histological changes is a distinct process. A biopsy can have only one grade and one stage, but may be scored for many individual histological features[10,11].

Keeping all these above facts in view, we conducted this study to determine the grading and staging of chronic hepatitis induced by hepatitis C virus, in our setup. This is first ever study that shows the data from whole rural Sindh, as the cases were collected from all the teaching hospitals attached with all the medical colleges of rural Sindh.

MATERIALS AND METHODS

This study was conducted at Medical Research Center Liaquat University of Medical and Health Sciences Jamshoro, Pathology Department Nawabshah Medical College for Girls Nawabshah and Biotechnology Department of University of Karachi, during August 2006 to June 2008.

This study was a multi-centric study covering all the interior of Sindh. The blood samples from 344 patients were collected from various medical wards of Liaquat University Hospital Jamshoro and Hyderabad, Nawabshah Medical College Hospital Nawabshah, Chandka Medical College Hospital Larkana, Civil Hospital Sukkur and Muhammad Medical College Hospital Mirpurkhas. The patients included in the study were 18-55 years of age, with persistent abnormal alanine aminotrasferase levels, and evidence of presence of HCV-RNA in serum of patient by PCR.

The suspected patients of chronic hepatitis were informed about the study, they signed a consent form and ELISA test for the presence of HCV antibodies was performed by ELISA kit of Biokit Spain.

The anti-HCV positive patients were submitted to a laboratory protocol. The questionnaire composed of clinical and epidemiological data (sex, age at biopsy, routes of contamination, age of infection, consumption of alcohol, and estimated duration of infection, defined as the time elapsed between the presumed date of infection and date of biopsy). The biochemical examinations included in the laboratory protocol were ALT and AST determination, number of times above normal level, gamma-GT (number of times above normal level), serum protein, serum albumin, serum bilirubin, blood urea, glucose, uric acid, cholesterol, triglycerides, serum electrolytes, coagulation (BT, CT, PT, APTT) and haematological (haemoglobin, TLC, DLC, Platelets) examinations. A sample of 10.0 ml of blood was collected in a tube with separating gel to obtain serum, which was stored at -80^{O}C for determination of hepatitis C virus by extracting HCV-RNA from plasma, amplified using reverse transcription and detected through the use of fluorescent reporter dye probes specific for HCV in the smart cycler^{O} (Cephid).

Histopathological evaluation

After completing the above mentioned protocol the patients were submitted to a percutaneous hepatic biopsy. A hepatic biopsy was performed in clinically indicated patients and the biopsy fragments were submitted to conventional histopathological procedures. The sample was taken and placed in 10% formalin, embedded in paraffin, cut into 4µm sections and stained with hematoxylin and eosin stain, supplemented by trichrome and reticulin special stains, observed under microscope. Histological diagnosis was made and results were tabulated.

Interpretation of results

The samples were considered adequate for analysis

when at least eight portal areas were seen. The criteria used for the chronic hepatitis classification included staging of fibrosis and grading of inflammatory activity[3,12]. The stage of fibrosis was evaluated as:
O = no fibrosis.
01 = portal fibrosis without septa.
02 = few septa.
03 = numerous septa delineating nodules without cirrhosis.
04 = cirrhosis.
The grading of activity was performed by taking into account the inflammatory activities in the portal tract, in the periportal and lobular regions:
0 = no histological activity.
01 = minimal lesion.
02 = mild activity.
03 = moderate activity.
04 = severe activity.

RESULTS

In this prospective study a total of 344 HCV-PCR positive patients with different genotypes were evaluated (239 mean and 105 females). Their ages ranged from 18-55 years with a mean age of 35.14 years. The baseline characteristics of study population are detailed in **Table I**. The histological necro-inflammatory activity of 344 patients of chronic hepatitis C patients is presented in **Table II**. According to histopathological analysis majority (43.89%) of the cases presented with grade 2. The histological fibrosis (staging) of 344 cases of chronic hepatitis C is presented in **Table III**. Majority of patients presented with fibrosis grade 2 and fibrosis grade 1 in 121 (35.17%) and 102 (29.65%) biopsy samples respectively.

TABLE I:
BASELINE CHARACTERISTICS OF THE STUDY POPULATION (n=344)

Chronic Hepatitis C patients With +ve HCV-PCR and genotype	344
Sex	
Men	239 (69.47%)
Women	105 (30.52%)
Age at Infection	
< 20 years	42 (12.20%)
21-40 years	221 (64.25%)
> 40 years	81 (23.55%)
Mean Age	35.14 years
Duration of infection in year	
< 2 years	140(40.69 %)
3 - 5 years	196 (56.9 %)
> 5 years	08 (2.32 %)

TABLE II:
INFLAMMATORY HISTOLOGICAL ACTIVITY (GRADING) OF CHRONIC HEPATITIS C PATIENTS (n=344)

Inflammatory Activity (Grading)	Number	Percentage
None (A0)	0	0
Minimal (A1)	38	11.04
Mild (A2)	151	43.89
Moderate (A3)	117	34.01
Severe (A4)	38	11.04

TABLE III:
HISTOLOGICAL FIBROSIS (STAGING) OF CHRONIC HEPATITIS C PATIENTS (n=344)

Stage of Fibrosis	Number	Percentage
No Fibrosis (F0)	14	4.06
Portal Fibrosis (F1)	102	29.65
Few Septa (F2)	121	35.17
Many Septa (F3)	77	22.38
Cirrhosis (F4)	30	8.72

DISCUSSION

Grading is used to describe the intensity of necroinflammatory activity while staging is an indication of architectural alteration thus signifying progression of the disease towards cirrhosis or end-stage liver disease. This histological activity is important for the patient and the clinician because it provides a measure of severity of the hepatitis at the time of biopsy, and this is not always matched by abnormal liver function test[1].
The concept of grading and staging have traditionally been applied to neoplasia; grading describes the degree of differentiation of a neoplasm, while staging describes the extent of its spread. The same principles have come to be applied, however, with some modifications to chronic hepatitis, the estimation of fibrosis progression and knowledge concerning associated factors in chronic hepatitis C is extremely important for understanding its natural history[3]. The application of this concept of grading and staging seeks to impute a prognostic value to biopsy assessment in chronic hepatitis. Therapeutic intervention typically has most of its effect on disease activity[5].
In current study we observed majority i.e. 151 (43.89%) of cases in grade A2 and 121 (35.17%) in stage F2, which is in line with another local study[13]. We observed 117 (34.01%) patients in grade A3 and 102

(29.65%) in stage F1; this finding is also observed by other workers[14] in grading, but some researchers show majority of cases in grade A0 followed by A1[15], and majority of cases in stage F1[14] and F0[15] followed by stage F2[14] and F0[15].

In our study no biopsy specimen revealed necro-inflammatory grade 0; and 14 (4.06%) cases were in stage F0. This is controversial with other workers who observed majority of cases in grade A0 and stage F0[15], Some studies in other parts of the world also observed cases in grade A0[14,16] and F0[17].

In this study we found 38 (11.04%) cases presented with grade A4 and 30 (8.72%) cases in stage F4, while a study in Islamabad shows majority of cases in stage F4 and F2[18]. This observation was also controversial with other studies, conducted at different parts of the world, who observed no any case in grade A4 and least cases in stage F4,[14,15] while we observed least cases in stage F0.

The controversies in the observation of grading and staging in our study and other studies conducted in developed countries may be multifactorial. As the role of HCV genotype is documented in the pathogenesis and progression of disease[19], so these may be linked with HCV genotyping. The other factors involved seems to be illiteracy and poverty, as the current study was conducted in interior of Sindh province of Pakistan where majority of population who resides in rural areas are poor and illiterate having little knowledge about the risk factors and also they come for medical assessment at a late stage. The third factor in this regard is treatment by quacks, as qualified medical graduate doctors are seldom present in villages of Sindh, and these helpless poor peoples are bound to get medical assessment from the quacks, who instead of treating the patients are the strong source of transmitting the HCV infection by reuse of syringes, frequent use of injections and drips, and using multi-dose injection vials.

CONCLUSION

In current study we observed majority of cases in A2, A3 grade of inflammation and F1, F2, F3 stages of fibrosis, which indicates that patients came late for medical assessment, which may cause difficulties in management. This warrants the necessity of HCV awareness programs in rural areas of Sindh.

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