

# LONG-TERM EFFECT OF INTERFERON THERAPY ON INCIDENCE OF CIRRHOSIS AND HEPATOCELLULAR CARCINOMA IN THAI PATIENTS WITH CHRONIC HEPATITIS B

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**Abstract.** The aim of this study was to assess the long-term effects of interferon (IFN) therapy on the incidence of disease progression to cirrhosis and hepatocellular carcinoma (HCC) in Thai patients with chronic hepatitis B. Sixty-seven patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B who received IFN therapy were retrospectively analyzed. The average duration of follow-up was 59.4±30.9 months (ranging from 20 to 119 months). Seventy-two untreated patients with comparable clinical data and mean duration of follow-up served as a control group. During follow-up, 24 (35.8%) treated and 7 (9.7%) untreated patients had a sustained loss of HBeAg. However, none of the treated patients or controls became negative for hepatitis B s antigen (HBsAg). Among treated patients, the response was independent of type and dose of IFN, as well as the presence of steroid priming. In addition, 1 of 24 (4.2%) sustained responders and 6 of 43 (14%) non-responders progressed to cirrhosis whereas 16 of 72 (22.2%) in the control group progressed to such sequelae. The overall incidence of new cirrhosis in sustained responders was significantly lower than in the control group ( $p=0.04$ ). HCC appeared in 11 cirrhotic patients: 9 (12.5%) in the control group and 2 (4.7%) of the non-responders, whereas none of the sustained responders developed HCC. The average period to detection of HCC was 70.5±12.4 months for non-responders and 65.3±27.6 months for the control group, with no significant differences between these groups. In conclusion, our data suggest that IFN therapy might prevent the progression of cirrhosis and the development of HCC in patients with chronic hepatitis B. These beneficial effects were particularly observed in those who achieved a sustained virological response to treatment.

## INTRODUCTION

Hepatitis B virus (HBV) infection is considered a leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) in Southeast Asia, China and sub-Saharan Africa (Lee, 1997). Currently, interferon (IFN) is widely used for treatment of chronic HBV infection in most countries. The aims of IFN therapy are to suppress or eradicate HBV, to diminish necroinflammation and to reduce the risk of cirrhosis and HCC (Perrillo, 1993). Among patients treated for 3-6 months, its therapeutic

efficacy rate is approximately 30-40% in terms of virological and histological remission (Wong *et al*, 1993; Hoofnagle and DiBisceglie, 1997). Furthermore, previous studies have suggested that loss of HBeAg in chronic HBV infection, whether due to IFN therapy or occurring spontaneously, is associated with improved clinical outcome (Niederau *et al*, 1996; Lau *et al*, 1997; Fattovich *et al*, 1997). However, it is still unclear whether IFN therapy could prevent the development of cirrhosis and HCC in patients with chronic hepatitis B. Thus, the main aim of the current study was to evaluate retrospectively the long-term beneficial effects of IFN therapy on the incidence of cirrhosis and HCC among Thai patients with chronic hepatitis B.

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## PATIENTS AND METHODS

**Patients receiving IFN therapy**

All adult hepatitis B e antigen (HBeAg)-positive patients having received IFN therapy in King Chulalongkorn Memorial Hospital and followed for at least 18 months after completion of treatment were analyzed retrospectively and included in this study. Patients with antibodies against hepatitis C virus (HCV) or human immunodeficiency virus (HIV) and patients with advanced cirrhosis (Child class B or C) were excluded. There were 67 patients (53 men and 14 women with a mean age of  $36.9 \pm 10.5$  years) treated with IFN between 1989 and 1997 who fulfilled these criteria. Most patients included in this study underwent liver biopsy prior to IFN treatment. Ultrasonography was also performed before commencing therapy to document the absence of space-occupying lesions suggesting HCC.

As for types and doses of IFN, 44 patients received 3 to 5 million units (MU) of interferon alfa-2b (Intron A, Schering-Plough Corporation) thrice weekly for 20 to 24 weeks (total dose 180 to 360 MU) (5 cases with prednisolone priming); 18 patients received 3 to 6 MU interferon alfa-2a (Roferon A, Roche) thrice weekly for 20 to 24 weeks (total dose 180 to 432 MU); 5 patients received 3 to 5 MU of human lymphoblastoid interferon (alfa-n1, Wellferon, Wellcome) thrice weekly for 20 to 24 weeks (total dose 180 to 360 MU). Of these patients, approximately 97% (65 of 67 patients) showed varied degrees of fever, chills and general malaise after the first injection of IFN. In addition, most patients were found with varying degrees of leukocytopenia and thrombocytopenia, and some patients required a reduction of IFN dosage. However, no patients discontinued the long term IFN therapy due to its serious side effects, which included depression, psychosis or severe bone marrow suppression.

The majority of patients were followed up in 2-4-week intervals during treatment and 6-12-month intervals or more frequently when indicated after completion of treatment. Peri-

odic evaluations included clinical assessment, conventional blood biochemical tests [blood cell counts, serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT)] and serological HBV markers (HBsAg, anti-HBs, HBeAg and anti-HBe, Auszyme, Abbott Laboratories, North Chicago, IL, USA). To screen for HCC, serum alpha-fetoprotein (AFP) and ultrasonography were performed one or two times per year with the majority of patients. HCC was diagnosed based on liver tumor features detected by imaging studies and confirmed by histology and/or AFP levels above 400 IU/ml. The development of new cirrhosis was diagnosed by clinical data (presence of esophageal varices, encephalopathy, ascites), histology or imaging features suggestive of cirrhosis.

Initial virological response to IFN was assessed in relation to the rates of seroconversion to anti-HBe within 12 months after IFN discontinuation. A sustained response was defined as the maintenance of a response throughout the follow-up period. Patients undergoing relapse had reappearance of HBeAg after an initial response. Patients with persistently detectable HBeAg during and after completion of IFN treatment were considered non-responders.

**Control group**

A comparison group comprised 72 untreated chronic hepatitis B and/or compensated cirrhosis (Child class A) patients who had been serum HBeAg-positive at the initial diagnosis (from 1989 to 1997) and had been followed-up for at least 24 months until the end of the observation period, which was updated to September 1999 or until the first day when HCC was detected. These patients were enrolled in the study at the first medical examination and were cancer-free for at least 12 months since enrolment. The reasons for not being treated were refusal of treatment or contraindications to IFN therapy for other reasons such as concomitant diseases or economic problems. The majority of the untreated patients were monitored as the treated cases in terms of number and intervals between

visits, blood tests and ultrasonography performed.

### Statistic analysis

All results are expressed as mean  $\pm$  SD. Comparisons between groups were made by the  $\chi^2$  or Fisher's exact test for categorical variables and by the Mann-Whitney test or Student's *t*-test when appropriate for quantitative variables. A *p*-value below 0.05 was considered significant.

## RESULTS

Table 1 summarizes the baseline clinical data of the treated patients at the time of initial therapy with IFN and those of untreated cases at the time of diagnosis of chronic hepatitis B. Among those receiving IFN therapy, mean serum AST and ALT were significantly higher compared to those without treatment (*p*=0.003 and 0.001, respectively). However, there were no significant differences between the two groups regarding age, sex, known duration of HBsAg positivity, the presence of preexisting cirrhosis, and other baseline biochemical liver function tests.

Twenty-seven (40.3%) of IFN-treated

patients showed an initial therapeutic response. Of those with initial response, 5 patients developed HBV reactivation with reappearance of HBeAg; 3 patients in the second year and the remaining two patients in the third year of the follow-up period. Another two initial responders displayed transient elevation of ALT levels, but without HBeAg reactivation, in the second year. In addition, during the mean follow-up period of 59.4 $\pm$ 30.9 months, late response developed in 2 of 40 (5%) initial non-responders within 3 years after IFN discontinuation. Therefore, the overall sustained response rate in IFN-treated patients was 35.8% (24 of 67 patients). In contrast, spontaneous seroconversion of HBeAg in untreated controls was found in 7 of 72 (9.7%) which is significantly lower than in the treated group (*p*<0.001). In our study, none of the treated patients or controls became negative for HBsAg.

The incidence of progression to new cirrhosis among patients with non-cirrhosis at entry was compared between treated patients and the control group. In the treated group, 1 of 24 (4.2%) sustained responders and 6 of 43 (14%) non-responders progressed to cirrhosis, whereas 16 of 72 (22.2%) in the control group progressed to such sequelae. The overall incidence of new cirrhosis in sustained responders was significantly lower than with

Table 1  
Baseline clinical characteristics of the patients at entry.

Characteristics	Interferon treated	Untreated	<i>p</i> -value
Number of patients	67	72	
Sex (M/F)	53/14	47/25	NS
Age (year)	36.9 $\pm$ 10.5	39.9 $\pm$ 13.7	NS
Known duration of HBsAg(+) (year)	5.7 $\pm$ 4.1	6.3 $\pm$ 4.9	NS
Known cirrhosis	12 (17.9%)	16 (22.2%)	NS
Duration of follow-up (month)	59.4 $\pm$ 30.9	60.1 $\pm$ 35.3	NS
Laboratory data			
Bilirubin (mg/dl)	0.7 $\pm$ 0.3	0.8 $\pm$ 0.4	NS
AST (IU/l)	114.1 $\pm$ 94.7	68.8 $\pm$ 58.1	0.003
ALT (IU/l)	180.7 $\pm$ 137.9	93.3 $\pm$ 114.4	0.001
ALP (IU/l)	196.5 $\pm$ 76.5	197.7 $\pm$ 114.3	NS
Albumin (g/dl)	4.7 $\pm$ 0.4	4.5 $\pm$ 0.7	NS
Prothrombin time (second)	12.8 $\pm$ 1.2	13.8 $\pm$ 2.1	NS

Table 2  
Clinical characteristics and outcomes of patients at the end of follow-up.

Characteristics	Interferon treated		Untreated
	Sustained response	Non-sustained response	
Number of patients	24	43	72
Sex (M/F)	20/4	33/10	47/25
Age (year)	37.0±13.3	35.8±9.9	39.9±13.7
Known cirrhosis at entry	4 (16.7%)	8 (18.6%)	16 (22.2%)
Pretreatment level of ALT (IU/l)	204.3±121.6	134.5±84.3 <sup>b</sup>	
Pretreatment level of HBV DNA (Meq) <sup>a</sup>	125.1±168.4	165.8±281.5	
IFN type (Intron/Roferon/Wellferon)	18/4/2	26/14/3	
IFN total dose	322.5±75.4	335±87.9	
Steroid priming prior to IFN	2 (8.3%)	3 (6.9%)	
HBeAg seroconversion	24 (100%)	0 (0%) <sup>b</sup>	7 (9.7%) <sup>b</sup>
New cirrhosis	1 (4.2%)	6 (14%)	16 (22.2%) <sup>b</sup>
HCC	0 (0%)	2 (4.7%)	9 (12.5%)

<sup>a</sup>Measurement by branched DNA signal amplification assay.

Data were presented as mean ± standard deviation.

<sup>b</sup>Statistical analysis compared to sustained response group:  $p < 0.05$ .

the controls ( $p=0.04$ ). In addition, HCC appeared in 11 cirrhotic patients during the follow-up period: 9 (12.5%) in the control group and 2 (4.7%) in the non-responders, whereas none of the sustained responders developed HCC (Table 2). The average time lapse until detection of HCC was  $70.5 \pm 12.4$  months for non-responders and  $65.3 \pm 27.6$  months for the control group, with no significant differences between both groups ( $p > 0.05$ ).

## DISCUSSION

Chronic HBV infection is still a global health problem that can lead to significant morbidity and increased mortality in infected individuals. Previous studies on its natural history have shown that ongoing HBV replication and liver inflammation are associated with unfavorable progression of the disease such as cirrhosis, end-stage liver disease and the development of HCC (Fattovich, 1996; Lee 1997). Thus, it is reasonable to speculate that a sustained virus suppression before irreversible liver damage could result in decreasing risks of such HBV-related complica-

tions. To achieve these ends a wide variety of antiviral and immunomodulatory therapies have been evaluated in the past decades. Among these, IFN was the first therapy approved for chronic hepatitis B in most countries. The principal mechanisms of action of IFN are thought to include antiviral, antiproliferative and immunomodulatory effects (Peters, 1989). Currently, although other antiviral agents such as nucleoside analogs are being extensively studied, IFN continues to play an important role in the treatment of chronic hepatitis B (Malik and Lee, 2000).

In the present study, long term effects of IFN therapy in Thai patients with chronic hepatitis B was assessed retrospectively, therefore, some degree of bias could not be avoided. In spite of these limitations, the observations in our study are of interest. Our data demonstrated that IFN therapy accelerated HBeAg clearance in initially HBeAg-positive patients to an approximately 25% higher than in cases of spontaneous HBeAg seroconversion (35% and 10%, respectively). These data were consistent with the results of a meta-analysis in which the seroconversion rates of HBeAg in patients treated with IFN and controls were

approximately 33% and 12%, respectively (Wong *et al*, 1993). Indeed, clearance of HBeAg, a marker of active viral replication, is one of the initial goals of antiviral therapy for chronic hepatitis B. Following initial clearance of HBeAg, the subsequent loss of HBsAg is expected as a long-term consequence of successful IFN treatment. However, such clearance of HBsAg was not detected in our study. This very low overall loss of HBsAg was in accordance to that observed among Chinese populations (Lok *et al*, 1993; Lin *et al*, 1999) but contrasting that reported from Western countries where the percentage of HBsAg clearance varied from 19-65% (Korenman *et al*, 1991; Carreno *et al*, 1992; Niederau *et al*, 1996). The exact reasons for these discrepancies among studies are unclear but may in part be related to immune tolerance induced by the acquisition of HBV infection during early childhood among Asian populations (Lok *et al*, 1993).

Retrospective multivariate analyses have indicated that low pretreatment HBV DNA levels, high ALT levels and evidence of active necroinflammation on liver biopsy are independent variables predicting a favorable response to therapy (Lee, 1997; Hoofnagle and Di Bisceglie, 1997). In our study, however, we did not demonstrate that initial levels of HBV DNA correlated with a beneficial response. Furthermore, use of steroid priming before IFN therapy did not result in significantly higher rates of HBeAg clearance. In fact, the benefit of steroid priming for treatment of chronic hepatitis B is still controversial. Most of the randomized trials revealed no difference between either presence or absence of steroid priming, although it may be useful in a subpopulation of chronic hepatitis B patients (Yokosuka, 2000). It also has been suggested that differences in IFN type and total dose significantly influence the response rate of chronic hepatitis B patients (Pestka *et al*, 1987). Nonetheless, our data showed that the differences in IFN type had no effect on the outcome of therapy. This observation was consistent with recent data on the long term response rate to IFN therapy to be independent of type,

dose or treatment regimens (Oliveri *et al*, 1999).

As previously mentioned, one major issue concerning the efficacy of IFN is whether treatment can modify the natural history of chronic hepatitis B. In this respect, the benefit of IFN therapy in prolonging survival in HBeAg-positive patients was first suggested by Wong *et al* using a mathematical simulation model (Wong *et al*, 1993), and subsequently confirmed by Niederau *et al* (1996) in a cohort of 103 treated patients. In the latter study, it was demonstrated that cumulative survival was significantly higher among patients in whom HBeAg was eliminated than among those who remained seropositive (100% vs 70% at six year). Furthermore, the frequency of severe clinical complications due to cirrhosis was significantly lower among the responders (Niederau *et al*, 1996). Indeed, this end point of reducing cirrhosis development could also be observed in our series. During the long-term follow up period, one of 24 (4.2%) patients who cleared HBeAg subsequently developed new cirrhosis compared with 6 of 43 (14%) patients with persistent HBeAg positivity, and 16 of 72 (22.2%) untreated cases. In addition, it is interesting to note that the frequency of pretreatment cirrhosis was comparable between responders and non-responders. This observation suggests that for the individual patient with chronic hepatitis B, the presence of compensated cirrhosis is not a major predictive factor for non-response to IFN treatment.

Moreover, our data suggest that the incidence of HCC tends to decrease among patients responsive to IFN therapy, supporting the concept that IFN might prevent hepatocarcinogenesis in chronic hepatitis B patients. Such an effect of IFN therapy was also demonstrated in recent non-randomized controlled trials. (Oon, 1992; Mazzella *et al*, 1996; Fattovich *et al*, 1997; Ikeda *et al*, 1998). Furthermore, Lin *et al* (1999) have recently conducted a randomized controlled trial to confirm the long-term effects of IFN therapy on HCC prevention in Taiwanese patients with chronic hepatitis B. In that study, 67 patients received IFN and 34 patients received pla-

cebo. Follow-up at 1.1 to 11.5 years after the end of therapy showed that the cumulative incidence of HCC was significantly higher in the control group compared to the treated group. In addition, IFN therapy could significantly prolong the survival in treated patients when compared to the control subjects. On multivariate analysis, it was shown that IFN therapy, preexisting cirrhosis and the patient's age were significant independent factors affecting both survival and HCC development. These predictive factors were also demonstrated in a recent study in Italy during which patients with chronic hepatitis B were observed for almost 8 years (Di Marco *et al*, 1999).

How IFN might reduce the incidence of HCC in patients with chronic hepatitis B is unknown. Indeed, IFN displays many immunological and biological functions such as inhibition of cell division via transcription factors, interferon regulatory factor (IRF), growth inhibitory actions through changes in signal transduction and activation of natural killer cells, resulting in tumor suppression (Swaminathan *et al*, 1992; Harada *et al*, 1993). Therefore, it is possible that IFN has direct effects on hepatocarcinogenesis as demonstrated in a randomized controlled trial performed on patients with advanced HCC (Lai *et al*, 1993). Also, it is noteworthy that the greatest reduction in risk for HCC development was observed in those with a virological response to treatment (Baffis *et al*, 1999). Such observations were confirmed in our study in that only non-responders and untreated patients were at risk of HCC development during the follow-up period. Taking into account that hepatocarcinogenesis in HBV-associated cirrhosis is accelerated by sustained necroinflammation of hepatocytes, it is speculated that IFN therapy may also diminish the risk of HCC indirectly through suppression of HBV replication (Ikeda *et al*, 1998). However, HBV-induced hepatocarcinogenesis has been attributed to a variety of mechanisms, including HBV integration into the host genome leading to genetic changes and subsequent tumor development (Okuda, 1992). In addition, the

viral 'X' gene encodes a transactivating protein that may promote hepatocyte transformation via up-regulation of cellular proto-oncogenes and inactivation of the *p53* tumor suppressor gene (Henkler and Koshy, 1996). Thus with HBV infection, both early cellular genetic alterations and the later development of cirrhosis are implicated in the formation of HCC. In this respect, further studies are necessary to elucidate the potential mechanisms and roles of IFN as to hepatocarcinogenesis in chronic HBV infection.

In conclusion, our data suggest that IFN therapy might prevent the progression of cirrhosis and the development of HCC in patients with chronic hepatitis B. These beneficial effects were particularly observed in those who achieved a sustained virological response to treatment. Nonetheless, additional studies are still required to confirm the long-term effects of IFN therapy on eventual clinical outcomes, particularly in terms of prevention of HCC and prolonged survival in patients with chronic hepatitis B.

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