DOI: http://dx.doi.org/10.18203/issn.0019-5359.IndianJMedSci20170484

Viral Infections Original Article

Effect of indigenous interferon - alpha on Hepatitis B virus deoxyribonucleic acid level in hepatitis b e antigen-positive chronic Hepatitis B patients

Pravin M. Rathi, Samit S. Jain

ABSTRACT

Background and Objective: HBeAg-positive chronic hepatitis B patients have high serum HBV DNA level showing high viral replication. Goal of treatment of hepatitis B is to prevent cirrhosis, hepatic failure and hepatocellular carcinoma by serum alanine transaminase (ALT) normalization, decrease in serum hepatitis B virus (HBV) deoxyribonucleic acid (DNA) and loss in hepatitis B e antigen (HBeAg). Interferons (IFNs) have antiviral, anti-proliferative, and immunomodulatory effects. IFN-α is effective in suppressing HBV replication and in inducing remission of liver disease. **Materials and Methods:** In this prospective, single treatment arm study, HBeAg-positive chronic hepatitis patients without decompensated liver disease were enrolled to receive indigenous recombinant IFN-α 2b in the dose of 5 MU daily for 6 days a week subcutaneously for 16 weeks. Quantitative HBV-DNA, HBeAg, and hepatitis B surface antigen (HBsAg) were assessed at baseline and at the end of treatment. ALT level assessment was done at baseline and during therapy at week 1, week 2, week 8, week 12, and week 16. **Results:** Out of 37 patients enrolled in the study, 8 patients (21.62%) did not complete study due to lost to follow-up (3 patients), discontinuation due to adverse event (3 patients), and consent withdrawal (2 patients). Among 29 patients who completed the study, 10 patients (34.48%) had clearance of HBeAg and 1 patient (3.44%) had lost HBsAg after 16 weeks of therapy. Mean ALT level started decreasing after 4 weeks of therapy but did not come to normal range till 16 weeks of therapy. At least 2 log decreases in HBV DNA was observed in 9 (31.03%) patients and at least 1 log decrease in 18 (62.06%) patients. Overall decline in HBV DNA level was observed in 62% patients after 16 weeks of therapy. **Conclusion:** IFN-α treatment does result in HBeAg and HBsAg loss and decreases HBV-DNA levels in chronic hepatitis B patients. Most of adverse events were mild to moderate in intensity. So, interferon-α therapy was well tolerated, safe, and efficacious to treat HBeAg-positive chronic hepatitis B patients without decompensated liver disease.

Key words: *Alanine transaminase level, chronic hepatitis B, hepatitis B virus deoxyribonucleic acid level, interferon-α, HBeAg-positive*

INTRODUCTION

Hepatitis B is a potentially life-threatening viral infection of the liver. Hepatitis B virus (HBV) is a deoxyribonucleic acid (DNA) virus with compact genome that primarily interferes with the functions of liver by replicating in hepatocytes. Worldwide, an estimated two billion people have been infected with the HBV, and more than 350 million have chronic infection.[1] It is a major health problem in India with average estimated carrier rate of 4.0%, with a total pool of approximately 36 million carriers.[2] Professional blood donors have HBsAg positivity rate of 14%. HBV is reported to be responsible for 70% of cases of chronic hepatitis and 80% of cases of cirrhosis of the liver.[2]

HBV transmission results from exposure to infectious blood or body fluids. Possible modes of transmission are unprotected sex, blood transfusion, contaminated needles and syringes, and vertical transmission during childbirth.[3] The risk of vertical transmission is high if mother is hepatitis B e antigen (HBeAg)-positive. HBV can also survive outside the body for prolonged period.[4,5]

After infection, the first virologic marker detectable in serum is hepatitis B surface antigen (HBsAg) that precedes elevation of serum aminotransferase activity and clinical symptoms. The most essential factor in patients with chronic hepatitis B is degree of HBV replication. Chronic HBV infection with ongoing viral replication is indicated by the presence of HBeAg and high HBV DNA level in serum. Many antiviral and immunomodulatory drugs have been tried, interferon alpha IFN-α in particular have been studied extensively. Recombinant IFN-α 2b increases the rate of elimination of HBeAg from low rate of spontaneous clearance of 5-10% without treatment to between 30-50% with treatment.[6] Seroconversion from HBeAg to anti-HBeAg antibodies leads to disappearance of serum HBV DNA level.[7-9]

MATERIALS AND METHODS

The study was prospective, open-label, single arm with an objective of to evaluate the safety and efficacy IFN-α 5MU daily for 6 days a week for 16 weeks subcutaneously in patients with chronic hepatitis B. The study was conducted from May 2006 to February 2007 B.Y.L. Nair Hospital, Mumbai, India. Patients were included in the study with following inclusion/exclusion criteria:

Main inclusion criteria

(1) Male or female patients above 18 years of age diagnosed with chronic hepatitis B. (2) Seroreactive for HBsAg for at least 6 months. (3) Seroreactive for HBeAg and serum aminotransferase >1.5 × upper limit normal. (4) Patients of chronic hepatitis with compensated liver disease with or without cirrhosis of liver. (5) white blood cell >3000/mm^3, neutrophil count >1500 mm^3 and platelet count >75000/mm^3.

Main exclusion criteria

(1) Abnormal levels of thyroid-stimulating hormone and T4. (2) Individuals infected with hepatitis A, C, D, and human immunodeficiency virus. (3) History of depression, suicidal ideation, suicidal attempt, and/or autoimmune disorders. (4) History of diabetes, seizure disorders, severe cardiac

Department of Gastroenterology, Topiwala Nair Medical College and BYL Nair Charitable Hospital, Mumbai, Maharashtra, India

Address for correspondence:
Dr. Pravin M. Rathi,
Department of Gastroenterology, Topiwala Nair Medical College and BYL Nair Charitable Hospital, Dr. AL Nair Road, Mumbai Central, Mumbai - 400 008, Maharashtra, India.
E-mail: rathipmpp@gmail.com

disease, retinopathy, cancer, renal disease. (5) History of hepatic encephalopathy, variceal bleeding, ascites, or other clinical signs of decompensation. Patients previously treated with IFNs.

Patients satisfying the eligibility criteria were enrolled in the study. Patients at each site were enrolled only after receiving approval from the respective institutional ethics committee and from regulatory authorities. All patients gave written informed consent prior to enrollment in the study.

Each of the patients enrolled on the study received recombinant IFN-α 2b (*ReliFeron*™) in the dose of 5MU daily for 6 days a week subcutaneously for 16 weeks. Quantitative HBV-DNA, HBeAg, and HBsAg were assessed at baseline and at the end of treatment. Alanine transaminase (ALT) level assessment was done at baseline and during therapy at week 1, week 2, week 8, week 12, and week 16.

HBV DNA purification, polymerase chain reaction (PCR) amplification, and quantification

4-5 ml blood samples collected in EDTA vacutainers or in plain vacutainers for serum. DNA was extracted from plasma of the patient samples using Qiagen RNA extraction kit.

Quantitative HBV PCR assay in pre-X region of the virus, serial dilutions of samples for viral load estimation was done. The lower limit of detection of the virus was 250 IU/ml (1250 copies/ml), and the assay sensitivity was 97-99%.

PCR was used to amplify pre-X region of HBV virus in the sample, using four serial dilutions (including original) of the DNA. HBV-specific primers were used to amplify the sample DNAs and subject to agarose electrophoresis. The intensity of the PCR products in comparison to defined quantitative ladder was used to quantitate HBV DNA in the samples. Additionally, quantitated positive control, negative control, and reaction control were used to validate the assay.

Statistical analysis

Data processing, tabulation of descriptive statistics, calculation of inferential statistics were performed primarily using SAS Version 9.1 for Windows. The HBV DNA and other laboratory values were expressed as mean, median, and percentage. Log change in HBV DNA value was tabulated in terms of counts and proportions along with 95% confidence interval.

Patient disposition and demography

Subjects were enrolled in single tertiary center. Thirty-seven subjects enrolled at baseline were qualified for this analysis. There were 31 (83.78%) male subjects and 6 (16.21%) female subjects in the study. The mean age of patients was 30 years, while mean body mass index was 21.04 kg/m^2 at baseline [Table 1].

At baseline, all patients were HBsAg and HBeAg-positive. Before start of therapy, serum HBV DNA was measured in all patients. In 3 (8.10%) patients, HBV DNA was not detected at baseline. Fourteen (37.83%) patients had >10^7 copies/ml of HBV DNA, and 19 (51.35%) patients had HBV DNA level between 10^4 and 10^7 copies per ml. Mean ALT level in 37 patients at baseline was 112.48 U/L ranging from 44.00 to 424.00 U/L [Table 2].

RESULTS

Efficacy results

Out of 37 HBeAg and HBsAg-positive patients enrolled in the study, 8 (21.62%) patients did not complete study due to lost to follow-up (3), discontinued due to adverse event (3), and consent withdrawal (2). Among 29 patients treated with IFN-α, 10 (34.48%) patients had clearance of HBeAg and 1 (3.44%) patient had lost HBsAg after 16 weeks of therapy [Table 3]. IFN-α-induced HBeAg clearance has been reported to be durable in 80% to 90% of patients after long-term follow-up of 4 to 8 years.[10-17] However, HBV DNA remains detectable in serum in most of the patients. Studies comparing the outcome of responders versus non-responders found that patient who cleared HBeAg had better overall survival and survival free of hepatic de-compensation.[10,11,13,18]

Serum HBV DNA was assessed at baseline and end of therapy (16 weeks). After therapy, only 6 (20.68%) patients had HBV DNA level >10^7 copies/ml as compared to 14 (37.83%) patients at baseline. Two log decrease in serum HBV DNA was observed in 9 (31.03%) patients (95% CI: 0.14, 0.48) and 1 log decrease in 9 (31.03%) patients. Overall, 18 (62.02%) patients showed decrease in serum HBV DNA level after 16 weeks of therapy. There were 6 (20.68%) patients in which no log change in HBV DNA level was seen and in 5 (17.24%) patients, serum HBV DNA level was found elevated after therapy from baseline [Table 4].

Liver cirrhosis and hepatocellular carcinoma are squealae of persistent and uncontrolled replication of HBV in the liver.[19,20] Since serum HBV DNA level is the direct reflection of intrahepatic viral replication, IFN-α-therapy have prevented hepatic complications by arresting viral replication.

ALT level assessment was done at baseline and during therapy at week 1, week 2, week 8, week 12, and week 16. Mean ALT level started decreasing after 4 weeks of therapy but remained out of range until 16 weeks of therapy (Graph 1).

Safety results

In the study of 37 patients, a total of 18 (48.64%) subjects in the study experienced adverse events. Out of which, 10 (27.02%) subjects had at least one adverse event related to the study drug. Three subjects were discontinued due to adverse events. For the known IFN-related adverse events, 7 (18.91%) subjects had pyrexia, 2 (5.40%) subjects had myalgia, and 2 (5.40%) subjects had headache. There were 4 (10.81%)

Table 1: Demography and baseline characteristics

Variable	*Interferon alpha*
Age	
N	37
Mean	30.0
Median	26.0
Range	(18.00, 64.00)
Gender	
Male	31 (83.78%)
Female	6 (16.21%)
BMI	
N	37
Mean	21.04
Median	20.41
Range	(16.98, 28.13)

Table 2: Baseline HBV DNA and ALT level

Variable	*HBeAg positive N (%)*
HBV DNA	
N	37 (100.00)
Below detectable limit (<1250 copies/ml)	3 (8.10)
10^3 to 10^4 copies/ml	0 (0.00)
10^4 to 10^5 copies/ml	1 (2.70)
10^5 to 10^6 copies/ml	7 (18.91)
10^6 to 10^7 copies/ml	12 (32.43)
>10^7 copies/ml	14 (37.83)
ALT level	
N	37
Mean	112.48
Median	96
Range	(44.00, 424.00)

HBV= Hepatitis B virus, DNA=Deoxyribonucleic acid, HBeAg=Hepatitis B e antigen, ALT= Alanine transaminase

Table 3: Clearance of HBeAg and HBsAg

	Baseline	*After therapy (at 16 weeks)*
N	37	29
HBsAg positive	37 (100.00%)	28 (96.55)
HBsAg negative	0 (00.00%)	1 (3.44)
HBeAg positive	37 (100.00%)	19 (65.51)
HBeAg negative	0 (00.00%)	10 (34.48)

HBeAg=Hepatitis B e antigen, HBsAg=Hepatitis B surface antigen

Table 4: Post therapy HBV DNA analysis

Variable	*HBeAg reactive N (%)*	
HBV DNA		
N	29 (100.00)	
Below detectable limit (<1250 copies/ml)	5 (17.24)	
10^3 to 10^4 copies/ml	3 (10.34)	
10^4 to 10^5 copies/ml	2 (6.89)	
10^5 to 10^6 copies/ml	7 (24.13)	
10^6 to 10^7 copies/ml	6 (20.68)	
>10^7 copies/ml	6 (20.68)	
	95% CI	
Log change		
N	29 (100.00)	
In HBV	9 (31.03)	(0.14, 0.48)
≥2 log decrease	9 (31.03)	(0.14, 0.48)
DNA		
1 log decrease	9 (31.03)	(0.14, 0.48)
No log change	6 (20.68)	(0.06, 0.35)
At least one log increase	5 (17.24)	(0.03, 0.31)

DNA=Deoxyribonucleic acid, HBV= Hepatitis B virus, HBeAg=Hepatitis B e antigen

subjects with at least one adverse event, of which 2 patients were discontinued from study due to event. The reason for seriousness was hospitalization [Table 5].

DISCUSSION

Chronic hepatitis B has transmission capacity, leads to high morbidity and mortality in the community. The intention of treatment of chronic hepatitis B is to achieve sustained suppression of HBV replication and remission of liver disease through ALT normalization, decrease in serum HBV DNA level, and loss of HBeAg. IFNs have antiviral, anti-proliferative, and immunomodulatory effects and effective in suppressing HBV replication and in inducing remission of liver disease.[21-27]

Many clinical studies have shown that IFN-α is effective to reduce the level of serological and virological markers in chronic hepatitis B patients.[21-26] This study was prospective, open-label, and conducted on Indian population. Thirty-seven patients were enrolled in the study, of which 29 patients completed the study. Analysis has been done on 29 patients as per protocol.

HBeAg is the most significant prognostic serological marker that coincides with high level of virus replication and reflects the presence of circulating intact virons and HBV DNA. The rate of elimination of HBeAg, HBsAg, and HBV DNA were markedly higher among the IFN-α - treated patients than untreated patient. IFN-induced HBeAg seroconversion resulted in 34.48% patients indicated that IFN-α therapy led in clearance of HBeAg and arrested the virus replication effectively and thus prevented the long-term severe complications. HBeAg seroconversion results are corresponding to other published studies [Table 6].

HBV DNA level assessment done at 16 weeks was an early time point to see the response on serum HBV DNA level, but our results indicate that IFN-α therapy has effectively cleared the HBV DNA after therapy. At least 2 log decreases was observed in 9 (31.03%) patients and at least 1 log decrease in 9 (31.03%) patients. Overall decline or response to IFN therapy on HBV DNA level was observed in 62% patients after 16 weeks of therapy. IFN-α therapy has prevented hepatic complications by arresting viral replication as serum HBV DNA level is the direct reflection of intrahepatic viral replication.

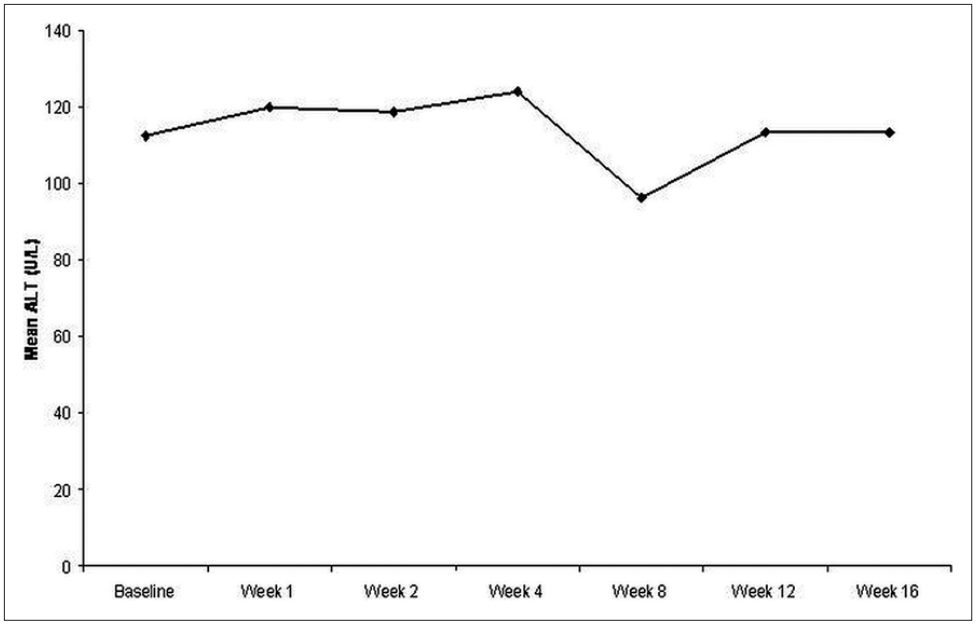
Table 5: Serious adverse events

Subject no.	*Age/sex*	*Adverse event term*	*Severity*	*Causality*	*Outcome*
3114	30/M	Respiratory infection	Moderate	Related	Resolved
3114	30/M	Neutropenia	Moderate	Related	Resolved
3301	56/M	Pneumonia	Severe	Unknown	Resolved
3508	58/M	Thrombocytopenia	Severe	Related	Resolved
3812	64/M	Suspected hepatocellular carcinoma	Mild	Unrelated	Not resolved

Table 6: Outcomes from controlled clinical studies

Study	*Treatment group (n)*				*Control group (n)*			
	Patients	*DNA*	*HBeAg*	*HBsAg*	*Patients*	*DNA*	*HBeAg*	*HBsAg*
RLS study	29	4	10	1	-	-	-	-
Williams *et al.*, 1990	23	9	9	3	7	2	2	0
Saracco *et al.*, 1989	33	26	23	8	31	15	12	1
Porres *et al.*, 1988	18	6	5	1	6	1	0	0
Muller *et al.*, 1990	30	9	9	1	28	3	3	0
Pastore *et al.*, 1988	14	8	8	0	14	4	2	0
Lok *et al.*, 1992	21	2	2	1	20	0	0	0

DNA=Number of patients that lost DNA, HBeAg=Number of patients that lost HBeAg, HBsAg=Number of patients that lost HBsAg, DNA=Deoxyribonucleic acid, HBeAg=Hepatitis B e antigen, HBsAg=Hepatitis B surface antigen



Graph 1: Mean ALT level during therapy

India spends only 5% annual gross domestic product (GDP) on health care.[28] Of this, most of the expenditure (about 80%) is private out-of-pocket. High out-of-pocket costs make health services inaccessible to a significant proportion of Indian households. Among those who decided not to seek medical care for an ailment, nearly 20% of urban and 28% rural households cited financial constraints as the limiting factor.[29] On considering the World Bank definition of poverty, according to estimates from the National Commission for Enterprises in the Unorganized Sector (NCEUS), 77% of Indians, i.e., about 836 million people, live on less than half a dollar a day.[30] In India, nearly 3.1 million additional households slip to levels below the poverty line ($1 per day) per annum as a result of hospitalization expenditure.[31] The total cost of the indigenous conventional IFN for duration of 16 weeks comes to approximately ₹ 92,000 to 95,000, whereas the cost of conventional IFN, marketed through multinational companies, is approximately ₹ 1,24,000 to 1,28,000. The per capita income at current prices during 2012-13 is estimated to be ₹ 68,747. Thus, patients can save approximately ₹ 30,000 to 33,000 on conventional IFN therapy. Even the Indian Supreme Court gave landmark judgment for promoting the generic drugs recently in the beginning of 2013.

Pegalyted-IFN has the advantages of more convenient administration, better pharmacokinetics, and more sustained viral suppression though clinical trials suggest that the efficacy of pegalyted-IFN is similar to or slightly better than standard IFN.

Our study has some limitations. First, the standard indigenous IFN has not been compared with any other IFN available in the market head to head. Second, the placebo arm was not there. Third, neither the study was blinded, nor the patients were randomized.

CONCLUSION

IFN-α treatment resulted in loss of HBeAg, HBsAg, and decreases serum HBV-DNA levels in chronic hepatitis B patients and thus prevented morbidity and mortality associated with disease. Most of adverse events were mild to moderate in intensity. So, IFN-α therapy is well tolerated, safe, and efficacious to treat HBeAg-positive chronic hepatitis B patients without decompensated liver disease.

ACKNOWLEDGMENTS

The authors thank Reliance Life Sciences for their valuable support. The authors also thank the Dean of T.N.M.C and B.Y.L. Nair hospital for allowing us to publish the paper.

REFERENCES

1. Hepatitis B. Available from http://www.who.int/mediacentre/factsheets/fs204/en/. [Last accessed on 2009 Nov 9]
2. Tandon BN, Acharya SK, Tandon A. Epidemiology of hepatitis B virus infection in India. Gut 1996:38 Suppl 2:S56-9.
3. Mast EE, Margolis HS, Fiore AE, Brink EW, Goldstein ST, Wang SA, *et al*. Advisory Committee onImmunization Practices (ACIP). A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: Recommendations of the advisory committee on immunization practices (ACIP) part 1: Immunization of infants, children, and adolescents. MMWR Recomm Rep 2005;54:1-31.
4. Bond WW, Favero MS, Petersen NJ, Gravelle CR, Ebert JW, Maynard JE. Survival of hepatitis B virus after drying and storage for one week. Lancet 1981;1:550-1.
5. Petersen NJ, Barrett DH, Bond WW, Berquist KR, Favero MS, Bender TR, *et al*. Hepatitis B surface antigen in saliva, impetiginous lesions, and the environment in two remote Alaskan villages. Appl Environ Microbiol 1976;32:572-4.
6. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positivechronic hepatitis B. A meta-analysis Ann Intern Med 1993;119:312-23.
7. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis 2005;25 Suppl 1:3-8.
8. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 1981;94:744-8.

9. Lok AS, Lai CL, Wu PC, Leung EK, Lam TS. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology 1987;92:1839-43.
10. Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, *et al*. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422-7.
11. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European concerted action on viral hepatitis (EUROHEP). Hepatology 1997;26:1338-42.
12. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971-5.
13. Lau DT, Everhart J, Kleiner DE, Park Y, Vergalla J, Schmid P, *et al*. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;105:1660-7.
14. Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. Gastroenterology 1993;105:1833-8.
15. Korenman J, Baker B, Waggoner J, verhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114:629-34.
16. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. J Viral Hepat 1998;5:389-97.
17. Carreño V, Castillo I, Molina J, Porres JC, Bartolomé J. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. J Hepatol 1992;15:102-6.
18. Van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Darwish Murad S, de Man RA, *et al*. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804-10.
19. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-45.
20. Evans AA, O'Connell AP, Pugh JC, Mason WS, Shen FM, Chen GC, *et al*. Geographic variation in viral load among hepatitis B carriers with differing risk of hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev 1998;7:559-65.
21. Williams SJ, Craig PI, Cooksley WG, Mason WS, Shen FM, Chen GC, *et al*. Randomised controlled trial of recombinant human inter-feron-oA for chronic active hepatitis B. Aust N Z J Med 1990;20:9-19.
22. Saracco G, Mazzella G, Rosina F, Cancellieri C, Lattore V, Raise E, *et al*. A controlled trial of human lymphoblastoid interferon in chronic hepatitis B in Italy. Hepatology 1989;10:336-41.
23. Porres JC, Carreño V, Mora I, Gutiez J, Moreno A, Ramon y Cajal S, *et al*. Different doses of recombinant alpha interferon in the treatment of chronic hepatitis B patients without antibodies against the human immunodeficiency virus. Hepatogastroenterology 1988;35:300-3.
24. Müller R, Baumgarten R, Markus R, Schulz M, Wittenberg H, Hintsche-Kilger B, *et al*. Treatment of chronic hepatitis B with interferon alfa-2b. J Hepatol 1990;11 Suppl 1:S137-40.
25. Pastore G, Santantonio T, Monno L, Milella M, Luchena N, Angarano G. Permanent inhibition of viral replication induced by low dosage of human leukocyte interferon in patients with chronic hepatitis B. Hepatogastroenterology 1988;35:57-61.
26. Lok AS, Wu PC, Lai CL, Lau JY, Leung EK, Wong LS, *et al*. A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. Gastroenterology1992;102:2091-7.
27. Chronic hepatitis B: Update 2009. Hepatology 2009;50:661-2.
28. Ministry of Health and Family Welfare. NationalHealth Accounts India. National Health Accounts Cell, Ministry of Health and Family Welfare. Government of India. New Delhi: 2005. p. 9.
29. National Sample Survey Organisation. Morbidity, Health Care and the Condition of the Aged. National Sample Survey Organization. Ministry of Statistics and Programme Implementation. Government of India. New Delhi: 2006. p. 19.
30. Report on conditions of work and promotion of livelihoods in the unorganised sector. National Commission for Enterprises in the Unorganised Sector. New Delhi: 2007. p. 1.
31. Van Doorslaer E, O'Donnell O, Rannan-Eliya RP, Somanathan A, Adhikari SR, Garg CC, *et al*. Effect of payments for health care on poverty estimates in 11 countries in Asia: An analysis of household survey data. Lancet 2006;368:1357-64.

How to cite this article: Rathi PM, Jain SS. Effect of indigenous interferon-alpha on hepatitis B virus deoxyribonucleic acid level in hepatitis B e antigen-positive chronic hepatitis B patients. Indian J Med Sci 2017;69:13-17.

Source of Support: Nil. **Conflict of Interest:** None declared.