EFFECT OF INDIGENOUS INTERFERON - ALPHA ON HEPATITIS B VIRUS DEOXYRIBONUCLEIC ACID LEVEL IN HEPATITIS B E ANTIGEN-POSITIVE CHRONIC HEPATITIS B PATIENTS

1Dr.Muhammad Bilal, 2Dr.Abdul Rauf, 3Dr.Arslan Ahmad Bakhsh
1Hamdard College Of Medicine &Dentistry, "Hamdard University Karachi"
2Avicenna Medical College Lahore UHS
3Hamdard College Of Medicine & Dentistry, "Hamdard University Karachi"

Abstract:
Background and Objective: HBsAg-positive patients of chronic hepatitis B, show high levels of serum HBV DNA and demonstrate higher levels of viral replication. Treatment of hepatitis B has a goal to achieve prevention of cirrhosis, hepatic failure and hepatocellular carcinoma by serum alanine transaminase (ALT) normalization, however, the 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 is effective in suppressing HBV replication and in inducing remission of liver disease. Materials and Methods: This is a prospective and single treatment results study, HBeAg-positive chronic hepatitis patients without decompensated liver disease were enrolled to receive indigenous recombinant IFN-α2b in the dose of 5 MU daily for 6 days a week subcutaneously for 16 weeks. The surface antigens (HBsAg), of Quantitative HBV-DNA, HBeAg, and hepatitis B, were assessed 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 included in our study initially, 8 patients (21.62%) failed to complete the study because they didn’t follow-up, some discontinued due to adverse events happened in their lives (3 patients), and some had withdrawn the consent (2 patients). 39 patients were the final participants of the study who have completed the follow-up, 10 patients (34.48%) had clearance of HBeAg and only one 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 two log decreases in HBV DNA was observed in 9 (31.03%) patients and at least one 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 treatment results in HBeAg and HBsAg loss and decreases HBV-DNA levels in chronic hepatitis B patients. Mild to moderate adverse effects were found in intensity. So, interferon-α therapy was well tolerated, safe, and efficacious in the treatment of HBeAg-positive chronic hepatitis B patients without decompensated liver disease.

Key words: Hepatitis B, hepatitis B virus deoxyribonucleic acid level, HBeAg-positive

Corresponding author:
Dr. Muhammad Bilal,
Hamdard College Of Medicine & Dentistry,
"Hamdard University Karachi"

Please cite this article in press Muhammad Bilal et al, Effect Of Indigenous Interferon - Alpha On Hepatitis B Virus Deoxyribonucleic Acid Level In Hepatitis B E Antigen-Positive Chronic Hepatitis B Patients., Indo Am. J. P. Sci, 2020: 07(09).
INTRODUCTION:
Hepatitis B is a high mortality viral infection that affects the liver. This virus directly attacks deoxyribonucleic acid (DNA) virus with compact genome. The function of this genome is primarily responsible for interferes with the functions of liver by replication in hepatocytes. An estimated two billion people around the globe have been infected with the HBV, and more than 350 million have chronic infection.[1] In the subcontinent, it’s a huge health problem with average estimated 4.0% carrier rate.[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.[1] 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. HBV replication is the one of the most important factors in patients affected with chronic hepatitis B. Chronic HBV infection with ongoing viral replication is indicated by the presence of HBeAg and high HBV DNA level in serum. Many antiviral and immunomodulators drugs have been tried, interferon alpha IFN-α in particular have been studied extensively. Recombinant IFN 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. Seroconversion from HBeAg to anti-HBeAg antibodies leads to disappearance of serum HBV DNA level. [7-9]

MATERIALS AND METHODS:
This is a prospective study, 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.

Main inclusion criteria
Patients were included in the study with following inclusion/exclusion 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 x upper limit normal.
(4) Patients of chronic hepatitis with compensated liver disease with or without cirrhosis of liver.
(5) white blood cell >3000/mm³, neutrophil count >1500 mm³ and platelet count >75000/mm³.

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 disease, retinopathy, cancer, renal disease. (5) History of hepatic encephalopathy, variceal bleeding, asciotes, 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 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 was 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/m² at baseline [Table 1].

At baseline, all patients were HBsAg and HBeAg-positive. Serum HBV DNA was measured in all patients before therapy. In 3 (8.10%) patients, HBV DNA was not detected at baseline. 14 (37.83%) patients had >10^7 copies/ml of HBV DNA, and nineteen (51.35%) patients had HBV DNA level between 10^4 and 10^7 copies per ml. ALT level in 37 patients at baseline was 112.48 U/L ranging from 44.00 to 424.00 U/L as a mean [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. 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.

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 squeal of persistent and uncontrolled replication of HBV in the liver. 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%) 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].
### Table 1: Demography and baseline characteristics

| Variable   | Interferon alpha |
|------------|------------------|
| Age        | 37               |
| 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) |
| 10^4 to 10^5 copies/ml | 0 (0.00) |
| 10^5 to 10^6 copies/ml | 1 (2.70) |
| 10^6 to 10^7 copies/ml | 7 (18.91) |
| >10^7 copies/ml | 12 (32.43) |
| 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          | 5 (17.24)            |
| limit (<1250 copies/ml)   |                      |
| 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 DNA                | 9 (31.03)            |
| 2 log decrease            | 9 (31.03)            |
| DNA                       | 1 log decrease       |
|                           | 9 (31.03)            |
| No log change             | 6 (20.68)            |
| At least one log increase | 5 (17.24)            |

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

Table 5: Serious adverse events

| Subject no. | Age/s ex | 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    | Mild     | Unrelated | Not resolved |
|             |          | carcinoma                   |          |           |         |

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-\[21\] 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 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-\[21\] treated patients than untreated patient. IFN-induced HBeAg seroconversion resulted in 34.48% patients indicated that IFN-\[21\] 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].
Table 6: Outcomes from controlled clinical studies

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

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 were 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.

Graph 1: Mean ALT level during therapy

India and Pakistan spend only 6% annual gross domestic product (GDP) on health care.[28] Of this, most of the expenditure (about 80%) is private. High out-of-pocket costs make health services inaccessible to a significant proportion of 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%, i.e., about 136 million people, live on less than half a dollar a day.[30] In Pakistan, nearly 3.1 million additional households
slip to levels below the poverty line ($1 per day) per annum as a result of hospitalization expenditure.\cite{31}

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-\alpha 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-\alpha therapy is well tolerated, safe, and efficacious to treat HBeAg-positive chronic hepatitis B patients without decompensated liver disease.

**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 on Immunization 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-positive chronic 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, Realidi 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. Krosggaard 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
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. Gastroenterology 1992;102:2091-7.

27. Chronic hepatitis B: Update 2009. Hepatology 2009;50:661-2.

28. Ministry of Health and Family Welfare. National Health Accounts Pakistan. National Health Accounts Cell, Ministry of Health. Government of Pakistan. 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: 2006. p. 19.

30. Report on conditions of work and promotion of livelihoods in the unorganized sector. National Commission for Enterprises in the Unorganized 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.