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Original Research Article

# Seroconversion and Seroprotection after Hepatitis B Vaccination using Recombinant Vaccine via Subcutaneous Route in Patients with Bleeding Diathesis.

**Mahamine Kaustubh[1], More Asmita[2], Gangurde Nita[3], Sharma Svasti[4], Vasa Manav[5], Singh Jeetendra[6]**

[1]Assistant Professor, Department of Medicine, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Adgaon, Nashik 422003, India.
[2]Assistant Professor, Department of Medicine, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Adgaon, Nashik 422003, India.
[3]Professor, Department of Microbiology, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Adgaon, Nashik 422003, India.
[4]Intern Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Adgaon, Nashik 422003, India
[5]Phase II MBBS, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Adgaon, Nashik 422003, India
[6]Professor, Department of Pharmacology and Therapeutics, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Adgaon, Nashik 422003, India

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**Corresponding author: Singh Jeetendra**
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**Abstract**

Patients of bleeding disorders are repeatedly exposed to blood and blood products and are thus at an increased risk of acquiring transfusion associated infection which like HIV, Hepatitis B and Hepatitis C. It is recommended that all patients of bleeding disorders should be vaccinated against hepatitis B virus as early as possible. Hepatitis B vaccine is conventionally administered via intramuscular route. Intramuscular injections are to be avoided in patients of bleeding disorders for the risk of bleeding and hematoma formation. It is advised to vaccinate them via subcutaneous route. Few studies have been done to study the efficacy of subcutaneous route.

The aim of this study was to measure the seroconversion and seroprotection after primary immunization with recombinant hepatitis B vaccine by subcutaneous route in patient with bleeding diathesis.

Cases and controls were screened for HBsAg, anti HBc (total) and anti HBs and ALT.

30 patients of bleeding disorders and 30 patients of were vaccinated against hepatitis B using recombinant vaccine via subcutaneous route and intramuscular route respectively at 0, 1, 6 month interval. HBsAg, anti HBc (total), ALT and anti HBs titre were estimated one month after every dose of vaccine that is at 1, 2 and 7 month interval. . The dose (10µg in age <10, 20µg for>10yrs age) and schedule (0,1,6 months) was same in both the arms of study.

A total of 24 cases received all three doses of the vaccine and the remaining 6 received only 2 doses. All 30 controls received 3 doses.

Seroconversion was defined as anti-HBs titre≥1mIU/ml and seroprotection as anti HBs titre≥10mIU/ml.

Seroconversion (anti HBs ≥1mIU/ml) was observed one month after first dose of hepatitis B vaccine in 19 out of 25 cases (76%) and all 30 controls (100%). The 'p' value obtained using 2 tailed Fischer exact test is significant at 0.0061.

Seroprotection (anti HBs≥10mIU/ml) was observed one month after first dose of Hepatitis B vaccine in 14 out of 25 cases (56%) and 12 out of 30 controls (40%) and the difference is statistically insignificant (p=0.285). Geometric Mean Titre (GMT) at 1 month were 11.48 and 12.39 mIU/ml in s.c. and i.m. arm respectively.
100% seroconversion was achieved at 2 month after first dose of hepatitis B vaccine in both the study groups.
Seroprotection was achieved in 18 of 19 (94.73%) cases and 29 of 30 (96.66) controls at 2 months after second dose of hepatitis B vaccine. GMT at second month were 217.38 and 196.12 mIU/ml in s.c. and i.m. arm respectively.
100% seroconversion was achieved after third dose of hepatitis B vaccine.
GMT at 7 months after first dose of vaccine was 706 and 650 mIU/ml for cases and controls respectively.
None of the cases or controls reported any side effects.
Thus, this study demonstrate that

1. Subcutaneous route of hepatitis B vaccination using the conventional dose (10µg in age <10yrs and 20µg in >10yrs) of recombinant vaccine achieves seroconversion and seroprotection rates similar to that of intramuscular route.
2. Subcutaneous route is safe in patients of bleeding disorders.

Based on these findings we recommend that all patients of bleeding disorders should be vaccinated against hepatitis B using a recombinant vaccine in the dose of 10µg for age <10 years and 20µg in age>10 years via subcutaneous route.

**Keywords:** Hepatitis B Vaccination, Subcutaneous Route, Hemophilia.



## Introduction

Bleeding disorders are characterized by deranged coagulation profile due to defects or deficit of clotting factors [1]. The treatment of bleeding disorders depends upon replacement of individual clotting factor to its optimal level. Repeated transfusion of blood and blood products expose them to the risk of acquiring transfusion associated infections like Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) [2,3]. Amongst these HBV infection is preventable by vaccination. A vaccine using HBs antigen produced by recombinant technology using yeast is currently available and widely used for primary prevention. The recommended schedule for this vaccine is to administer 3 doses 20µg (1 ml) each intramuscularly in deltoid at 0-1-6 month interval [2]. In patients with bleeding disorders there lies a risk of haematoma formation by the intramuscular injections and hence is not the accepted route for Hepatitis B vaccination. There is a need to explore alternative routes of vaccination like intradermal and subcutaneous.

Subcutaneous route of administration is practiced for these patients with bleeding disorder. This is based on few studies from west which report varying degree of seroconversion. There is paucity of data on seroconversion after Hepatitis B vaccination by subcutaneous route in patients with bleeding disorder in Indian setup. This study aims to measure the seroconversion and seroprotection afforded by the subcutaneous route as compared to the intramuscular group.

## Materials and Methods

### Study Design:

The study was conducted in the Hemophilia Centre (HDCC) at a tertiary care centre. Clearance was obtained from

institutional protocol ethics committee. It was a prospective open labelled interventional study. The study population included 30 patients with bleeding disorder and 30 healthy volunteers undergoing hepatitis B vaccination.

**Inclusion Criteria**

1. All cases with known bleeding diathesis and requiring Hepatitis B vaccination.
2. All patients with bleeding diathesis vaccinated earlier but not seroprotected.
3. Patients of all age groups were included.

**Exclusion Criteria**

1. Individuals with clinical evidence of liver disease.
2. All patients infected with Hepatitis B virus.
3. Individuals fully vaccinated for Hepatitis B.
4. Individuals with previously known hypersensitivity to recombinant HBV vaccine.
5. Individuals suffering from acute severe febrile illness.
6. Patients who have an overt active bleeding.

**Selection of Cases**

Patients of bleeding diathesis who reported at hospital were screened and 40 such patients who were negative for HBsAg, anti HCV, anti HBc (total) and anti HBs and had serum ALT within normal limits were enrolled for the study and were given the first dose of recombinant hepatitis B vaccine. Out of 40, 2 patients suffered from moderate Hemophilia B and 38 patients suffered from Hemophilia A.

10 patients never followed up and were excluded from the study. Five patients of the remaining 30 who went on to receive second dose lost to follow up. One patient died in a road traffic accident. He had received two doses of the vaccine For 5 patients only baseline investigation and investigations after the last dose was available. There data was included in the study. None of the cases were given booster dose.

**Selection of Controls**

A set of 30 healthy persons (with no bleeding diathesis) undergoing Hepatitis B vaccination were included as control after screening them for HBsAg, anti HCV, ALT, anti HBc(total), anti HBs. They received recombinant hepatitis B vaccine 20µg (1ml) via the intramuscular route at 0,1 and 6 month interval. Anti HBs titres were followed up at 1,2,7 month interval. HBsAg, anti HBc(total) and serum ALT was also followed up. None of the controls were given booster dose.

**Methodology**

A written informed consent was taken from all the patients and controls prior to their inclusion in study . All patients were subjected to a detailed history through a pre - designed Proforma .

Patients were assessed for clinical details including previous history of any liver disease and were subjected to a baseline laboratory work up including a screening coagulogram , biochemical liver function and hepatitis B status by conducting following tests:

1) Tests of Haemostasis
   a) PT, aPTTK
2) Liver Function Tests
   a) ALT
3) Viral markers
   a) HBsAg
   b) anti HBc total
   c) anti Hbs titre
   d) anti HCV

**Vaccination**

**Vaccine**

We used Recombinant hepatitis B vaccine, Revac-B manufactured by Bharat Biotech, manufacturing date December 2009, Expiry date November 2012. Vaccine was stored at 4-8$^0$C temperature in the

refrigerator as prescribed by the manufacturer.

**Schedule and dose**

Vaccination was done as per standard protocol-Dose 20µg (1ml) Hepatitis B vaccine by subcutaneous route 0, 1 and 6 month interval in children and aduts >10years

Children below 10 years of age were given 10 g (0.5ml) as per standard protocol on 0,1 and 6 month interval.

Individuals not achieving the desire anti HBs titre of > 10mIU/ml at 7 months would receive a booster dose.

**Method of Injection**

**Subcutaneous route (for Case Subjects)**

- Explain to the patient what you are about to do.
- Put on gloves and draw up the vaccine dose to be given using the 22-gauge needle. Expel air from the syringe and attach the 26-gauge needle.
- Swab the skin into a fold in a suitable site (we preferred forearm in all patients.)
- Pinch the skin gently into a fold.
- Insert the needle horizontally into the fold of skin.
- Draw back on the needle to ensure that you are not in a vein.
- Slowly inject the vaccine. Withdraw the needle and wipe the area with cotton.

**Intramuscular route (for Control subjects)**

- Explain to the patient what you are about to do.
- Put on gloves and draw up the medication to be given using the 22-gauge needle.
- Expel air from the syringe and if necessary attach a 24-gauge needle.
- Swab the deltoid area.
- Insert the needle to full depth perpendicular to the skin.
- Draw back on the syringe to ensure that it is not in a vein.
- Slowly inject the medication. If the patient complains of discomfort, stop for a few seconds until the pain eases, then continue more slowly.
- Withdraw the needle and wipe the area with cotton.

**Recording of Side Effects**

Patients were interrogated regarding any side effects after every vaccination like

1. Fever,
2. Local reaction such as erythema at the site of injection,
3. Sore and swollen arm,
4. Headache
5. Nausea
6. Anaphylaxis
7. Malaise
8. Lymphadenopathy
9. Others

**Follow Up Studies**

- Anti HBs titre were performed one month after each dose of vaccination. Thus anti HBs titre were done on 1, 2, 7 months respectively.
- Serum ALT, anti HBc (total) and HBsAg were also followed up.
- Seroconversion was defined as anti Hbs titre 1-10mIU/ml and seroprotection as anti Hbs titre>10mIU/ml.

**Statistics**

Statistical Analysis was done using Independent ‘t’ test, Fischer’s exact test using SPSS software version 17.0.

**Observation and Results**

This study was conducted over a period of 14 months wherein we screened the patients of bleeding diathesis attending the Centre for clinical details including previous history of any liver disease and

were subjected to a baseline laboratory work up including a screening coagulogram , biochemical liver function and hepatitis B status by conducting HBs Ag , anti HCV, anti HBc (total), Alanine transaminase and anti HBs titre. A prior written consent was obtained from all the patients and guardians in case of minors.

Forty patients of bleeding disorder who were negative for the markers of hepatitis B and C infection and with normal S. ALT levels were enrolled in this study. These patients were to receive three doses of recombinant hepatitis B vaccine at 0,1, and 6 month interval and followed up as mentioned in materials and methods. Out of these forty, ten patients lost to follow up after the first visit and hence were excluded from the study. Out of the remaining thirty, five stopped following after second dose of vaccine due to personal reasons and one patient died in a road traffic accident. He had received two doses of the vaccine. Other twenty four patients completed the vaccination schedule (for five of these patients the baseline and data at seventh month of vaccination was available). None of the patients opted out of the study due to any adverse reaction or side effect of vaccine.

On the similar lines thirty healthy male volunteers undergoing hepatitis B vaccination, who did not have any bleeding disorder and were negative for markers of hepatitis B and C and normal serum alanine transaminase were included in the study as control. They were vaccinated and followed up as mentioned in materials and methods. All of them tolerated the vaccine well and did not report any side effect.

All cases and controls were males. No one amongst the cases or controls had history of jaundice, previous hepatitis B vaccination. All the cases and controls were carefully looked for any contraindication for hepatitis B vaccination namely Known hypersensitivity to recombinant hepatitis B vaccine, acute febrile illness and overt bleeding. Clinical and laboratory data was received in a proforma and analysed. Seroconversion was defined as anti HBs titre ≥1mIU/ml and Seroprotection was defined as anti HBs titre≥10mIU/ml

Henceforth patients of bleeding disorders who received hepatitis B vaccine by subcutaneous route will be referred to as “Cases” and the healthy volunteers who received the vaccine by intramuscular route will be referred as “Controls”.

**Patient Characteristics**

**Table 1: Patient Characteristics**

| | | Cases n=30 | Controls n=30 |
|---|---|---|---|
| Age | | 12.85 ± 10.616 | 35.77± 11.392 |
| Sex | | 30 (100%) Males | 30 Males |
| Bleeding disorder | Hemophilia A | 28 | 0 |
| | Hemophilia B | 2 | 0 |
| PT/ aPTT | | 30 (100%) Deranged | -- |
| ALT | | 21.966± 6.47 | 24.53±5.90 |
| HBsAg | | All Non-reactive | All non-reactive |
| Anti HBs | | All negative | All negative |
| Anti HBc (T) | | All negative | All negative |
| Anti HCV | | All non-reactive | All non-reactive |

Out of the 30 patients, twenty eight patients had Hemophilia A, out of which sixteen patients (53%) had severe disease (Factor VIII<1%), and 7 (23.33%) others had moderate disease

(Factor VIII=1-5%), two (6.66%) suffered from Hemophilia B and had moderate disease (Factor IX- 1-5%). (Fig1). Factor levels were not known for 5 patients(16%).

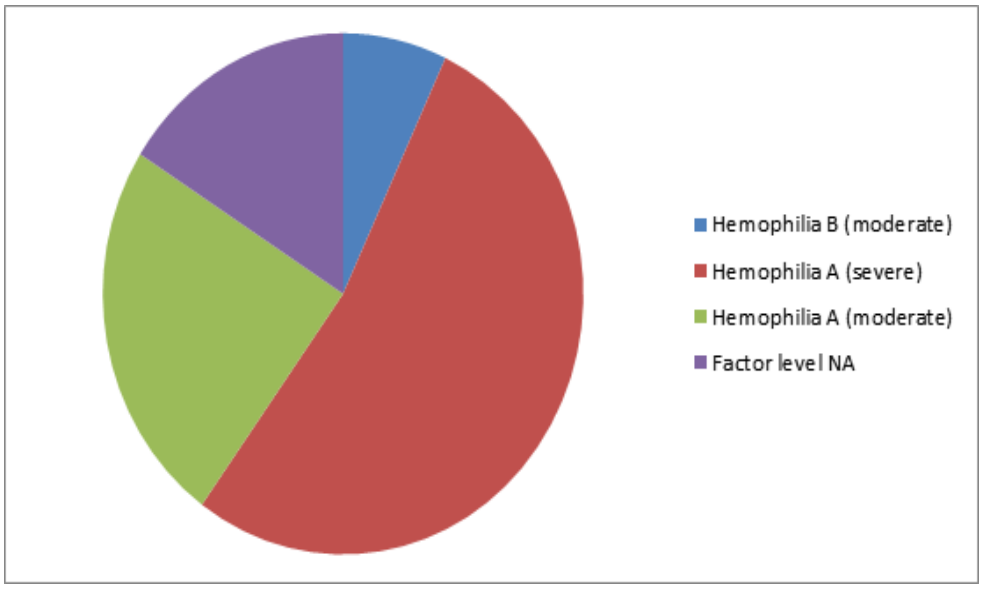


**Figure 1: Severity of disease in patients**

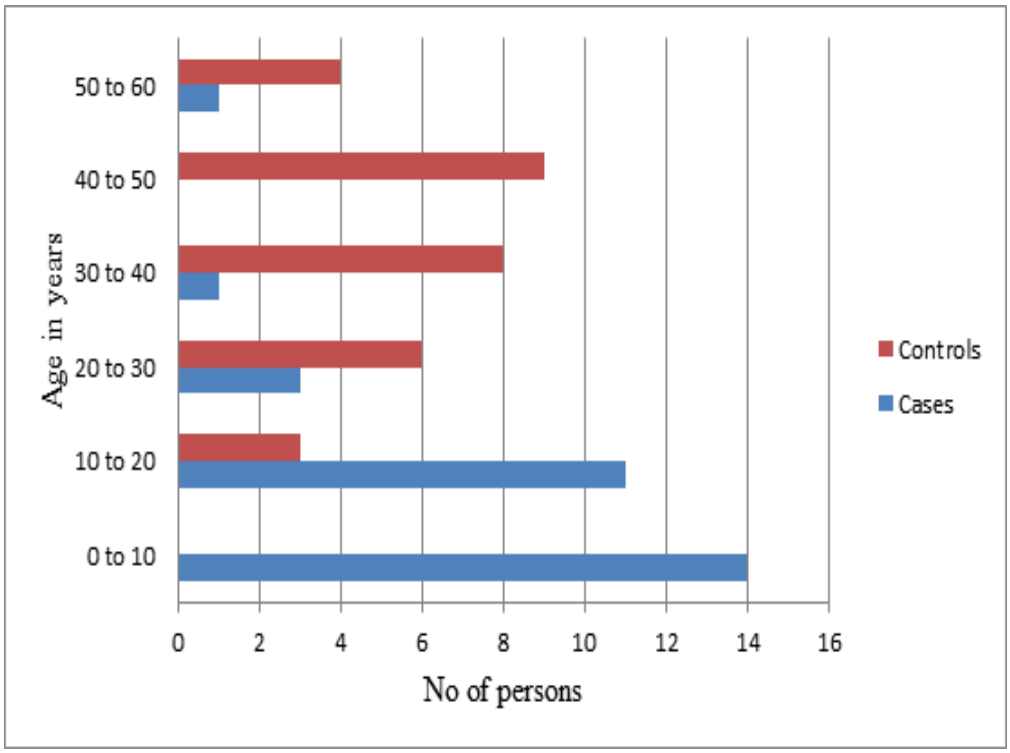


**Figure 2: Age distribution of cases and controls.**

The mean age of the cases was 12.85± 10.616, Median=10.5 and mode=8.0. The youngest case was 18 month old and oldest being 52 year old at the time of enrolment. Mean age for controls was 35.77±11.392, Median=36.5, Mode=30. Range of Age for controls was 17-56 years. Fig 2 depicts the frequency distribution of age for cases and controls.

None of the controls had history of transfusion of blood or blood products. Amongst the cases the mean RBC(η=30) transfusion was 0.766±1.887, mean FFP(η=30) 24.966±64.723, mean Factor

VIII(η=28) 9483.92 ± 15735.31, mean Factor IX (η=2) 3000 ± 0. Table 2 shows the transfusion of blood and blood products as per severity of disease.

**Table 2 Total Transfusion of blood and blood products received previously amongst the cases**

| Severity of disease | RBC unit Mean±S.D.# | FFP unit Mean±S.D.+ | Factor VIII Mean±S.D. | Factor IX Mean±S.D. |
|---|---|---|---|---|
| Sev Hemophilia A *η=18* | 0.944 ± 2.363 | 39 ± 80.868 | 12155.56± 18703 | -- |
| Mod Hemophilia A *η=10* | 0.6 ± 0.843 | 0.7 ± 1.636 | 4675 ± 6487.22 | -- |
| Mod Hemophilia B *η=2* | 0 | 20 * | -- | 3000 ** |

#-one RBC unit containing 300 ml
+-one FFP unit containing 250ml

*-one patient received 40 units and other did not receive FFP

** both the patients received 3000 units each

**Follow Up Studies**

The cases and controls were sampled at 1, 2 and 7 month interval after first dose of hepatitis B vaccine. ALT, HBsAg, anti HBc(t) and anti HBs titre was estimated as per the protocol.

**Table 3 Follow up studies in the hepatitis and seroconversion parameter**

| Month after First dose | 1 | | | 2 | | | 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Test | Case n=25 | Control n=30 | P value | Case n=19 | Control n=30 | P value | Case n=24 | Control n=30 | P value |
| ALT | 24 ±6.02 | 24.73 ±5.82 | 0.650 | 22.4 ±4.63 | 23.40 ±4.53 | 0.631 | 22.20 ±4.63 | 21.80 ±3.9 | 0.73 |
| HBsAg | All NR | All NR | All NR | All NR | All NR | All NR | All NR | All NR | All NR |
| Anti HBc(t) | All NR | All NR | All NR | All NR | All NR | All NR | All NR | All NR | All NR |
| Anti HBs | | 19.80 ±22.41 | 0.042* | 316.1 ±234.7 | 261.72 ±186.5 | 0.400 | 776.91 ±276.28 | 720 ±259.28 | 0.448 |

*p<0.05, Statistically significant.

The mean anti HBs titre 1 month after the first dose of hepatitis B vaccine were found to be higher in cases (p<0.05). (see table 3 & 4). There was no statical difference between the mean anti HBs titres between the two groups at 2 and 7 month after first dose of Hepatitis B vaccine.

**Table 4: Anti HBs titre after one month of first dose of hepatitis B vaccine**

| Route | Minimum titre mIU/ml | Maximum titre mIU/ml | Arithmetic Mean titre mIU/ml±S.D. | Geometric Mean titre mIU/ml |
|---|---|---|---|---|
| Subcutaneous η=25 | <1 | 247 | 40.728±61.279 | 11.148 |
| Intramuscular η=30 | 1 | 92.43 | 19.805±22.416 | 12.3893 |

**Table 5: Anti HBs titre after second dose of hepatitis B vaccine**

| Study Group | Minimum titre mIU/ml | Maximum titre mIU/ml | Mean titre± S.D. mIU/ml | GMT |
|---|---|---|---|---|
| Cases | 3.9 | 913 | 316.1±234.71 | 217.38 |
| Control | 7.8 | 702 | 261.73±186.50 | 196.12 |

**Table 6: Anti HBs titre after third dose of hepatitis B vaccine**

| Study group | Minimum titre mIU/ml | Maximum titre mIU/ml | Mean titre± S.D. mIU/ml | GMT |
|---|---|---|---|---|
| Cases | 119 | 1100 | 776.91±276.23 | 706 |
| Control | 118 | 1100 | 720±259.28 | 650 |

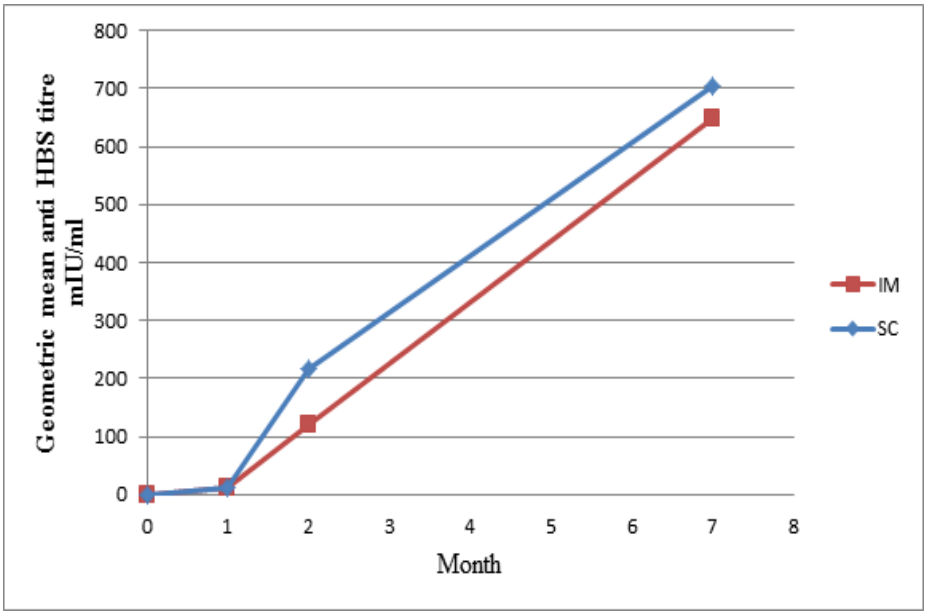


**Figure 3: compares the Geometric Mean titres at 0,1,2 and 7 months after the first dose of hepatitis B vaccine.**

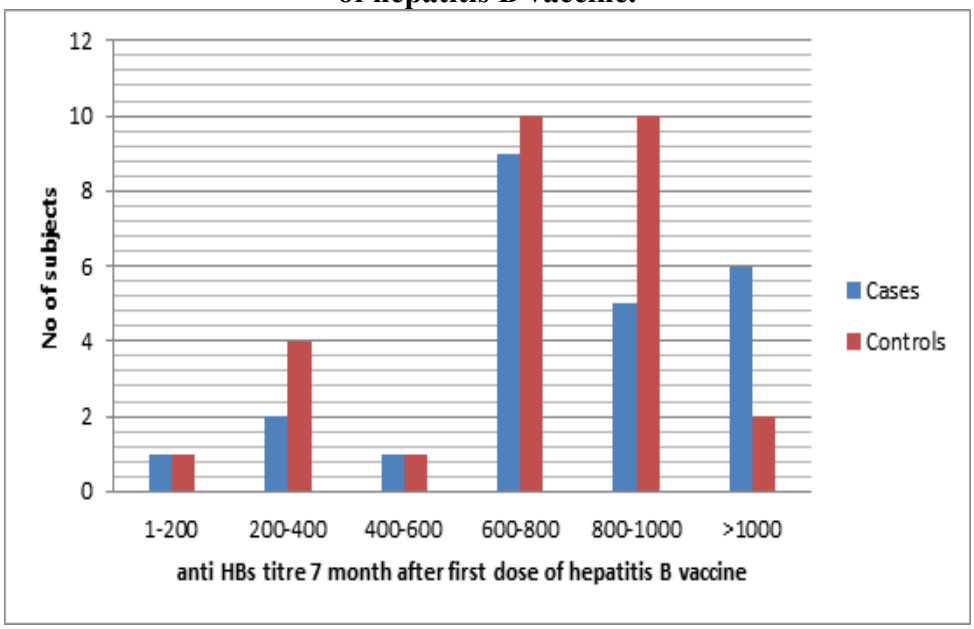


**Figure 4: Maximum titre attained 7 months after the first dose of vaccination**

Table 7 and Fig 5 analyse the age wise distribution of GMT.

**Table 7. Age wise distribution of GMT**

| Study group | Age group | No | GMT1 | GMT2 | GMT7 |
|---|---|---|---|---|---|
| Cases | 0-10 | 14 | 9.421 | 274.83 | 736.67 |
| | 10-20 | 11 | 7.28 | 283.71 | 574.94 |
| | >20 | 5 | 1.33 | 60.28 | 883.41 |
| Controls | 0-10 | 0 | 0 | 0 | 0 |
| | 10-20 | 3 | 17.30 | 255.50 | 919.74 |
| | >20 | 27 | 11.94 | 190.44 | 625.63 |

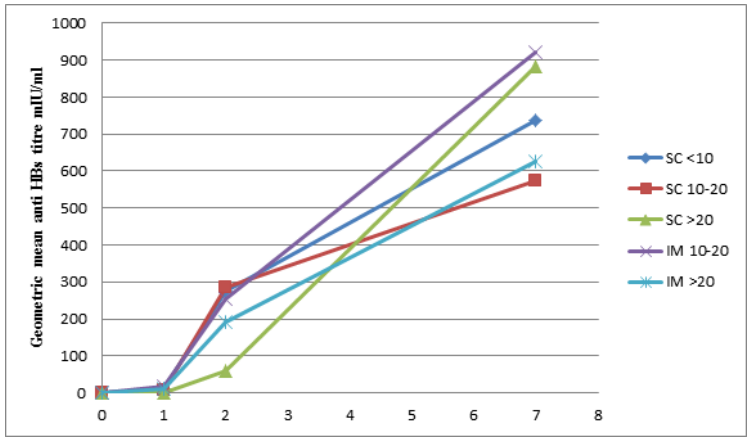


**Figure 5: Geometric mean titres with age distribution**

Seroconversion and Seroprotection Data

**Table 8: Seroconversion and Seroprotection after first dose**

| Route | No seroconversion Anti HBs <1 | Seroconversion Anti HBs≥1 | P value | No seroprotection Anti HBs <10 | Seroprotection Anti HBs≥10 | P value |
|---|---|---|---|---|---|---|
| Cases η = 25 | 6 | 19 | 0.0061 | 11 | 14 | 0.285 |
| Controls η = 30 | 0 | 30 | | 18 | 12 | |

**Table 9. Seroconversion and seroprotection one month after second dose of hepatitis B vaccine.**

| Route | No seroconversion Anti HBs <1 | Seroconversion Anti HBs≥1 | P value | No seroprotection Anti HBs <10 | Seroprotection Anti HBs≥10 | P value |
|---|---|---|---|---|---|---|
| Cases η = 19 | 0 | 19 | 1.00 | 1 | 18 | 1.00 |
| Controls η = 30 | 0 | 30 | | 1 | 29 | |

Seroconversion (anti HBs ≥1mIU/ml) was observed one month after first dose of hepatitis B vaccine in 19 out of 25 cases (76%) and all 30 controls(100%). The 'p' value obtained using 2 tailed Fischer exact test is significant at 0.0061. (see Table 8)

Seroprotection (anti HBs≥10mIU/ml) was observed one month after first dose of

Hepatitis B vaccine in 14 out of 25 cases (56%) and 12 out of 30 controls (40%) and the difference is statistically insignificant (p=0.285). (see Table 8)

100% seroconversion was achieved at 2 month after first dose of hepatitis B vaccine in both the study groups.

Only 1 case and 1 control failed to achieve anti HBs titre ≥10mIU/ml after second dose of vaccine, thus were labelled as not seroprotected

At 7 month after first dose of the hepatitis B vaccine all cases and controls who received 3 doses of the vaccine, 24 cases and 30 controls achieved protective levels of the anti HBs titre. Fig 3 shows seroprotection patterns after each dose of the vaccine.

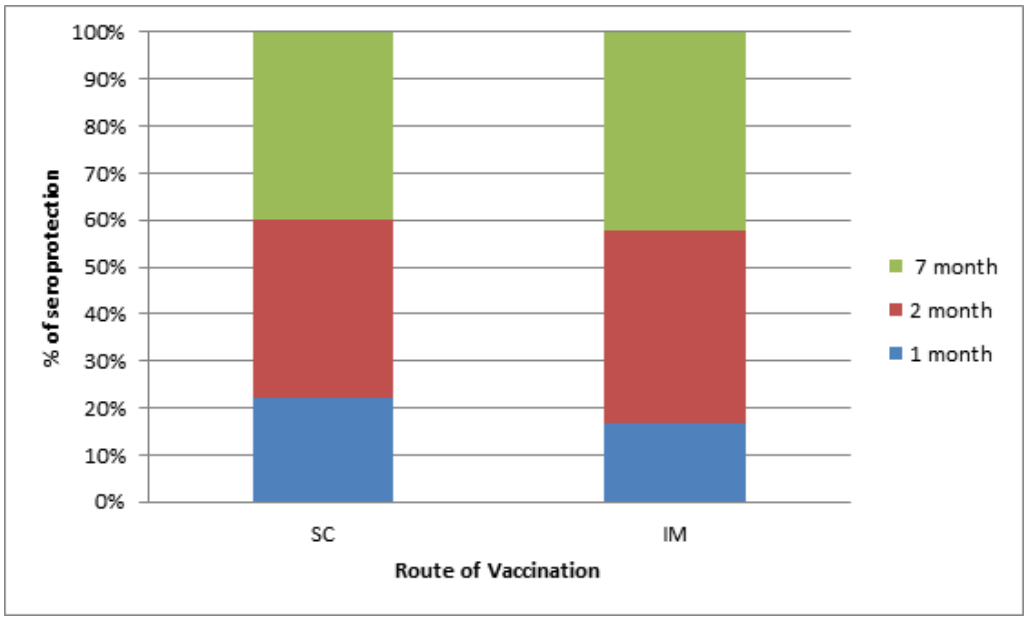


**Figure 6 : Seroprotection at 7 month after first dose of vaccination.**

Thus it can be concluded that

1. Individuals vaccinated by recombinant hepatitis B vaccine via subcutaneous route achieve protective antibody titres comparable to those vaccinated via intramuscular route
2. Subcutaneous route is safe.

**Discussion**

Patients of bleeding disorders are receiving multiple transfusion of blood and blood products and are constantly at risk to acquire hepatitis B virus infection. This makes it imperative to vaccinate them against hepatitis B. The risk of bleeding makes use of conventional intramuscular route challenging. This study has shown that subcutaneous administration of recombinant hepatitis B vaccine achieves comparable rates of seroconversion and protective levels of anti HBs titres.

In this study we vaccinated patients of bleeding disorders (Hemophilia A-28 and Hemophilia B-2) with recombinant hepatitis B vaccine via subcutaneous route and other set of healthy volunteers (30) were given the same vaccine via intramuscular route. The dose (10µg in age <10, 20µg for>10yrs age) and schedule (0,1,6 months) was same in both the arms of study. Antibody produced against HBs antigen was measured.

A total of 24 cases received all three doses of the vaccine and the remaining 6 received only 2 doses. All 30 controls received 3 doses.

Seroconversion was defined as anti-HBs titre≥1mIU/ml and seroprotection as anti HBs titre≥10mIU/ml.

19 out of 25(76%) in s.c. arm and all 30 (100%) in i.m. arm achieved

seroconversion (p=0.0061) 1 month after forst dose of vaccine which is statistically significant. Individuals attaining protective levels of anti HBs (≥10mIU/ml) one month after first dose of vaccine were 14 out of 25 in s.c. arm (56%) and 12 out of 30 (40%) in i.m. arm (p=0.285) which is statistically insignificant. Geometric Mean Titre (GMT) at 1 month were 11.148 and 12.39 mIU/ml in cases and controls respectively.

All cases and controls achieved seroconversion one month after second dose of hepatitis B vaccine. One out of 19 cases in s.c. arm (5.2%) and one in 30 (33.3) cases (p=1) failed to attain titres >10mIU/ml after second dose of vaccine. GMT at second month were 217.38 and 196.12 mIU/ml in cases and controls arm respectively. [4]

All cases and controls ultimately achieved seroprotection after third dose of vaccine. GMT at seventh month were 706 and 650 mIU/ml in s.c. and i.m. arm respectively.

No adverse reaction was noted in any of the individual who received the vaccine. There was no incident of bleeding or hematoma formation in any of the hemophilia patients who received the vaccine by s.c. route.

A similar study was done by Wahl M. and Hermodsson S. [5] in 1987 in 39 healthy students received 3 doses of plasma derived hepatitis B vaccine in doses of 20µg via i.m, and 2µg via s.c or i.d. route. Significantly lower seroconversion rates were observed in s.c. route two months after second dose and at 7 months (42% and 63%) vs i.m.route (88% and 94%) and i.d. route (100%).

Another study by Hayashi J. et al [6] completed in 1989, 44 mentally challenged individuals were vaccinated with 3 doses of recombinant hepatitis B vaccine at 0-, 1- and 6-month interval either by 10µg via s.c. route. Seroconversion rates for subcutaneous route at 1 month and 6 months were 9 out of 44 (20.5%) and 37 out of 44 (84.1%).

This observation contradicts our findings and suggests that lowering the dose of hepatitis B vaccine given via subcutaneous route reduces its efficacy to produce seroconversion and seroprotection.

An Italian study by A.R.Zanetti et al [7] done in 1986 wherein out of 113 hemophiliacs were vaccinated via s.c. route at 0,1,2 and 14 month, 111 (98%) achieved the desired anti HBs titre of >10mIU/ml.

Similarly in an American study by Robert Janco [8] done in 1995, 28 out of 29 (96%) hemophiliacs showed positive anti HBs titre after 3 doses of recombinant hepatitis B vaccine at 0,1and 6 month interval.

A Czech study by Roznovsky L. et al [9] completed in 2010 achieved seroprotection (anti HBs >10mIU/ml) in 50 out of 51 (98%) patients of hemophilia patients who received standard doses of hepatitis B vaccine. In the follow up they found waning of protective anti-HBs antibodies was detected in 4 % and 24 % of patients within 5 and 10 years after vaccination.

To study the long term efficacy of s.c. route an american study by John M. Maris et al followed the anti HBs titre after primary hepatitis B vaccination and observed that 11 of 55 hemophilia patients lost detectable anti HBs titre after primary adequate response over a median follow up of 6.1 years. Waning of titre below the protective level has probably little bearing on the host's resistance to hepatitis B infection because of prolonged immunologic memory.

Another Italian Study by Elena Santagostino et al [10], 1992 demonstrated that rapid seroconversion can be achieved using 3 doses of recombinant hepatits B vaccine in a dose of 20µg via subcutaneous route in the deltoid region at 0,2 and 6 weeks attaining 20%, 53%, 87%

and 93 % seroconversion rate at 2,6,10 and 24 week respectively. To maintain the antibody response a booster dose was given at 24-week interval in absence of which the antibody response fell to 76% at 1 year. We observed 100% seroconversion by 12 weeks after 2 doses of the vaccine. [11]

**Table 10 Studies demonstrating seroconversion after subcutaneous hepatitis B vaccination in Patients of Bleeding disorders**

| Investigators | Place | Year | No of cases | Dose Age | Schedule | Seroconversion |
|---|---|---|---|---|---|---|
| A.R. Zanetti et al | Italy | 1986 | 113 | 20µg | 0, 1, 6* | 98% |
| Robert Lanco | US | 1995 | 29 | 20µg | 0, 1, 6* | 96% |
| Santagostino et al | Italy | 1992 | 85 | 20µg | 0, 2, 6, 22+ | 96% |
| Roznovsky L. et al | Czech | 2010 | 51 | 20µg | 0, 1, 6* | 98% |
| This Study | India | 2011 | 30 | 20µg | 0, 1, 6* | 100% |

*Months interval

+ weeks interval

Considering the observations made by these studies and results of our study we would like to make a statement that primary vaccination by recombinant hepatitis B vaccine via s.c. route is safe and achieves anti HBs titre levels similar to that of i.m. route when used in standard prescribed dose 10µg for age<10years and 20µg for age> 10 years at 0-, 1- and 6-month interval.

## Recommendation and Conclusions

The purpose of this study was to assess the seroconversion and seroprotection after primary immunization with recombinant Hepatitis B vaccine (10µg<10 years, 20µg for age>10 years) by subcutaneous route in patient with bleeding diathesis and compare it to the intramuscular route We found that the seroprotection afforded by the subcutaneous route was similar to that of intramuscular route. In fact in our study, 100% of the subjects who received S.C. vaccine were seroprotected at 7 months after first dose. This is a higher rate of seroconversion and seroprotection than the previous studies which used a lower dose of 2µg of the vaccine but similar to the studies which employed the same dose and regime (20µg at 0,1,6 month interval). The subcutaneous route is also as safe as intramuscular route.

Based on the results of this study we recommend,

All patients of bleeding diathesis should be vaccinated against hepatitis B using the recombinant vaccine 10µg in age <10 years and 20µg in age >10 years via subcutaneous route at 0, 1, 6 month interval.

This study conducted on a cohort of 30 patients of bleeding disorder hence larger trial is needed to derive a conclusion. Also subjects who received S.C. recombinant hepatitis B vaccine should be followed up for a long time to look for waning of anti HBs titre. Larger trials might expose the non-responders. A different strategy must be devised to deal with the non-responders.

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