CHAPTER V

DISCUSSION
AND
CONCLUSION


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The present work was carried out to study the prevalence of HBV, HCV [HBsAg, anti-HBc and anti-HCV] and their co-existence [anti-HBc and anti-HCV] in vaccinated ESRD patients on maintenance hemodialysis and high-risk control individuals. The vaccine response is also estimated in these individuals to study immunogenicity and efficacy of hepatitis B vaccine.

A large survey of HD centers in 1974, in United States, found HBV incidence rates of 6.2% among patients and 5.2% among staff.99 As a result of segregation, universal precautions, vaccination, reduced blood transfusions, and screening of organs before transplantation, the incidence of HBV infection decreased to 0.08% for patients and 0.05% for staff within dialysis units in the United States by 1996 99 These achievements were also supported by better blood-bank screening-measures, the introduction of which dramatically decreased the risk of transfusion-associated HBV infection. The western data are suggestive of a prevalence of HBV in 5 – 10 % in dialysis subjects of whom more than 50 % remained persistently infected.17

**HBsAg**

Indian data in this regard appear scanty and reports from different centers have great variation. The data in Indian studies ranges from 3.4 % to 42 %15 and Mani et al have reported the prevalence to be as high as 77 %.16 Sisodia D P et al reported 18.9 % HBsAg prevalence in 1990.112 Shreeprakash et al observed remarkable drop in HBsAg
reactivity rate from 18.9 % to 7.0 % over 9 years period from 1990 to 1999 and thereafter a steady rate of 7.5 % HBsAg prevalence in a hemodialysis center in Central India.  

The observed HBsAg (5.0 %) prevalence in the present study is lowest of so far available data. This reflects strict implementation of vaccination and universal precautions in the hemodialysis unit under study.

With the use of Epo (erythropoietin), to correct the anemia in the ESRD patients, the frequency of blood transfusion has been considerably reduced. Still the ESRD patients require blood transfusion on many occasions. The involved blood units are stringently screened for HBsAg, HIV [I+II] antibodies and Anti HCV antibodies.

Recent studies have revealed that HBV may not be cleared even after the disappearance of serum HBsAg.  

The replication of HBV is ongoing in a substantial proportion of healthy blood donors who are HBsAg negative but have anti-HBc as the only marker of HBV infection. In the developed countries, where the transfusion-transmitted hepatitis has been reduced greatly, screening of blood for Anti-HBc antibodies is mandatory. The blood or blood product positive for Anti-HBc antibodies is not used, in many developed countries, for transfusion purpose.

The regional surveillance centers established by the government of India since 1984 have shown HBsAg positivity ranging from 3 – 15 % in general population, 1 – 8 % among voluntary blood donors and 5 – 36 % among professional blood donors.
In a study in Lucknow, Saraswat et al found that on transfusion of HBsAg negative blood, 14.6% of recipients developed transfusion associated hepatitis, of these 67% were due to HBV and 33% due to HCV.\textsuperscript{114}

Testing of blood units for Anti-HBc of the blood units is not mandatory in India. Exposure to HBsAg negative, but Anti-HBc and HBV-DNA positive blood unit may result in HBV infection if the patient does not have protective titer of neutralizing antibody at the time of exposure.

The doctors and the staff members of the dialysis unit are at high risk of exposure to HBV. Hepatitis was noted in 15.7% of patients and 10.5% in staff by V N Acharaya et al\textsuperscript{116} in the Dialysis Unit in Bombay. P Thomas et al reported 23% HBV infection in the staff members in a Dialysis Unit in South India in 1997.\textsuperscript{117}

None of the doctors or staff members of the dialysis unit in the present study was HBsAg positive. This achievement was probably due to strict implementation of Hepatitis B vaccination and adaptation of universal precautions in the dialysis unit.

**Anti-HBc**

The presence of anti-HBc antibodies in the absence of circulating HBsAg indicates past HBV infection in clinical or sub-clinical form. Higher (33.6%) Anti-HBc prevalence was observed among ESRD patients compared to (7.4%) the control group.
Very scanty data is available regarding Anti-HBc prevalence in ESRD patients in India. Shreeprakash et al \(^{17}\) observed very high prevalence of Anti-HBc (80\%) in chronic hemodialysis patients in central India.

In the present study the observed prevalence of Anti HBc is 33.6\%. This prevalence of Anti-HBc in ESRD patients is very high compared to 7.4\% prevalence in the control group. This suggests more frequent exposure of ESRD patients to HBV than the control subjects. This suggests presence of very low levels of HBV in blood of patients positive for Anti-HBc responsible for the nosocomial spread of HBV in the dialysis unit.

The situation becomes grim in Anti-HBc positive ESRD patients with dormant infection who are also at risk of reactivation HBV infection during post renal-transplant immunosupression or any exacerbation.

**HBV DNA**

The negative results for HBV-DNA, done by qualitative PCR in ESRD patients with strongly positive Anti-HBc, implies presence of very low levels of HBV-DNA in such patients that is not detected by qualitative PCR methods or the presence of pre-core mutants of HBV in the ESRD patients. More sensitive procedures may be applied to detect HBV-DNA in the sera of such individuals.

**HCV**

Hepatitis C Virus [HCV], another parenterally transmissible hepatitis virus, frequently associated with HBV, is also commonly reported in ESRD patients on maintenance dialysis.
In the present study the prevalence of Anti-HCV positive individuals in ESRD patients is 31.1 %.

Worldwide data of HCV infections in dialysis patients varies from < 5% to 68 % of CRF \(^\text{19,20}\) subjects. Indian reports indicate 12 – 45 % \(^\text{21,22}\) range of HCV infection in CRF patients. Shreeprakash et al reported 30 % Anti HCV positivity in CRF patients in Central India \(^\text{17}\).

The prevalence of Anti-HCV in ESRD patients in the present study is very high compared to 2.2 % prevalence in control group.

Despite stringent screening measurements by the blood bank before blood donation, high prevalence of HCV infection in the dialysis patients, suggests nosocomial spread of HCV in dialysis unit. Nevertheless it has been observed by some researchers that the HCV infections can occur in dialysis patients who have not received any blood transfusion. \(^\text{118,119,120}\) Molecular genotyping has, in fact, established the nosocomial spread of HCV infections in the dialysis units. Finding of HCV-RNA in patients who are negative for Anti-HCV antibodies by some workers further supports the possible nosocomial spread. As suggested by Jose et al \(^\text{121}\) HCV is responsible for some unexplained ALT elevations despite the absence of anti-HCV positivity. They also confirmed that currently available serodiagnostic tests underestimate HCV prevalence in dialysis patients and gave a possible explanation for this that the immunosuppression that characterizes hemodialysis patients may be responsible for the inability to express detectable amounts of serum anti-HCV. This study questioned the isolation criteria that are used to
prevent HCV transmission in hemodialysis units based on the anti-HCV status. Periodic Anti-HCV determinations remain mandatory, since cost and technical difficulty make PCR analysis inviable as a screening method.

**Co-infection [HBV-HCV]**

HBV-HCV co-infection have been observed by many other researchers. The HBV-HCV co-infection is responsible for morbidity among the chronic dialysis patients.

Silent infection in hepatitis C: Hepatitis B is frequently presented when assessed by PCR even in the absence of the usual serological HBV markers. Uchida speculated that HBV promoted replication of HCV in this situation. In a more recent investigation 22% of HBsAg negative patients had HBV by PCR. A particular situation is the constellation of ‘Anti-Hbc alone’. Forty percent of such patients are anti-HCV positive and 40-90 % are also RNA positive.

In the present study the co-infection rate among Anti-HCV positive patients is 48.5% [anti-HBc positive]. So the chances of these patients having silent HBV infections are very high.

**Duration of hemodialysis and prevalence of HBV & HCV**

In the present study seroconversion of ESRD patients on maintenance hemodialysis to Anti-HCV was associated with number of dialysis, [duration of hemodialysis treatment.] The Anti-HCV and co-infection with HBV showed similar behavior as the number of dialysis reach over 500. The present study indicates that about 57.1 % of the ESRD
patients acquire dual infection with HBV and HCV over a time period of five years of dialysis treatment.

**Hepatitis B Vaccine Immunogenicity**

Hepatitis B vaccine has been recommended for both hemodialysis patients and staff members since the vaccine became available in 1982. The recommended primary series of hepatitis B vaccine induces a protective anti-HBs response (defined as $\geq 10$ milli-International Units [mIU]/mL) in 90%–95% of adults with normal immune status. The major determinant of vaccine response is age. Compared with adults with normal immune status, the proportion of hemodialysis patients who develop a protective antibody response after vaccination (with higher dosages) is lower. (Range: 34%–98%)\textsuperscript{128,129,130,131}

**In Control group**

According to the present study protective titers were observed in the 86.0 % individuals in the control group irrespective of their age and time of vaccine inoculation. The vaccine offered 100 % protection up to first year of vaccine inoculation. The antibody titers with different vaccination time over 8 years interval was done. The weighted average protection offered by the vaccination up to 8 years is 86.51%.

**In ESRD patients**

The immunogenicity among the ESRD patients in the present study was poor as the vaccine induced protective titers only in 58 % of
patients while 42% remained non-protective [Anti-HBs titers < 10 mIU/ml].

Vaccine immunogenicity among high-risk control individuals was independent of age group in the present study but among ESRD patients vaccine induced protection (%) showed decline from age group- up 30, 31-40 and > 40 years.

**Hepatitis B Vaccine Efficacy**

**In control group**

For persons with normal immune status, controlled clinical trials have demonstrated that protection from acute and chronic HBV infection is virtually complete among those who develop a protective antibody response after vaccination\(^{132,133}\)

This observation is seconded by the present study as none of the high-risk group individual was having demonstrable HBsAg in the blood.

In some persons, the only HBV serologic marker detected is anti-HBc (i.e., isolated anti-HBc). Among most asymptomatic persons in the United States tested for HBV infection, an average of 2% (range: <0.1%--6%) test positive for isolated anti-HBc\(^ {134}\) among injecting-drug users, however, the rate is 24%.\(^ {135}\) In general, the frequency of isolated anti-HBc is directly related to the frequency of previous HBV infection in the population and can have several explanations. This pattern can occur after HBV infection among persons who have recovered but whose anti-HBs levels have waned or among persons...
who failed to develop anti-HBs. Persons in the latter category include those who have circulating HBsAg at levels not detectable by current commercial assays. However, HBV DNA has been detected in <10% of persons with isolated anti-HBc, and these persons are unlikely to be infectious to others except under unusual circumstances involving direct percutaneous exposure to large quantities of blood (e.g., transfusion)\textsuperscript{136}

The observation in the control subjects, that about 90% of the individuals positive for Anti-HBc antibody had Anti-HBs > 10 mIU/ml, indicates that the individuals who were exposed to HBV during the period, overcome the infection and successfully eliminated the HBsAg from the blood with production of free neutralizing anti-HBs that creates immunity from future infection.

Only 10% (1/10) anti-HBc positive vaccinated individuals in the control group. These are susceptible individuals and as a precautionary measure need revaccination (booster).

So, therefore, in healthy vaccinated individuals protection from acute and chronic HBV infection is virtually complete among those who develop a protective antibody response after vaccination.

**In ESRD patients**

In immunosuppressed persons (including hemodialysis patients), most newly acquired HBV infections result in chronic infection. Although the consequences of acute hepatitis B can be severe, most of the serious sequelae associated with the disease occur in persons in whom
chronic infection develops. Although persons with chronic HBV infection are often asymptomatic, chronic liver disease develops in two-thirds of these persons, and approximately 15%--25% die prematurely from cirrhosis or liver cancer.\textsuperscript{137,138}

Among the ESRD patients 66.7% of the HBsAg positive individuals were among hepatitis B vaccine non-responders [Anti-HBs < 10 mIU/ml] while 33.3% were vaccine responders [Anti-HBs > 10 mIU/ml]. This explains the HBV infections with positive HBsAg among the vaccine non-responders.

Among vaccine responders [titers > 10 mIU/ml] (33.3%) were HBsAg positive. This is difficult to explain and probably needs further details regarding any other immunodeficiency in such individuals or false positive HBsAg. The test for HBsAg should be repeated in such individuals.

Among hemodialysis patients, controlled clinical trials conducted in different countries demonstrated efficacy of 53%--78% after preexposure immunization.\textsuperscript{139,140} However no efficacy was demonstrated in one trial performed in the United States.\textsuperscript{141}

Reinfection or reactivation of latent HBV infection has been reported among certain groups of immunosuppressed patients, including those who have undergone renal transplant and those infected with human immunodeficiency virus (HIV).\textsuperscript{142,143} These patients were positive for antibody to hepatitis B core antigen (anti-HBc), with or without
antibody to HBsAg (anti-HBs), and subsequently developed detectable levels of HBsAg.

Presence of 42.5% of Anti-HBc positive individuals in the vaccine non-protected group, despite occasional re-vaccination, suggests that such Anti-HBc reactive ESRD patients could be chronic carriers of HBV infections and are at risk of reactivation of HBV infection during immunosupresion following renal transplantation or by any exacerbation.

HBV exposure in such individuals without protective anti-HBs levels can lead to infection and the viral clearance may not be complete leading to chronic hepatitis B. Presence of low virus load in the blood may be responsible for nosocomial spread of HBV in the dialysis unit.

The prevalence of Anti-HCV did not show any relation with Anti-HBs titers although more percentage of Anti-HCV positive individuals were among the vaccine protected group.

No statistical significance was observed in the prevalence of HBV-HCV co-infection with the anti-HBs titers as equal distribution of prevalence was found among the protected as well as non-protected group.
CONCLUSION:

The present study concludes that:

Though the prevalence of HBsAg positive ESRD patients in the dialysis center in the present study is low, the rate of HBV and HCV exposure is significantly higher among End-stage renal disease patients on maintenance hemodialysis than the high-risk individuals.

The high prevalence of HBV may be because of lower vaccine protection among ESRD patients and higher rate of exposure during the process of hemodialysis or other parenteral route.

The high serological prevalence of HBV and HCV and their co-infection markers is associated with the duration of hemodialysis treatment. (Number of hemodialysis)

Presence of anti-HBc in patients with anti-HCV indicates silent HBV infections in these patients.

The recommended primary series of hepatitis B vaccine induces a protective anti-HBs response (defined as $\geq$10 milli-International Units [mIU]/mL) in all adults with normal immune status. The immunogenicity of hepatitis B vaccine is 100 % till first year of vaccination and the immunogenicity persists in 86.51 % up to 8 years of last vaccine inoculation.

Compared to adults with normal immune status, the proportion of ESRD patients on maintenance hemodialysis, who develop a protective antibody response to hepatitis B vaccine, is significantly
lower and this puts ESRD patients at greater risk of acquiring hepatitis B, either nosocomially or during blood transfusion.

Age is one of the determinants of vaccine response in ESRD patients and preexposure vaccination at an early age may offer better protection in the ESRD patients.

The study emphasizes the need for revaccination of vaccine non-responders among the ESRD patients by more frequent periodic estimation of their anti-HBs levels and taking extra precaution of vaccine non-responders before subjecting them to blood transfusion or renal transplantation.

Anti HBc testing of donors should be made mandatory before blood transfusion. This will further reduce the exposure of vaccine non-responders among ESRD patients to probable presence of HBV in HBsAg negative but anti-HBc positive blood.

More sensitive techniques, to detect very low viremia, should be applied in ESRD patients who have anti-HBc with or without protective titers of anti-HBs. This may help in detection of chronic carriers in the dialysis unit with very low HBsAg [viral load] that is not detected by the presently available kits. Studies should be carried out for prophylactic treatment with antiviral drugs in such patients to prevent reactivation of dormant infection after renal transplant. Isolation HBVDNA positive patients by using dedicated dialysis machines would further bring down the nosocomial spread of HBV in the dialysis unit.
HCV spread in the dialysis unit begins much before Anti-HCV antibodies rise to detectable levels in hemodialysis patients. There is a need to develop a test to detect HCV antigen before the seroconversion for the diagnosis of early HCV infection. Isolation of such patients may help in bringing down the spread of HCV in the dialysis units.

Renal transplantation facility is available at many nephrology centers in our country. Prevention of HBV and HCV infection before the patient is subjected to hemodialysis till the patient is taken for transplantation will result in longer and better graft and patient survival after renal transplantation.