Liver Disease in Renal Transplant Recipients

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INTRODUCTION
Liver disease is an important cause of morbidity and mortality in renal transplant patients. The spectrum of illnesses in these patients ranges from asymptomatic rise in transaminases, seen in 7-24% of renal transplant patients; to liver failure in 8-28% of the long-term survivors. Many factors contribute to the occurrence of liver disease in renal transplant patients. Some of the causes of liver disease are enumerated in Table 1. It is important to note that most of the causes listed result in acute hepatitis. Hepatitis B and C viruses and alcohol are the only causes of chronic liver disease. Azathioprine and cyclosporin can also result in chronic hepatitis. This distinction should be clearly made, when approaching any case of liver disease in a renal transplant recipient. This discussion will be restricted to hepatotropic viruses.

HOW DO THESE PATIENTS ACQUIRE THESE INFECTIONS?
Prior to the development of vaccination, hepatitis B was a major hazard for patients undergoing haemodialysis. Table 2 summarizes the extent of hepatitis infection in these patients. The relationship between duration of treatment and amount of blood exposure and development of hepatitis B is well documented. In the dialysis units, blood always remains in the extracorporeal circuit and HBV can thus be easily transmitted. Contamination by blood from gloves, dialysis machine surfaces and other environmental surfaces can lead to transmission of infection. Infection can also be transmitted through a graft from an infected donor.

Hepatitis C has now become more common in haemodialysis patients. The prevalence varies from 36% in North America and 39% in South America to 54% in Europe and 51% in Asia. However, with stringent blood banking protocols, this prevalence rate of HCV infection is expected to fall. Several risk factors have been identified for development of hepatitis C infection in haemodialysis patients. Studies have shown that patients who have been transfused more units of blood are at a higher risk of HCV infection. The duration of end stage renal disease, and logically, the duration of dialysis, also determine the risk of infection. Patients on peritoneal dialysis are at a much lower risk of acquiring hepatitis C infection than those on haemodialysis. Pretransplant tests in our dialysis unit showed a HBV DNA positivity of 12.1%. The HCV antibody positivity in the pretransplant patients was 8%. After transplantation, the HCV antibody prevalence doubled to 15%. The antibody response to HCV is poor in dialysis patients and there may be an underestimation of the actual incidence of disease.

BEHAVIOUR OF HEPATITIS VIRUSES UNDER IMMUNOSUPPRESSION
The role of immunosuppressants in liver disease has undergone a sea change. Prior to the identification of the hepatitis viruses, corticosteroids were used in the treatment of severe chronic hepatitis. However, subsequent studies found that immunosuppressants were not useful but deleterious to the patients with documented hepatitis B infection.

Hepatitis B
The behaviour of hepatitis B virus with concomitant use of corticosteroids has since then been well studied. With the use of

| Table 1: Causes of Liver Disease in Renal Transplant Patients |
|-------------------|-------------------|
| Infections
| Hepatotropic viruses | Hepatitis B, Hepatitis C, Hepatitis D |
| Other viruses | CMV, Herpes virus, EB virus, Varicella zoster |
| Drug toxicity | Azathioprine, Cotrimoxazole, cyclosporin, lipid lowering agents |
| Other causes | Alcohol abuse, hemosiderosis |
corticosteroids, there is an increased expression of hepatitis core antigen in the liver, an increase in serum HBV DNA levels and a decrease in ALT levels. Interestingly the levels of anti HBc seemed to decrease in a group of patients on prednisolone, reflecting depression of host immune responses. The host immune system determines persistence or clearance of hepatitis B, and the effect of corticosteroids on this system is of paramount importance. Evidence points out that T cell mediated immune mechanisms are involved in the pathogenesis of hepatitis B. In vitro studies on cell lines have shown increasing HBV DNA expression with the use of corticosteroids. Interestingly, this effect was not seen in the culture system with cyclosporin and azathioprine. These in vitro findings seem to suggest a glucocorticoid response element (GRE) in the HBV genome. The use of steroids enhances HBV replication through this element.

The long-term mandatory immunosuppression following renal transplantation can be detrimental to the hepatitis virus infected patient. The immunosuppression leads to enhanced HBV replication and impaired T cell function. The pathogenesis of the liver disease will become a cytotoxic virus mediated liver disease rather than an immune mediated liver disease.

Hepatitis C

T cell mediated immune mechanisms are also involved in the pathogenesis of hepatitis C. Similar to the effects on hepatitis B patients, long term use of corticosteroid therapy results in increased HCV viremia, with decreased ALT and hepatitis activity. However, no ALT flares developed after cessation of therapy. HCV RNA levels increase greatly after renal transplantation in patients with HCV infection, but the higher serum level of HCV RNA is not associated with more severe liver disease.

**Table 2 : Hepatitis B Infection In Patients Undergoing Dialysis**

| Prevalence of current or past infection | 50–67% |
| Incidence of new infections during first year | 40% |
| Clinically apparent acute illness | 32% |
| Chronic infection | 60–90% |
| HBSAg positive chronic infection | 53–61% |

**COURSE OF LIVER DISEASE IN RENAL TRANSPLANT PATIENTS**

Liver disease after renal transplantation is an important cause of morbidity, and its impact on survival of the transplanted patients needs to be emphasized.

**Hepatitis B**

A few important points need to be clarified before discussing the course of hepatitis B induced liver disease. The immunosuppression in the post transplant setting results in clinical and biochemical latency, with abnormalities being picked up only when the disease has progressed. Clinical and biochemical changes do not reflect the degree of histological change. It is thus important to have a liver biopsy at the time of transplantation, as this forms a basis for further histological surveillance.

The course of hepatitis B in renal transplant patients differs from that in immunocompetent individuals. As compared to 95% clearance of HBSAg in immunocompetent individuals, none of the patients clear this antigen in the post transplant setting.

Sequential biopsies taken at the time of renal transplant, one year later and three years later, show definite worsening of liver lesions. Liver histology was studied in 46 HBSAg positive patients. An early biopsy within 2 months following transplant showed evidence of chronic liver disease in 59% of patients. Biopsy done later, within a mean time of 43 ± 6 months, showed evidence of chronicity in 91%.

Serial biopsies in 19 HBSAg positive patients, followed up after renal transplant, showed progression to fibrosis in 12 patients. In a follow up study of 22 HBSAg positive immunosuppressed renal transplant patients, seven patients developed cirrhosis, six developed chronic active hepatitis and five developed chronic persistent hepatitis.

HBV DNA is deemed to be the best marker of viral replication. Reactivation of infection, marked by HBV DNA positivity occurred in 11 out of 12 patients who were initially HBV DNA negative. An increase of DNA was noted in 6 out of 11 patients who were HBV DNA positive initially. This is a direct evidence of increase in viral replicative activity in renal transplant patients while on immunosuppressive therapy.

The mortality of patients with hepatitis B is higher than that of patients without the infection. In 405 post renal transplant patients, the mortality was 45% in the group with hepatitis as compared with 16% in the non hepatitis group. The French series however, had a lower mortality of 17.5% in HBSAg positive patients as compared with 7.6% in the HBSAg negative patients.

Hepatoma, which is associated with hepatitis B infection, has also been reported in the transplant recipients. This is an additional cause of morbidity and mortality in these patients.
Hepatitis C
As in the case of hepatitis B, biochemical tests do not reflect the nature of HCV infection. Only 20% of virucenic renal transplant patients were found to have elevated ALT levels\(^5\). Similarly, in a follow up study, 51% of HCRNA positive renal transplant patients, maintained normal biochemistry throughout their follow up\(^5\).

Correlating with histological evidence of liver disease, only 26% of patients with abnormal liver function tests had evidence of chronic hepatitis on biopsy. Conversely, 60% of patients with normal liver function tests had chronic liver disease\(^6\). Again the obvious conclusion is that raised ALT level is not a good surrogate marker for worsening histological disease.

Liver biopsy shows a range of histological lesions, ranging from mild non-significant lesions, to severe lesions and even cirrhosis. The liver biopsy is usually performed in patients with abnormal liver function tests. In such a group of patients, prevalence of cirrhosis was found to be 23%\(^7\). On the other hand, if liver biopsy is done routinely, irrespective of liver function tests, the prevalence of cirrhosis is only 4%\(^8\). The other factor affecting the type of histology, is the timing of liver biopsy. In the patients studied by Hestin et al, the post transplantation time was longer with more severe hepatitis (Knoddel >5) as compared with those with mild hepatitis (Knoddel <5) (58±26 months vs 55±29 months). This indirectly reflects the duration of immunosuppression and subsequent duration of active viral replication.

The role of hepatitis C in patients’ survival is the key question in the management of these patients. Several studies have shown that hepatitis C infection does not alter survival of renal transplant recipients\(^10\)-\(^18\). Contrary to these studies, Pereira and colleagues reported a higher mortality in HCV infected renal transplant patients (41% vs 15%) and a 3.3 times risk of death in this group as compared with patients who were HCV negative before renal transplant \(^19\). Interestingly, the major cause of death was sepsis (78%) and not liver cell failure (11%). This study throws up the all-important question on whether one should offer transplant to hepatitis C patients. This question was addressed by Knoll et al who compared survival in 33 HCV positive patients who underwent transplant with that in 25 patients who were on maintenance haemodialysis\(^8\). The survival in the former group was significantly higher than that in the latter group (p=0.043). In India long term maintenance haemodialysis is difficult and costly, and transplantation may be the only option.

The other important question that arises, is the role of HCV infection on graft survival. In one study the graft loss was more in the anti HCV positive renal transplant recipients than in the anti HCV negative recipients (87% vs 74%)\(^9\). This finding has not been corroborated by other studies\(^12\) and the general consensus is that graft survival is not affected by HCV infection.

**PREVENTION OF INFECTION: ROLE OF VACCINATION**

The development of recombinant hepatitis B vaccine has provided the platform for effective prevention of hepatitis B after transplantation. However, there are many impediments to efficient vaccination. Hepatitis B vaccine is weakly immunogenic in the setting of renal failure, possibly due to inherent immunosuppression associated with the disease. As compared to protection of more than 95% in the immunocompetent persons\(^12\), the sero-responsiveness in patients undergoing haemodialysis was only 50%\(^9\). In the post renal transplant scenario, the concomitant use of maintenance immunosuppression affects response to the vaccine. The response rate was found to be only 17.6% in stable renal transplant recipients who received 3 doses of recombinant hepatitis B vaccine\(^9\). It is believed that there is a T cell defect in non-responders to hepatitis B vaccine\(^9\). Non responding cell cultures also did not secrete Interleukin 2 (IL2) in response to HBsAg stimulation\(^10\). However, addition of recombinant human IL2 (HuIL2) as an adjuvant to the hepatitis B vaccine did not result in a significant change in uremic patients who were previously non-responders to the vaccine\(^10\). Other adjuvants, which have been tried, are granulocyte macrophage colony stimulating factor (GM-CSF) in haemodialysis patients. Seven out of fifteen patients with concomitant GM-CSF showed significant increase in antibodies\(^11\).

Enhancement of the efficacy of vaccination has been attempted by increasing the number of doses. A seroconversion of 73% has been reported in haemodialysis patients with 4 doses of the vaccine\(^14\). Intra-dermal vaccine protocols have also been tried to increase rates of seroconversion\(^15\).

**THERAPY OF CHRONIC HEPATITIS IN RENAL TRANSPLANT PATIENTS**

**Interferon**

Interferon is the most widely studied therapeutic agent for chronic hepatitis. It has an antiviral action, probably by inhibition of viral protein synthesis. Interferon increases intracellular levels of2', 5', oligoadenylate synthetase that activates ribonuclease to cleave viral RNA\(^16\). It can also increase the expression of HLA antigens on infected hepatocytes, induce cytokine gene expression and also enhance the effector function of cytotoxic T-lymphocytes, natural killer cells and macrophages\(^17\). These actions act as a double-edged sword in the post transplant setting. Interferon can lead to graft rejection and can be potentially hazardous.

The effect of interferon and its role in graft rejection was first recognized in renal transplant patients receiving prophylactic interferon therapy for CMV infection\(^18\). Rostaing et al studied 16 renal transplant
patients with chronic hepatitis\(b\). Interferon was given in a dose of 3 MU thrice weekly. Six of these patients (27%) developed acute or subacute renal failure. Baseline creatinine levels went up from 105±31 mmol/L to 207±63 mmol/L in these patients. Renal biopsies showed diffuse interstitial oedema, dilatation of peritubular capillaries and scattered mononuclear cell infiltration. None of these were classical of cellular or vascular rejection. It is thus probable that interferon results in deterioration of graft function by not only stimulating rejection but by other hitherto unknown mechanisms as well.

This same group of investigators looked at viral kinetics in transplant recipients before and after alpha interferon therapy\(c\). They found a significant decrease in HCV RNA, one month after starting therapy. However, HCV RNA reappeared in all the patients after stopping therapy.

The effect of interferon in chronic hepatitis B is also dismal. There are isolated reports of successful interferon therapy\(d\). A long-term study on a mixed group of renal transplant patients was reported recently\(e\). Of the 42 patients, 11 had HCV infection alone, one had HBV infection alone, 3 with HBV and HDV, 12 with HBV and HCV and 2 patients with HBV, HCV and HDV infections. All these patients received interferon at a dose of 3 MU thrice weekly. None of these patients cleared the HCV infection. Four patients cleared the HBeAg. Even in this study acute rejection episodes occurred in seven patients and five patients lost their grafts.

In our institution we have started interferon on eight patients with chronic hepatitis, four with hepatitis B and four with hepatitis C. Careful in-patient monitoring was done initially on all these patients. None of the patients rejected their graft. Creatinine values remained stable in all these patients. However, none of these eight patients cleared their infection after 6 months of therapy.

Lamivudine

Newer approaches to treatment of hepatitis B include the use of nucleoside analogues that inhibit viral DNA replication. Lamivudine is the (−) enantiomer of 3′ thiocytidine. It is relatively non-toxic and reduces hepatitis B DNA levels dramatically, often to undetected levels\(f\).

Lamivudine as monotherapy for one year was tried on 358 immunocompetent patients\(g\). All these patients had pre and post treatment liver biopsies. At a dose of 100 mg per day there was a 98% reduction in HBV DNA levels, 16% conversion to anti-HBe status and a significant reduction in progression to fibrosis.

Six HBV DNA positive cadaveric renal transplant patients were given lamivudine 100mg a day for a period of six months\(h\). There was a rapid disappearance of HBV DNA in all these patients, normalization of ALT in four patients and no change in renal function and proteinuria. When lamivudine was stopped, biochemical and virological relapse occurred in all the patients. Similar results were also reported in a study of eight renal transplant recipients who were HBV DNA positive\(i\). None of these patients had any renal complications and no modification of immunosuppression was required.

A combination therapy of interferon and lamivudine has been tried in chronic hepatitis B and has not been found to be effective\(j\). Lamivudine treatment decreases viral DNA levels dramatically. This is associated with improved T cell responsiveness to HBeAg and HBeAg\(k\). It is believed that by decreasing the viral load, lamivudine breaks the state of hyporesponsiveness that is seen in the chronic hepatitis B patients. The possible mechanism of this hyporesponsiveness is deletion of the T cells by exhaustion, especially in the setting of high viral load.

Interferon is an inhibitor of T cell growth. Therefore, the concomitant use of both these drugs will decrease the beneficial effects of lamivudine. Marinos et al. found that the impaired T cell response was not restored in patients who were on the combination therapy with interferon and lamivudine as compared to those on interferon alone\(l\). Probably sequential therapy with lamivudine and interferon will provide us the answers to the efficacy of this combination therapy.

The long term effects of a prolonged lamivudine use and the development of resistance by mutations in the HBV polymerase gene are areas of concern. Resistance to lamivudine has been reported in liver transplant patients treated with lamivudine\(m\). The usual site of mutation is in the YMDD motif of the DNA polymerase gene\(n\). In immunocompetent individuals there is a 98% reduction in DNA levels with no rise in levels of the mutated virus. This indicates a reduced capacity of the mutated virus to replicate. This is not so with concomitant use of immunosuppression as in the post liver transplant situation. On lamivudine therapy there is an initial fall of DNA levels followed by breakthrough of viral replication to levels higher than pretreatment values\(o\). This may occur in the renal transplant patients who also are on prolonged immunosuppression. The long-term effects of prolonged lamivudine therapy in renal transplant patients have not been studied.

Ribavirin

Ribavirin is a synthetic guanosine analogue with a broad spectrum of activity over several viruses. Its mode of anti-viral action is unclear\(p\). It is also postulated to have several immunomodulatory actions and probably enhances the Th1 response\(q\). Treatment of patients with ribavirin alone has no effect on serum HCV RNA levels, but leads to transient decreases in ami-notransferase levels\(r\).

Two large controlled trials on the combination therapy of Ribavirin and interferon have been reported. The first trial randomized 912 patients to either inter-
feron alone or a combination of Interferon and Ribavirin. Each of these groups was further subdivided into 24-week and 48-week treatment periods. The sustained response after 24 weeks of therapy was 31% in the 24-week combination group and 38% in the 48-week combination group. In the combination group the sus-
tained response in the 24-week Interferon alone group was 6% as compared with 13% in the 48 week Interferon group. The second trial randomised 100 patients to each Interferon for 24 weeks or the com-
bination therapy for 24 weeks41. The sus-
tained response was 18% and 36% respec-
tively. Ribavirin and Interferon combina-
tion therapy appears to be an exciting pros-
tpect in the treatment of hepatitis C. There are no reported data on the use and effect of this combination in renal trans-
plant patients.

CONCLUSION
Liver disease in the renal transplant pa-
tient poses an important challenge to the treating physician. The risks of acquisi-
tion in the pre-transplant setting need to be re-emphasized. Stringent blood bank-
ing protocols, infection control in the di-
alysis units and active immunization of all chronic renal failure patients will go a long way in reducing the incidence of infection. Protocols for the treatment of chronic hepatitis infection control in the dialysis units and active immunization of all chronic renal failure patients will go a long way in reducing the incidence of infection. Treatment of chronic hepatitis in the renal transplant recipient is dismal especially in the setting of concomitant immunosuppression. Newer nucleoside analogues and possible combination thera-
pies will likely influence greatly the future of therapy of chronic hepatitis in renal transplant patients.

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