A STUDY ON SEROCONVERSION RATE FOLLOWING HEPATITIS B VACCINATION IN CHRONIC KIDNEY DISEASE POPULATION WITH CURRENT IMMUNIZATION STRATEGY

Dissertation submitted in partial fulfilment of

the requirements for the degree of

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CERTIFICATE

This is to certify that this Dissertation entitled, **"A Study On Seroconversion Rate following Hepatitis B Vaccination in Chronic Kidney Disease Population with Current Immunization Strategy"** is the bonafide original work done by Dr. E. Indhumathi, under our guidance and supervision in the Department of Nephrology, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai submitted as partial fulfillment for the requirement of D.M., (Nephrology) examination Branch III Nephrology, August 2014 of the Tamilnadu Dr.M.G.R Medical University Chennai.

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DECLARATION

I, Dr E.Indhumathi , solemnly declare that the dissertation **"A Study On Seroconversion Rate following Hepatitis B Vaccination in Chronic Kidney Disease Population with Current Immunization Strategy"** is the bonafide work done by me at Department of Nephrology, Madras Medical College under the expert guidance and supervision of **Dr.N.GOPALAKRISHNAN MD.,DM.,FRCP.** Professor of Nephrology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfilment of requirement for the award of D.M. Degree (Branch III) in Nephrology.

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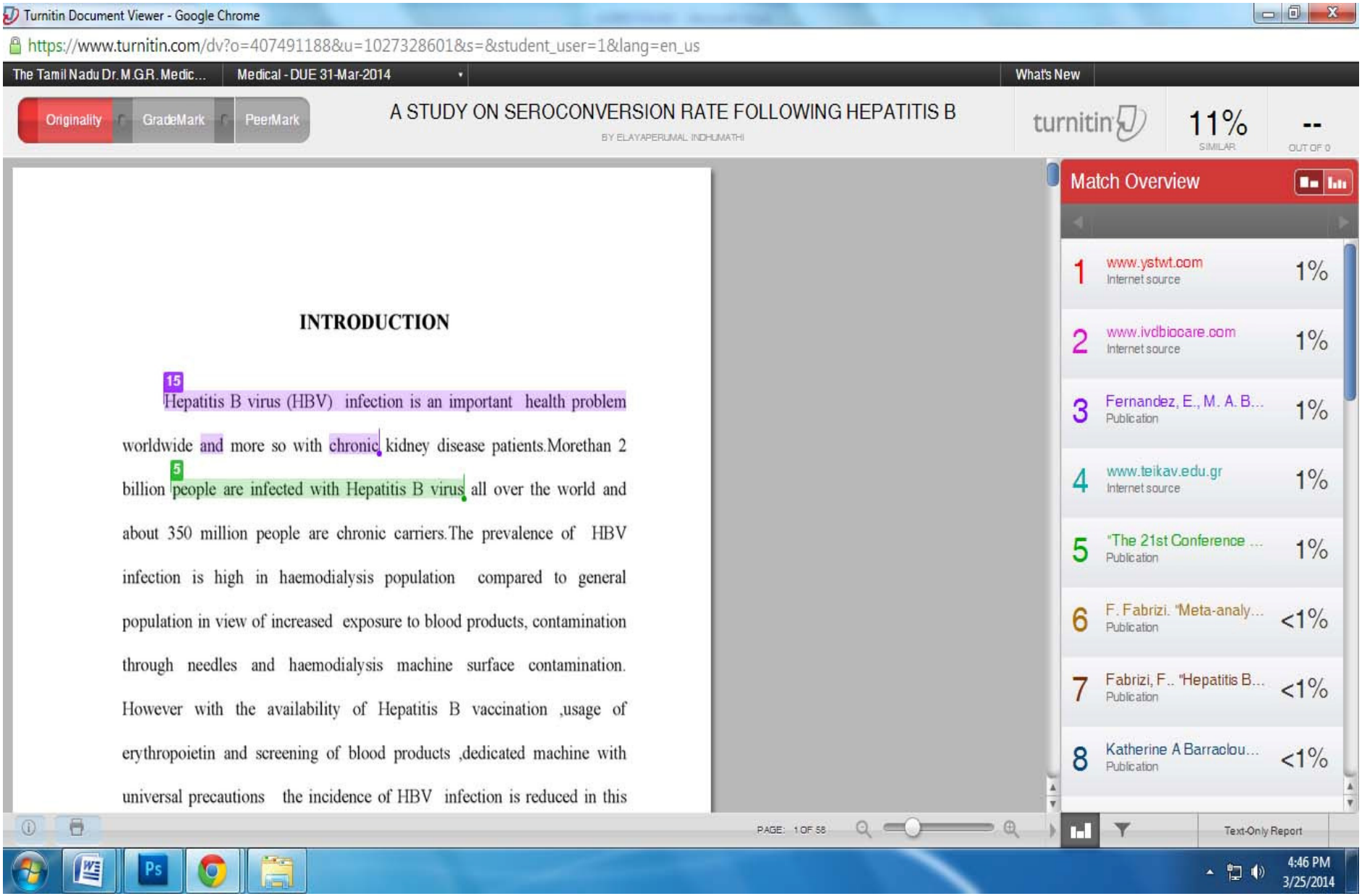
INTRODUCTION

Hepatitis B virus (HBV) infection is an important health problem worldwide and more so with chronic kidney disease patients.Morethan 2 billion people are infected with Hepatitis B virus all over the world and about 350 million people are chronic carriers.The prevalence of HBV infection is high in haemodialysis population compared to general population in view of increased exposure to blood products, contamination through needles and haemodialysis machine surface contamination. However with the availability of Hepatitis B vaccination ,usage of erythropoietin and screening of blood products ,dedicated machine with universal precautions the incidence of HBV infection is reduced in this population.Still HBV infection is a persistent problem and the immune response to vaccination is impaired.The seroconversion rate following Hepatitis B vaccination in healthy individuals is morethan 90% but in patients undergoing haemodialysis it is about 50% -60% . The centre for disease control (CDC) and prevention recommends Hepatitis B vaccination for patients with chronic kidney disease not requiring dialysis 3 doses at 0,1 and 6 months with 20 µg of each dose and for patients undergoing dialysis 4 doses at 0,1,2 and 6 months with 40 µg.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INDEX

INTRODUCTION

Hepatitis B virus (HBV) infection is an important health problem worldwide and more so with chronic kidney disease patients. More than two billion people are infected with Hepatitis B virus all over the world and about 350 million people are chronic carriers[1,2]. The prevalence of HBV infection is high in haemodialysis population compared to general population in view of increased exposure to blood products, contamination through needles and haemodialysis machine surface contamination [3]. However with the availability of Hepatitis B vaccination, usage of erythropoietin and screening of blood products, dedicated machine with universal precautions the incidence of HBV infection is reduced in this population.Still HBV infection is a persistent problem and the immune response to vaccination is impaired.The seroconversion rate following Hepatitis B vaccination in healthy individuals is morethan 90% but in patients undergoing haemodialysis it is about 50% -60% [4] . The centre for disease control (CDC) and prevention recommends Hepatitis B vaccination for patients with chronic kidney disease not requiring dialysis 3 doses at 0,1 and 6 months with 20 µg of each dose and for patients undergoing dialysis 4 doses at 0,1,2 and 6 months with 40 µg [5,6].

This study was done to measure the antiHBS antibody titre following 4 doses of 40 µg of Hepatitis B vaccine in haemodialysis patients and compare with 3 doses of 20 µg in healthy controls.

AIMS AND OBJECTIVES

1. To find out the sero conversion rate following hepatitis B vaccination in Chronic kidney disease population with current immunization schedule and compare it with adult healthy volunteers.

2. To analyze the impact of various factors on immune response following hepatitis B vaccination.

REVIEW OF LITERATURE

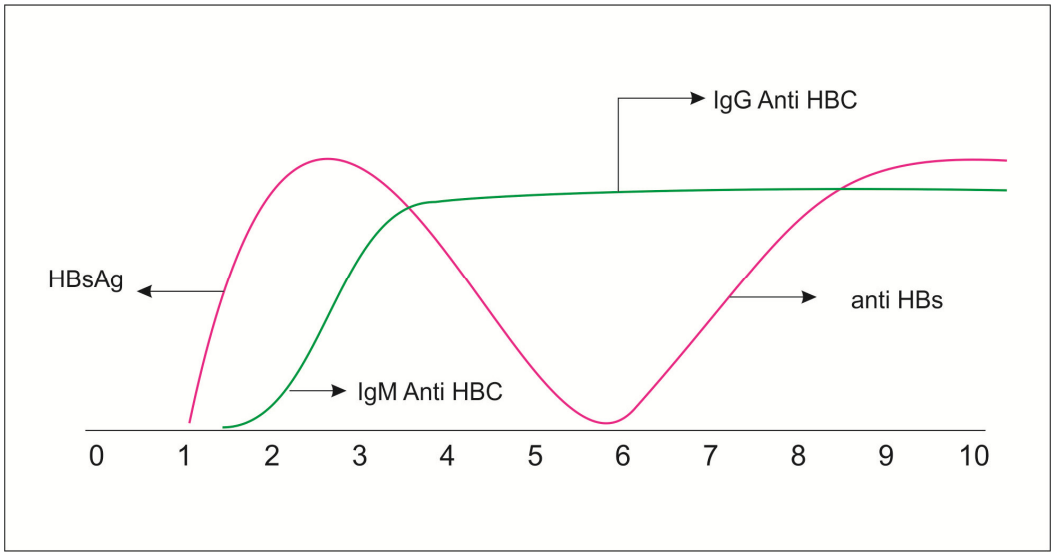
Infection is the second most common cause death in chronic kidney disease next to cardiovascular cause.This is because chronic kidney disease patients (CKD) are immunosuppressed with impaired immune response of both cellular and humoral immunity to infections. CKD patients when they undergo haemodialysis they are much more prone for infections than general population in view of exposure to blood .Infection due to hepatitis B virus (HBV) is one of the common infection that occurs in dialysis patients as they are blood borne pathogens.

HBV infection is a problem throughout the world and more than 2 billion people are affected. India is classified as having intermediate endemicity for HBsAg carrier state (2–7%) [(1)]

Hepatitis B Virus is a small DNA Virus with 7 genotypes. It's diameter is 45nm. The major route of transmission is through direct contact with blood or secretions. Also vertical transmission can also occur. In India the common route of HBV infection is vertical followed by horizontal transmission. The major genotypes are A&D with reports of C from eastern part of India [(7,8)]. The virus can live upto one week in the environment. After infection with Hepatitis B virus first antigen to appear in the blood is Hepatitis B surface antigen (HBsAg). This occur even before elevation of transaminases. This is present for 1-2 months commonly and rarely upto 6

months following which (anti HBS) antibody to HBsAg occurs which persists throughout life. During the window period, i.e. between the disappearance of HBsAg and appearance of anti HBS, the antibody to core antigen (anti HBC) present and that will be the only evidence for HBV infection. Initially IgM anti HBC antibodies appeared 1-2 weeks after HBsAg occurred followed by IgG anti HBC antibodies which persists life long. HBeAg, a serologic marker of active viral replication started appearing shortly after HBsAg and becomes undetectable before the disappearance of HBsAg. HBcAg is not detectable in the serum. Anti HBs is a protective antibody with the recovery from acute infection. HBsAg disappeared and antiHBs persists.Wherein,the persistence of HBsAg for more than 6 months is taken as chronic infection and anti HBs titer is not detected or present in very low level. [9,10]

Hepatitis B infection



Months after exposure

Hepatitis B infection can cause acute hepatitis, chronic hepatitis, cirrhosis liver and hepatocellular carcinoma. Hepatitis B virus infection is haemodialysis patients. Compared to earlier date, the incidence of HBV infection in haemodialysis unit has come down markedly due to more usage of erythropoietin rather than blood transfusion, routine screening of blood before transfusion and following universal precautions. But still the incidence is haemodialysis patients is more than general population. It is due to repeated exposure to blood and blood products, contamination through haemodialysis machine and usage of multidose vial in HD Unit.

Incidence:

Incidence of HBV infection in haemodialysis unit in developed countries has come down to < 1 % following universal precautions and intensive vaccination schedule and in less developed countries the incidence is 10-20%. [4,11] . Jha et al reported the incidence in India in haemodialysis patients as 20 – 45%.

In haemodialysis patients once infection occurs it is usually asymptomatic and liver enzymes are not elevated. 50-60% of them became chronic carriers and acts as a reservoir spreading the infection to other patients and medical staff personnel.

Prevention of HBV Infection in Haemodialysis Unit: (HD)

1. Universal Precautions:

According to CDC guidelines universal precautions have to be followed strictly in haemodialysis . Proper hand washing between patients, wearing eye goggles, gowns and gloves and changing of gloves between patients are essential in HD unit.

2. Segregation of HD Machine:

Patients infected with Hepatitis B virus infection should be dialyzed in a separate machine in a segregated room as per CDC guide lines. This is done to avoid contamination of HD machine surface to other machines and hence patients. Also multidose vials and sharing of stationary things between HBV positive and negative patients should be strictly avoided.

3. HBV Vaccination:

For the effective control of HBV infection in both general population and in HD unit, HBV vaccination plays an important role. Krugman in 1970 first detected that HBsAg is immunogenic and antibodies against HBsAg are protective. Thus HBV vaccination was found out. Initially it was obtained from the plasma of patients infected with Hepatitis B infection. But it increases the transmission of other infections also. Now, using recombinant technology, HBV vaccine is obtained. It has high patient safety

profile and highly immunogenic and when it given deep intramuscularly 3 doses at an interval of 0, 1, 6 months, the immune response will be more than 90%. [12,13]

In HD unit where the incidence of HBV infection is more, it is mandatory to give HBV vaccine to all HBV negative serology patients. CDC (Centre for Disease Control and Prevention) recommends HBV vaccination to be strictly adhered to as per intensified schedule.

Accordingly, 40 ug of HBV vaccine deep intra muscularly with 20 ug in each arm on 0,1,2 and 6 months to be given. Recently updated CDC guideline also recommends HBV vaccine to diabetic patients who are younger than 60 years old. Anti HBS titer to be checked 4 weeks after the last dose of vaccine and if less than 10mIU/m a booster dose has to be given. Periodically anti HBs titer to be measured and the titer should be maintained more than 10mIU/ml.

In spite of 40ug of HBV vaccine with four doses the immune response rate in haemodialysis patients is less 50-60% [14-16]. Even in patients who had immune response is >10mIU/ml, the anti HBs titer fall rapidly and thus protective for short duration only. The peaking level of anti HBs titer was also lower in HD patients compared to general population.[17,18]

If the needle used in HBV positive patients was inadvertently pricked the other patient or medical staff, immediately we need to check the anti HBs titer which is a protective antibody as the rate of transmission via needle stick is 30%. If the anti HBs titer is less than 10mIU/ml, they have to be given a booster dose of HBV vaccine. Also immunoglobulin need to be given immediately.

HBV infection can also be transmitted from medical staff if they are HBV positive. Hence it is mandatory that all medical staff in HD room should be vaccinated with 3 doses of 20ug each on 0,1 and 6 months and the titer should be checked periodically and if not protective level booster dose has to be given. [19, 20]

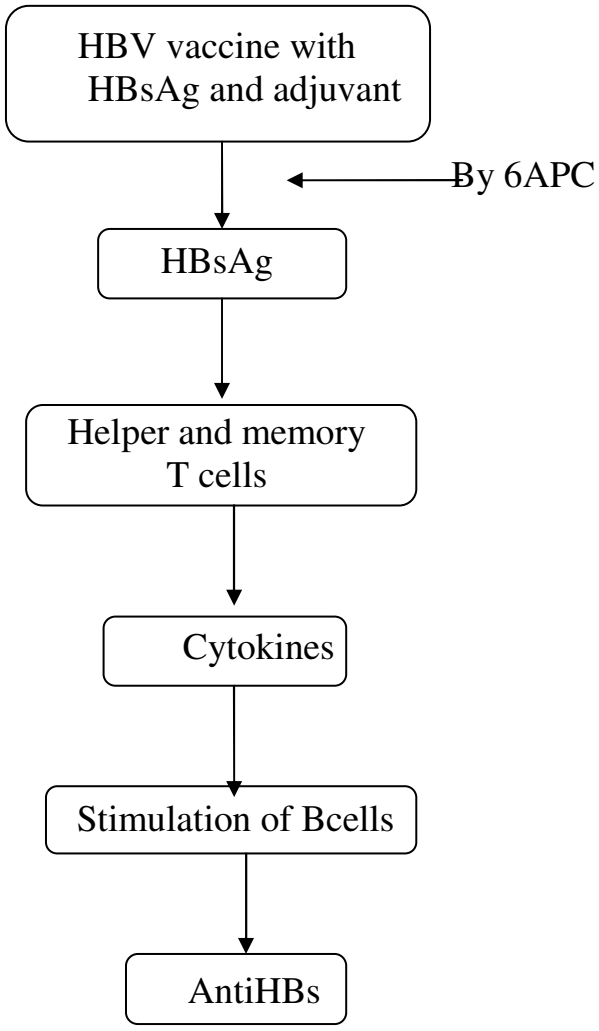
Immune response following HBV vaccination in haemodialysis patients:

Normally, following vaccination, the antigen in the vaccine is presented to T and B cells through antigen presenting cells such as dendritic cells. When T cells come in contact with HBsAg with increasd expression of costimulation ,T cells get activated and proliferation with the release of cytokines occur.The cytokines stimulate B cells which produces antibodies to HBsAg.

The adjuvant used in HBV vaccine is aluminium. The exact mechanism is unclear. It increases the uptake of antigen by antigen presenting cells, increases cytokine release from activated 'T' cells and increased proliferation of antigen specific 'T' cells. [21,22]

HBV vaccine mechanism

In normal persons



This normal immune response following vaccination is impaired in dialysis patients. [21] There is poor response following HBV vaccination even with intensified schedule and hence there is difficulty in controlling the infection in HD unit. The exact mechanism for the poor immune response is not known. However, Lim WH et al [22] in their study had shown that the function of the dendritic cell which is the antigen presenting cell presenting antigen to T cell is impaired in uraemia. It was also found that there is reduced activation of memory cells and the immune response has to depend only on circulating T cells in chronic kidney disease patients.[11,12,24]

Following vaccination in dialysis patients, the immune response is less with only 50-60% immune responders. The peak concentration of antiHBs titer is less and there is rapid fall of the titer in CKD patients. Also rapid seroconversion is impaired in such population and hence more susceptible for HBV infection. Hence patients need to be monitored frequently and booster doses have to be given repeatedly until the seroprotective rate of $\geq$ 10mIU/ml.[25-27]

Serez S et al and Ramezani A et al in their studies had shown that the antiHBs titer of $\geq$ 100 mIU/ml was protective and persisted for atleast one year, so that repeated booster doses can be avoided.[28-30]

Factors influencing the immune response:

There are various factors that negatively influence the immune response in CKD patients following vaccination.

1. Diabetic Mellitus and Immune response:

Diabetes mellitus is an immunocompromised state with reduced immune response to HBV vaccination. DM in CKD patients undergoing dialysis has an important factor that negatively influences the seroconversion.

In diabetes mellitus, there is reduction in the number of helper T cells and also impaired antigen presentation by dendritic cells. [30,31]

Sarah F. Schillie et al in their meta analysis including 2 randomized trials involving patients with and without chronic kidney disease on dialysis reported the immune response rate as 41.8% to 85.5% with mean of 60.1% in diabetics compared to 61.8% to 87.5% with mean of 75.1% in non-diabetic patients undergoing haemodialysis. Douvin et al in their studies had shown the immune response in diabetics following HBV vaccination was 94.4% using 4 doses of 0, 1, 2, 12 months of 20ug. Where in Bouter et al showed the response rate of 75.1%. The difference in response between the studies is due to difference in age group involved in both studies. Patients were much older in the study group with less immune response compared to

those with good immune response. In this meta analysis it was concluded that the older diabetic patients have less immune response compared to young diabetic individuals undergoing dialysis. There was association between the blood sugar control and duration of dialysis with immune response.[32, 33]

2) Malnutrition and Immune response:

The incidence of malnutrition of varying degrees in haemodialysis patients is high compared to non-dialysis patients. Many studies have proven beyond doubt that malnutrition negatively influences the immune response in dialysis patients. Malnutrition leads to inability to form antibodies following vaccination.[34,35]

Fernandez et al [36] showed the negative impact of malnutrition on immune response following HBV vaccination in dialysis patients. 64 patients undergoing haemodialysis were included and nutritional assessment done by measuring serum albumin level. The immune response rate was about 70% in patients with serum albumin of 4-4.5 gm/dl compared to only 12.5% in those with albumin of 3.01 to 3.5 gm/dl. In this study he also showed that the mortality is high in patients with low albumin and non-responders.

Fabrizi et al [37] did meta analysis of seven studies on the impact of nutritional status on HBV vaccination response in CKD patients. Study results showed that the poor nutritional status as estimated by serum albumin levels was an independent and adverse factor on immune response after HBV vaccination in chronic kidney disease patients.

Age and Immune response:

It has been shown in many studies that the immune response was low in advanced age in dialysis patients.With aging both cellular and humoral immunity reduced following antigenic stimulation.This is because of age related changes in immune system. [38,39]

Hans Kohler in his study reported that the association of less immune response following vaccination in dialysis patients was seen only in males and not in females. [40]

Fabrizi et al in the meta analysis of immune response in dialysis patients had found the negative correlation of age and immune response with overall relative risk was 0.74 in older dialysis patients.[41]

Some studies showed no association of advanced age and reduced immune response of older and younger dialysis patients.[42-44]

4) Immune response in early CKD:

It is well proven in many studies that the immune response following HBV vaccination was better in patients with early CKD staging compared to patients undergoing dialysis.[45]

Da Roza G et al [46] in a study of about 165 patients evaluated various factors influencing the immune response. The patients included were predialysis group with varying levels of GFR. The sero conversion rate in this group was 82% compared to vaccination to patients already initiated on haemodialysis, the response rate was 40-70%. In this study, it was shown that the higher the GFR, the higher the immune response. Agrawal S.K. et al in their study [47] showed a significant difference in sero conversion rate between patients with mild and severe renalfailure based on serum creatinine levels.

One Indian study by Shireen Siddique et a [48] analysed the sero protective rate following HBV vaccination in CKD patients with varying levels of serum creatinine stratified as mild, moderate chronic kidney disease and end stage renal disease. They found that the seroprotection was found in 90-100% of patients with mild and moderate CKD and 54.5% to 71% in patients with end stage renal disease. Therefore it is better to start HBV vaccination in CKD stage III itself when the immune response is good.

5) Obesity and Immune Response:

In obese individuals it was found that the immune response was lesser compared to non-obese patients. Reduced blood flow to adipose delay the antigen presentation to T cells leading to less immune response. Also the needle length may be shorter so that it may not pass through the fat and reach the muscle.[17]

6) Gender difference and immune response:

Most of the studies did not reveal any significant difference in immune response between different gender following vaccination. [26,49]

Hans Kohler had observed in his study of HBV vaccination response in dialysis patients that the response was more in female patients than in males but it was not statistically significant.[40]

Other factors such as native kidney disease, duration of dialysis and haematocrit had no effect on immune response following vaccination.[48]

Strategies to improve seroconversion rate:

In spite of higher doses of HBV vaccine in CKD patients on dialysis. the immune response rate was low and hence various methods have been adopted to increase immunogenicity.

1. Adjuventation:

Adjuvants are the substances that are added to the vaccine to increase immunogenicity. In the standard HBV vaccine, the adjuvant used is aluminium and the mechanism of which is unclear. Probably it stimulates T cells to increase cytokine release and hence increase B cell stimulation of antibody production.

Newer vaccines have been developed using different adjuvants that increase the antibody production rapidly, at a higher level and also persistently.

HBASO4:

NCT Kong in this study [50] evaluated the immune response in dialysis patients following adjuvant HBV vaccine HBV-AS04 and compared the response to 4 doses of 40ug of standard HBV vaccine. The immune response rate was rapid, higher and more persistent requiring less booster doses compared to standard vaccine. The antibody was persistent upto 42 months.

HBAS04 consists of recombinant HBV vaccine, aluminium phosphate and lipopolysaccharide content. This was obtained from the cell wall of Salmonella Minnesota [51] which was detoxified and then monophosphoryl lipid is obtained. This lipid content is added to the

HBAS04 vaccine.HBAS04 has been approved in Europe since 2005 for primary vaccination in patients with CKD of both dialysis and non-dialysis patients above the age of 15 years.

In this study NCT Kong had included 165 patients with 82 and 83 patients were given HBAS04 and standard HBV vaccine respectively. Single dose of HBAS04 and double the dose (40ug) of standard vaccine were given on 0, 1, 2 and 6 months to dialysis patients. They were followed up for 42 months. The sero protection rate at 1 month after completion of the vaccine schedule was similar 92.4 % vs 87% between 2 groups and there was no statistical difference between the groups.

These patients when followed up till 42 months had shown decline in anti HBs titer level but it was less with HBAS04 than standard vaccine so that the seroprotective level of titer (>100mIU/ml in this study) was present in 54.1% compared to 29% in standard vaccine at the end of 42 months. Therefore the requirement of booster doses ws less with HBAS04. The reactions that occurred with booster doses included minor symptoms like fatigue and pain at the infection site. Serious adverse effects like death and all happened rarely due to cardiovascular events.

HBAS02:

Another adjuvented vaccine in use is HBAS02. It consists of recombinant HBsAg with monophosphoryl lipid and Qs21. Qs21 increases the immunogenicity and it is a purified product obtained from the bark of a tree Quillaja Saponavia. HBAS02 is an oil based emulsion and does not contain aluminium.[51]

Murielle surquin in his study [52] included about 300 dialysis patients and compared 3 doses of HBAS02 with HB-AS04. The author reported that the sero protection rate (>100 mIU/ml) was rapid and higher at 1,2 and 7th month of vaccination. In terms of persistence of anti HBs titer, it was more with HB-AS02 at the end of 12 months (93.6%) compared to 78.6% with HB-AS04.

Monophosphoryl content of these adjuvented vaccines acts by binding to Toll like receptor-4 on antigen presenting cells. This leads to increased maturation and activation of the APCs and also enhances the expressionof costimulatory molecules on these cells. This further causes increased activation and proliferation of T cells with increased cytokines and hence enhanced antibody production.

Katherine A. Barraclough[53] analysed the immune response following HBAS04, HBAS02 and standard HBV vaccine in dialysis patients and

concluded by mentioning that the ability of adjuvented vaccines to improve the seroprotection rate of dialysis patients needs to be proven.

Also meta analysis done by Fabrizio Fabrizi et al [54] to evaluate the efficacy and safety of adjuvantation for HBV vaccine in patients with chronic kidney disease. He analysed ten studies and only prospective randomized trials were included.It did not reveal any significant increase in seroprotection rate with adjuvanted vaccine compared to control group of standard HBV recombinant vaccine.The results do not support for adjuvantation as a strategy to increase the seroprotective rate of HBV vaccine in this high-risk population.

2. Route of Administration:

HBV vaccine is given deep intramuscularly in all patients and in general population. Analysis in most of the studies was done following intramuscular route of administration of HBV vaccine. There are few clinical trials with intra dermal administration HBV vaccination as a way of increasing the immunogenicity is CKD patients.

In the skin there is higher concentration of both resident as well as recruited antigen presenting cells which can increase the immune response following vaccination more than in skeletal muscle. That is the rationale of giving intra dermal route rather than intramuscular route.

Katherine Barraclough [53] in their study reported that in dialysis patients who failed to respond to primary HBV vaccination with intramuscular rate, when given repeated doses of the vaccine intra dermally, was found to have better immune response rate. It was also shown that not only seroconversion rate but also the peak antibody titer and persistence of the antibodies were all found to have been more with intra dermal administration rather than intramuscular rate.

One Indian study from CMC, Vellore done by U. Anandh et al [55] had compared twice a week intra dermal administration to once a week intradermal administration of HBV vaccine. It was a randomized controlled trial. 85 patients were included and 77 completed the study. It was found that the sero protection rate was 77.8% with a twice a week ID administration compared to once a week ID vaccine. The immune response was found to be increased in patients receiving erythropoietin than those not receiving erythropoietin(78.1% vs 60%). The highest response rate was those getting twice a week ID vaccine as well as EPO also (86.7%).

In another study from Thailand [16], where they compared the ID administration with IM of HBV vaccination. It was reported that at 7th month the sero protective rate with ID route was 92% compared to 69% with IM route.

However the current guide lines advice against ID vaccination in view of insufficient data to support.

3) Addition of Growth Factor:

It has been found that addition of growth factor such as granulocyte macrophage colony stimulating factor (GM CSF) to HBV enhances the immune response. The exact mechanism is not clear but the proposed mechanism is by giving GM-CSF macrophages got activated, increased MHC class II expression on antigen presenting cells and enhance T and B cells activation and hence immune response. The dose of GM-CSF varies from 20-300ug.[56]

4) Role of Erythropoietin:

Erythropoietin plays a role in enhancing the immune response to HBV vaccine in dialysis patients by its effect on humoral and cellular immune system. Liu et al in their study showed that the immune response to HBV vaccine was enhanced by recombinant EPO.[57,58]

Also in the study by U. Anandh [55] showed that seroprotective rate was more in those getting EPO (78.1%) compared to those not getting EPO (60%).

It was also shown that intravenous iron therapy reduces the immune response.

Role of Levamisole

Levamisole is an immune modulator which increases the proliferation of natural killer cells and activated T cells enhancing the immune response following vaccination.[59,60]

Alavian and Tabatabaei in their metaanalysis had shown that when Levamisole was used as an adjuvant to HBV vaccine, the seroprotection rate was enhanced.[61]

To summarize, the incidence of HBV infection is still high in HD and it is highly recommended that intensified schedule of HBV vaccine to be given to all haemodialysis patients. It should be started in early CKD before the initiation of dialysis so that the response will be good. Anti HBs titer monitoring and regular booster doses are essential to maintain the seroprotectivity. Addition of adjuvants are novel strategies but need to be proven beyond doubt before regular use. Addition of growth factors, intra dermal administration may increase the immune response.

MATERIALS AND METHODS

This is a prospective comparative study comparing the sero conversion rate using 4 doses of 40µg of recombinant Hepatitis B virus vaccine (HBV vaccine) administered to chronic kidney disease Stage-V patients undergoing haemodialysis to healthy adult volunteers with 3 doses of 20µg recombinant HBV vaccine.

Study period was between Feb.2012 to January 2014.

Inclusion Criteria:

1. All patients with CKD-V undergoing maintenance haemodialysis at Madras Medical College & Rajiv Gandhi Govt. General Hospital who were given 4 doses of 40 µg of HBV Vaccine.
2. Healthy adult volunteers who were .given 3 doses of HBV vaccine.

Sero conversion rate was studied in both the groups 1 month after completion of vaccination schedule.

Exclusion Criteria:

1. Patients undergoing haemodialysis with HBSAg serology positive.
2. Patients already initiated on vaccination elsewhere and on irregular schedule.

- HBV vaccine was initiated on all patients undergoing maintenance haemodialysis with HBSAg negative serology at Madras Medical College & Rajiv Gandhi Govt. General Hospital with 40µg of recombinant HBV vaccine on 0, 1, 2, 6 months. (20 µg deep intramuscular in each deltoid).
- The control group included normal healthy adult volunteers negative for HBSAg serology.They were given 20µg of HBV vaccine deep intramuscular on 0, 1 and 6 months.
- Basic demographic data such as age, gender, BMI, duration of dialysis comorbid conditions such as DM, HT, CAD, CVA, history of smoking were collected. Clinical examination and laboratory parameters such as complete haemogram, blood sugar, urea, serum creatinine, serum electrolytes, serum total proteins, serum albumin, AST, ALT, bilirubin, lipid profile, Hepatitis C and HIV serology and calculation of URR were done.
- Hepatitis B (HBsAg) serology was done by HBsAg-card test which is a rapid, qualitative one-step immunoassay with a combination of monoclonal-dye conjugate (colloidal gold) with polyclonal solid phase antibodies to identify HBsAg .This test has a high degree of sensitivity. The whole blood was added directly to the sample pad. When the sample flowed through, the labelled antibody-dye

conjugate bound to HBsAg resulting in an antibody-antigen complex. The pad was in contact with a chromatographic test strip that contains a region of immobilised polyclonal anti-HBsAg antibody in the test line. The antibody-antigen complex formed a pink line of immobilised complex by the presence of antibody in the test line,showing the presence of HBsAg in the sample ;otherwise the test line will remain clear.

- Estimation of anti HBs titer four weeks after HBV vaccination was done by ELISA method.It was a one-step incubation with double antigen sandwich principle for quantitative detection of antibodies to hepatitis B surface antigen in serum .The anti-HBs ELISA kit used had polystyrene microwell strips pre-coated with recombinant HBsAg. Patient's serum was then added to the microwell with another recombinant HBsAg conjugated with Horse radish peroxidase (HRP) . With the presence of anti-HBs in the sample, both the antigens would bound to the antibody and during incubation, the specific immune complex formed was captured on the solid phase.After washing to remove unbound conjugates, chromogen solutions with tetramethylbenzidine and urea peroxide were added to the wells. In the presence of the antigen-antibody-antigen "sandwich" complex, the colourless chromogens are

hydrolyzed by bound HRP conjugate to blue coloured one. The blue colour turned into yellow after stopping the reaction with sulfuric acid. The amount of colour can be measured and was proportional to the amount of antibody in the sample. Wells containing samples negative for anti-HBs remained colourless.

- Primary outcome measure,the sero conversion is defined as anti-HBs antibody titer greater than 10mIU/ml one month after completion of vaccine schedule.

Statistical Analysis:

The data obtained were entered into SPSS 16 and analyzed.Categorical data were analyzed using chisquare test and continous variables with T test. P value less than 0.05 was taken as a significant one.

Ethical Clearance

Obtained from Institutional Ethics Committee, Madras Medical College Chennai.

Conflict of Interest : Nil

Financial Support : Nil

Limitation : Small Sample Size

RESULTS

Our study was a prospective comparative study comparing the sero conversion rate following 4 doses of 40µg of recombinant Hepatitis B virus vaccine (HBV vaccine) administered to chronic kidney disease Stage-V patients undergoing haemodialysis (N=34) to healthy adult volunteers with 3 doses of 20µg recombinant HBV vaccine (N=30). This study was conducted in the Department of Nephrology ,Madras Medical College between Feb.2012 to January 2014. Anti HBs titre was estimated 4 weeks after the last dose of vaccination.Patients with antiHBs titre ≥ 10mIU/ml were considered as immune responders and those with ≤ 10mIU/ml were considered as non responders.

DEMOGRAPHY

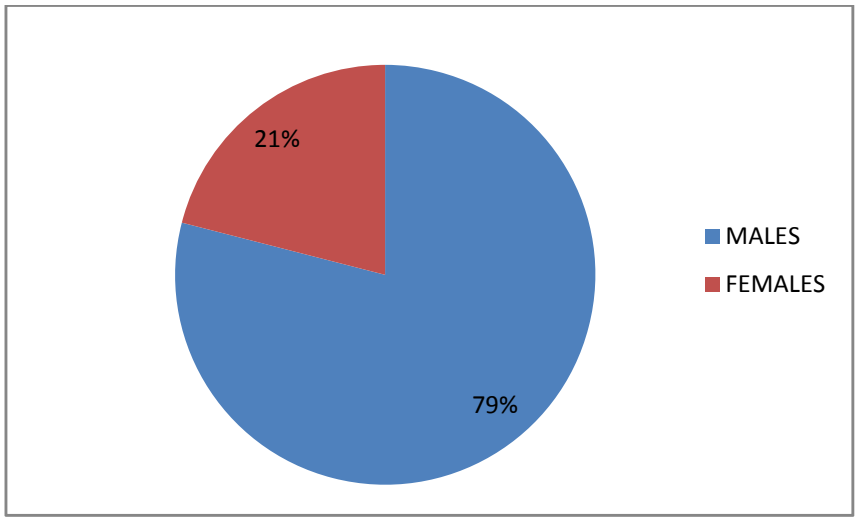
SEX DISTRIBUTION

Gender			AntiHBS		Total
			Non immune	Immune	
Gender	Female	Count	1	6	7
		Percentage	14.3%	85.7%	100.0%
	Male	Count	5	22	27
		Percentage	18.5%	81.5%	100.0%
	Total	Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P =0.793

Among 34 patients in the study group, 7 were females(20.6%) and 27 were males (79.4%).Thus men were the predominant group in this study.86% of females and 82% of males were found to be immune responders and there was no statistically significant difference between them.

SEX DISTRIBUTION



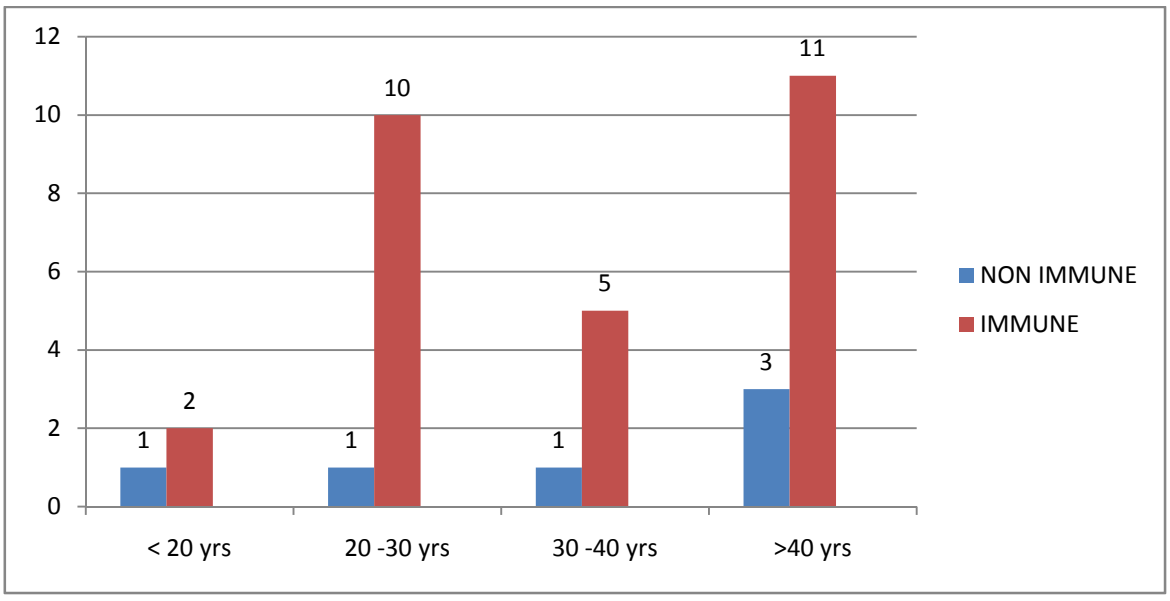
AGE DISTRIBUTION

AGE			AntiHBS		Total
			Non immune	Immune	
Age	< 20	Count	1	2	3
		Percentage	33.3%	66.7%	100.0%
	20 -30	Count	1	10	11
		Percentage	9.1%	90.9%	100.0%
	30- 40	Count	1	5	6
		Percentage	16.7%	83.3%	100.0%
	> 40	Count	3	11	14
		Percentage	21.4%	78.6%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P=0.752

Age varied from 18 to 64 years with age of 35.88±12.13.Majority of them were more than 40 years old (41.2%) followed by 20-30 years age group(32.4%).There was no significant difference regarding immune response between different age groups.

AGE DISTRIBUTION

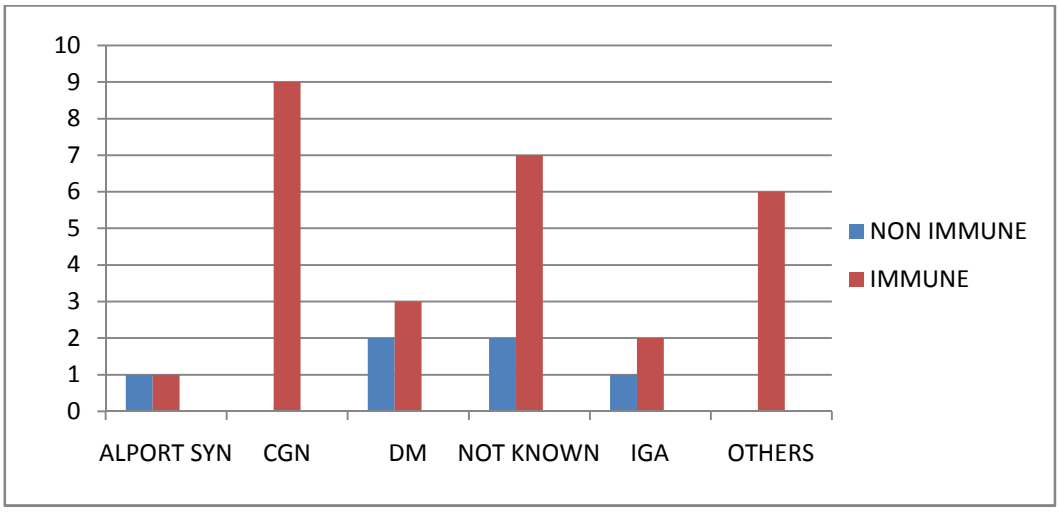


NATIVE KIDNEY DISEASE:

Native kidney disease			AntiHBS		Total
			Non immune	Immune	
NKD	ALPORT	Count	1	1	2
		Percentage	50.0%	50.0%	100.0%
	CGN	Count	0	9	9
		Percentage	0.0%	100.0%	100.0%
	DM	Count	2	3	5
		Percentage	40.0%	60.0%	100.0%
	NOT KNOWN	Count	2	7	9
		Percentage			100%
	IGA	Count	1	2	3
		Percentage	33.3%	66.7%	100.0%
	OTHERS	Count	0	6	6
		Percentage	0.0%	100.0%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P=0.247

Chronic glomerulonephritis was the most common cause for chronic kidney disease in this study group(26.5%) followed by diabetic nephropathy (14.7%).Etiology was not known in 26.5% of patients. None of the disease was found to be significantly associated with immune response following HBV vaccine.p=0.247.



DURATION OF DIALYSIS:

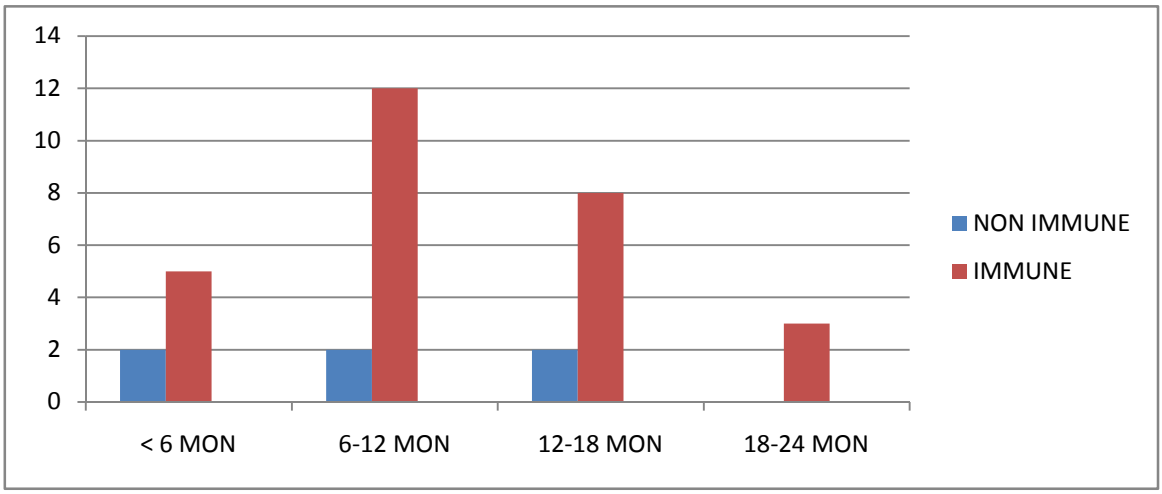
Duration of dialysis varied from 5 months to 23 months with mean of 11.8±7.07 and most of them had undergone 6- 8 months (70.5%) of dialysis. This study did not show significant association between duration of dialysis and immune response.(p=0.714).

Duration of Dialysis

DURATION OF HD IN MONTHS		Anti HBS		Total
		Non immune	Immune	
≤ 6	Count	2	5	7
	Percentage	28.6%	71.4%	100.0%
6 -12	Count	2	12	14
	Percentage	14.3%	85.7%	100.0%
12 - 18	Count	2	8	10
	Percentage	20.0%	80.0%	100.0%
18 - 24	Count	0	3	3
	Percentage	0.0%	100.0%	100.0%
Total	Count	6	28	34
	Percentage	17.6%	82.4%	100.0%

P=0.714

Duration of Dialysis



P=0.714

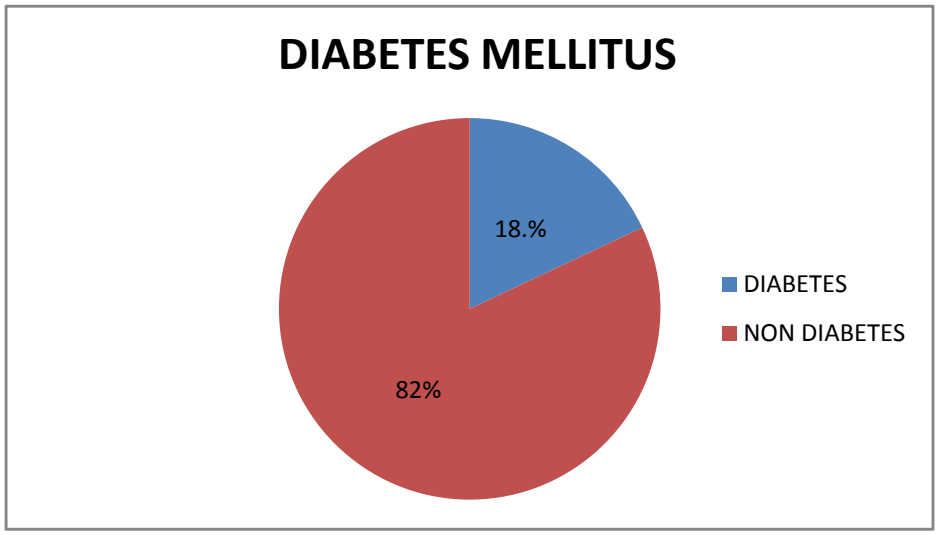
COMORBID CONDITIONS:

DIABETES MELLITUS

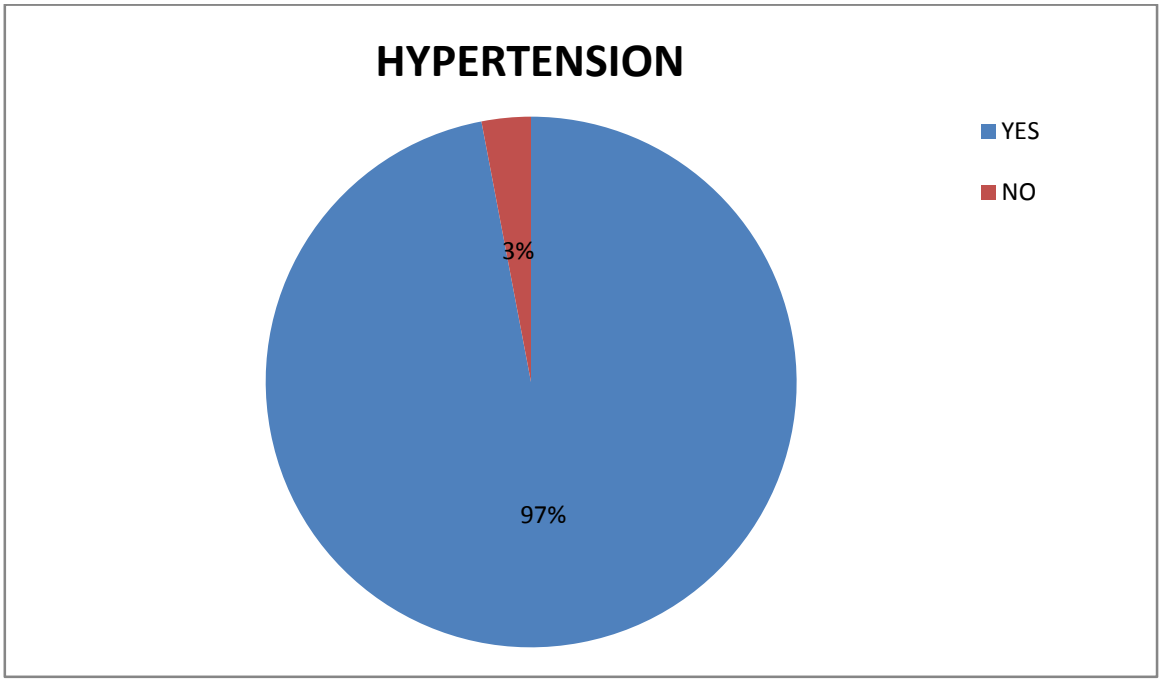
	Diabetic status	AntiHBS		Total
		Non immune	Immune	
NON DM	Count	4	24	28
	Percentage	14.3%	85.7%	100.0%
DM	Count	2	4	6
	Percentage	33.3%	66.7%	100.0%
Total	Count	6	28	34
	Percentage	17.6%	82.4%	100.0%

P=0.267

Most of the patients in this study group were non-diabetic (82.4%)) and only 17.6% were diabetic. Among 6 diabetic patients diabetic nephropathy was the underlying cause for chronic kidney disease in 5 of them. 85.7% of patients among non diabetics were immune responders compared to 66.7% among diabetics.



HYPERTENSION:



P=0.638

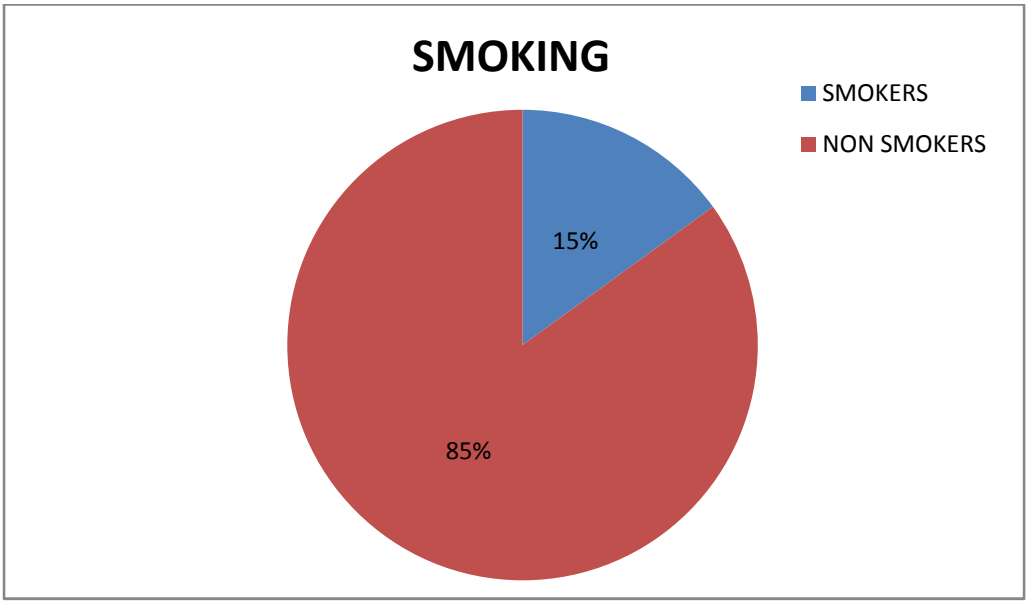
Almost all the patients in the study group had hypertension (97%) requiring 1 to 4 antihypertensives except one (3%).

SMOKING:

SMOKING		AntiHBS		Total
		Non immune	Immune	
Non smokers	Count	5	24	29
	Percentage	17.2%	82.8%	100.0%
Smokers	Count	1	4	5
	Percentage	20.0%	80.0%	100.0%
Total	Count	6	28	34
	Percentage	17.6%	82.4%	100.0%

P=0.881

History of smoking was present in 5 patients (15%) and the remaining had denied the history of smoking (85%).Immune response rate was 83% and 82% between smokers and non smokers and it was statistically not significant.



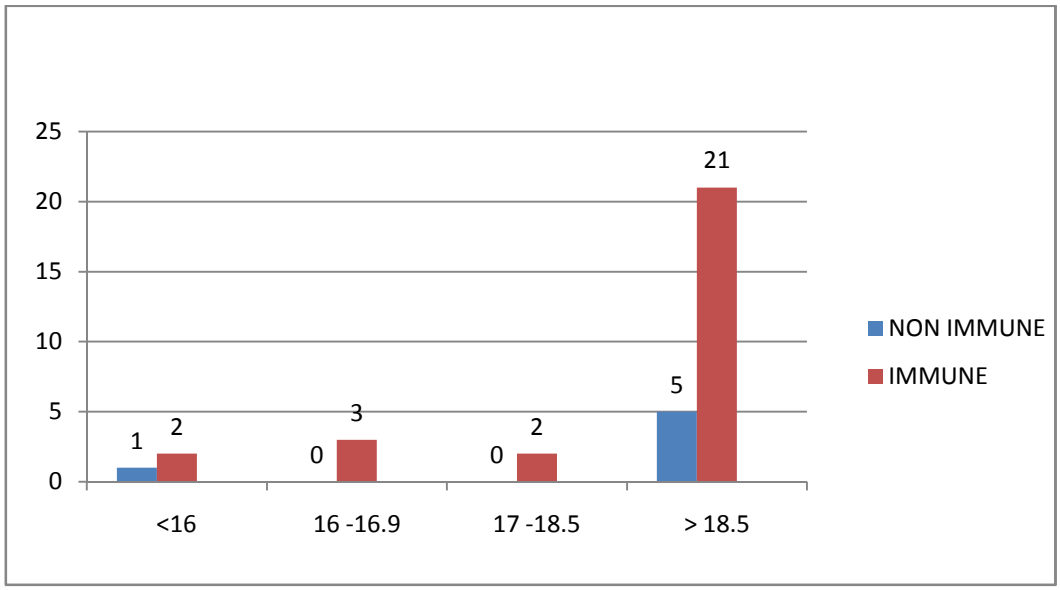
BODY MASS INDEX:

BMI		AntiHBS		Total
		Non immune	Immune	
< 16	Count	1	2	3
	Percentage	33.3%	66.7%	100.0%
16 -16.9	Count	0	3	3
	Percentage	0.0%	100.0%	100.0%
17 - 18.5	Count	0	2	2
	Percentage	0.0%	100.0%	100.0%
> 18.5	Count	5	21	26
	Percentage	19.2%	80.8%	100.0%
Total	Count	6	28	34
	Percentage	17.6%	82.4%	100.0%

P = 0.654

Body mass index varied from 12.4 to 28.1 with mean of 20.1 ± 3.24. 8 patients were malnourished with three of them had severe malnutrition (BMI < 16). Immune response was only 66.7% in severely malnourished patients compared to 80.8% in patients with BMI more than18.5

BODY MASS INDEX



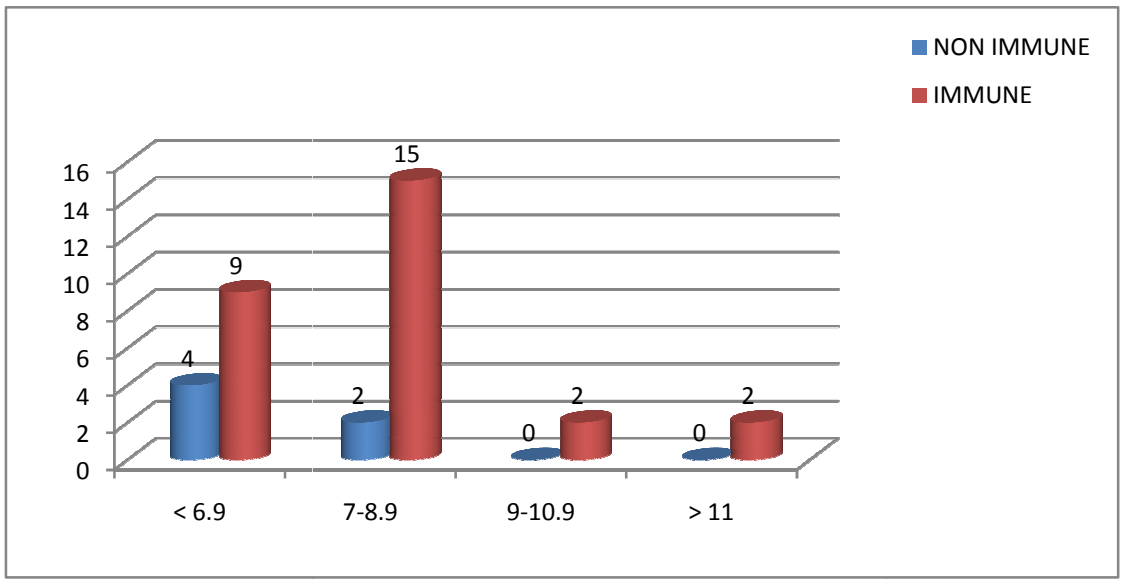
BLOOD HAEMOGLOBIN:

Most of the patients (94%) were anemic with hemoglobin ranged from 4.7 to 11.6 gm/dl with mean of 7.28± 1.66. Only two patients (6%) had hemoglobin more than 11.gm/dl. Immune response was 100% in patients with hemoglobin more than 11.gm/dl

HB LEVEL		AntiHBs		Total
		Non immune	Immune	
< 6.9	Count	4	9	13
	Percentage	30.8%	69.2%	100.0%
7 - 8.9	Count	2	15	17
	Percentage	11.8%	88.2%	100.0%
9 -10.9	Count	0	2	2
	Percentage	0.0%	100.0%	100.0%
> 11	Count	0	2	2
	Percentage	0.0%	100.0%	100.0%
Total	Count	6	28	34
	Percentage	17.6%	82.4%	100.0%

P=0.423

HAEMOGLOBIN LEVELS



BLOOD UREA:

Blood Urea level varied from 58 mg/dl to 272 mg/dl with mean of 134.08± 55.80. 21patients had blood urea of more than 100mg/dl.

SERUM CREATININE

CREATININE LEVEL			AntiHBS		Total
			Non immune	Immune	
CREATININE	< 5	Count	1	4	5
		Percentage	20.0%	80.0%	100.0%
	> 5	Count	5	24	29
		Percentage	17.2%	82.8%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P = 0.88

Serum creatinine varied from 2.6 to 13.9 mg/dl with the mean of 7.45 ± 2.66. 29 Patients (85.3%) had Serum creatinine of more 5 mg/dl. There was no significant difference regarding immune response between creatinine less than 5 or more than5 mg/dl.

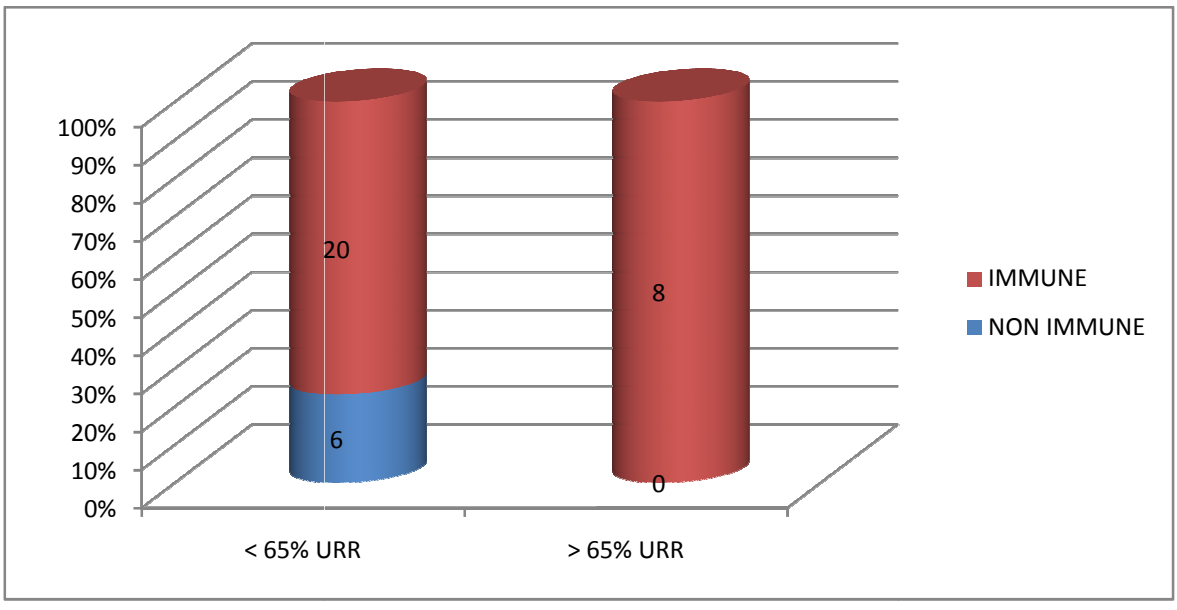
ADEQUACY OF DIALYSIS:

ADEQUACY			AntiHBS		Total
			Non immune	Immune	
URR	< 65	Count	6	20	26
		Percentage	23.1%	76.9%	100.0%
	> 65	Count	0	8	8
		Percentage	0.0%	100.0%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P = 0.13

Most of the patients (76.5%) were inadequately dialyzed with Urea reduction ratio (URR) of less than 65%. Immune response was 100% when URR was more than 65%.

ADEQUACY OF DIALYSIS



SERUM ALBUMIN:

ALBUMIN			AntiHBS		Total
			Non immune	Immune	
ALBUMIN	< 4	Count	5	24	29
		Percentage	17.2%	82.8%	100.0%
	> 4	Count	1	4	5
		Percentage	20.0%	80.0%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P = 0.88

85% of patients undergoing dialysis had hypoalbuminaemia (< 4gm/dl) with only 15% had albumin level > 4gm/Dl. However the immune response rate was almost similar between the groups.

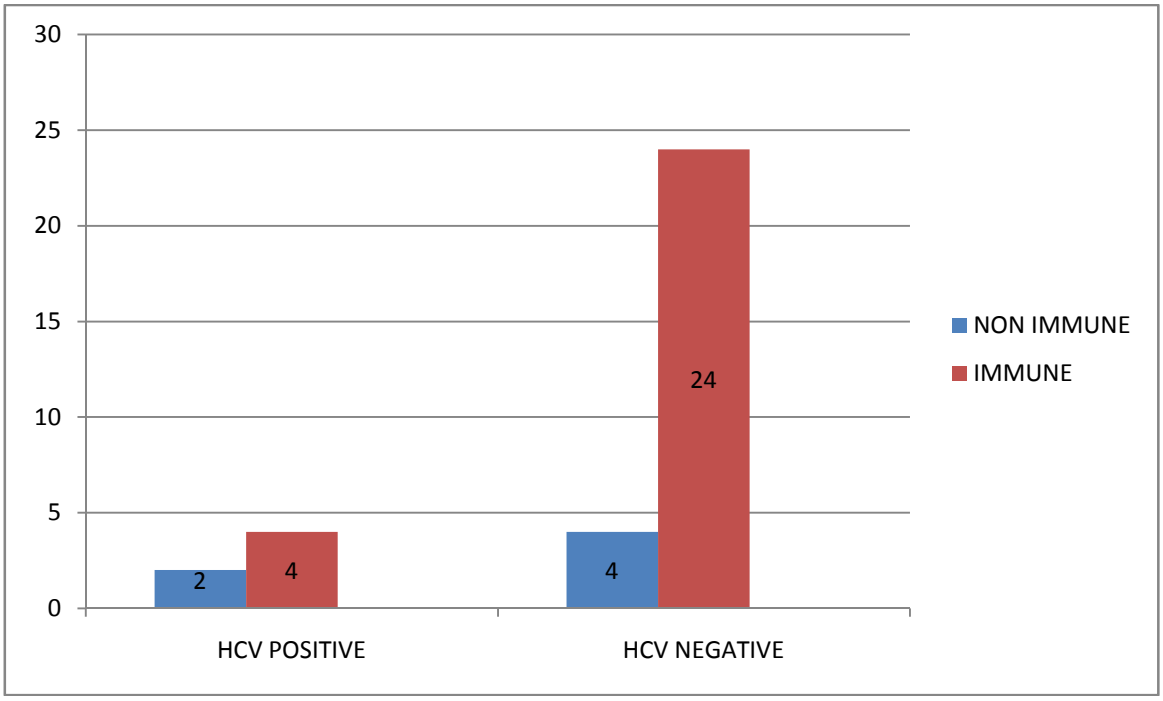
HCV SEROLOGY:

HCV STATUS			AntiHBS		Total
			Non immune	Immune	
HCV	Negative	Count	4	24	28
		Percentage	14.3%	85.7%	100.0%
	Positive	Count	2	4	6
		Percentage	33.3%	66.7%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

P=0.881

Among the study group patients 6 were (17.6%) HCV positive. Majority of HCV negative Serology patients (87.5%) have developed protective HBV immunity (>10mIU/ml) compared to 66.7% in HCV positive patients.

HCV STATUS



ACCESS INFECTION

ACCESS INFECTION			AntiHBS		Total
			Non immune	Immune	
ACC INFECTION	Absent	Count	6	24	30
		Percentage	20.0%	80.0%	100.0%
	Present	Count	0	4	4
		Percentage	0.0%	100.0%	100.0%
Total		Count	6	28	34
		Percentage	17.6%	82.4%	100.0%

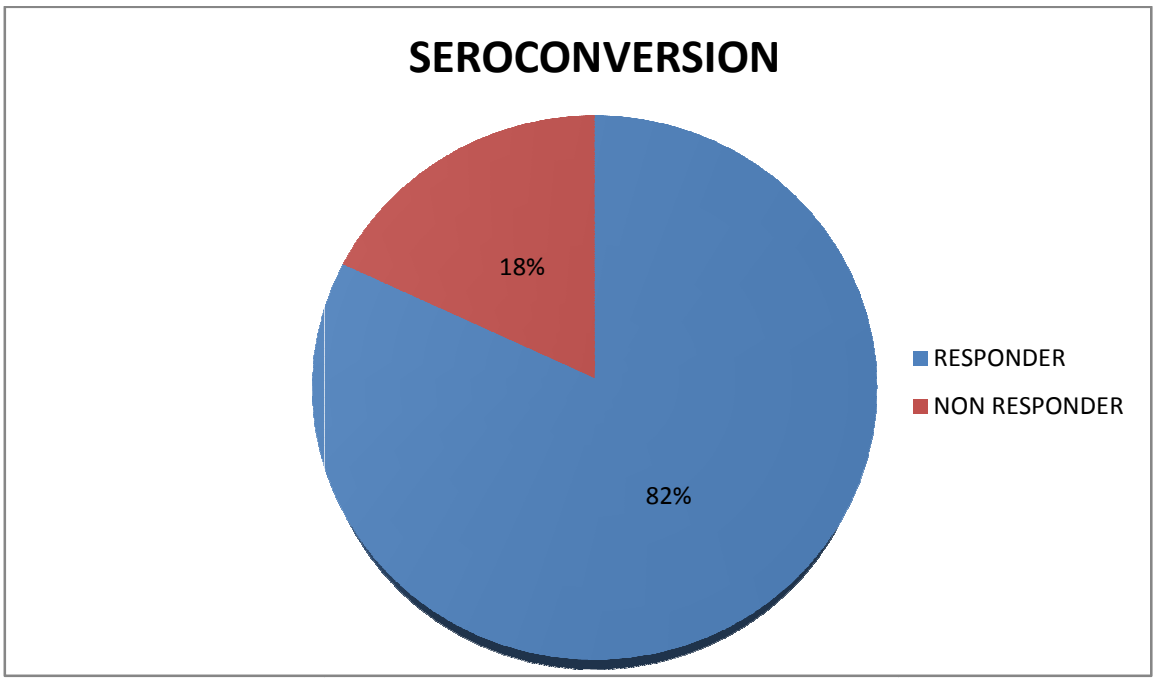
P = 0.324

Access Infection was present in 4 patients (11.8%). But all of them had antiHBs titers more than 10mIU/ml.

ANTIHBs TITER:

IMMUNE STATUS		Frequency	Percent
Valid	Non immune	6	17.6
	Immune	28	82.4
	Total	34	100.0

AntiHBs titer varied from 5.7 to 79.4 mIU/ml with mean of 24.71±16.09 in dialysis patients. Six of them (17.6%) had antiHbs titer < 10mIU/ml and 28 (82.4%) of patients had titer > 10 mIU/ml.



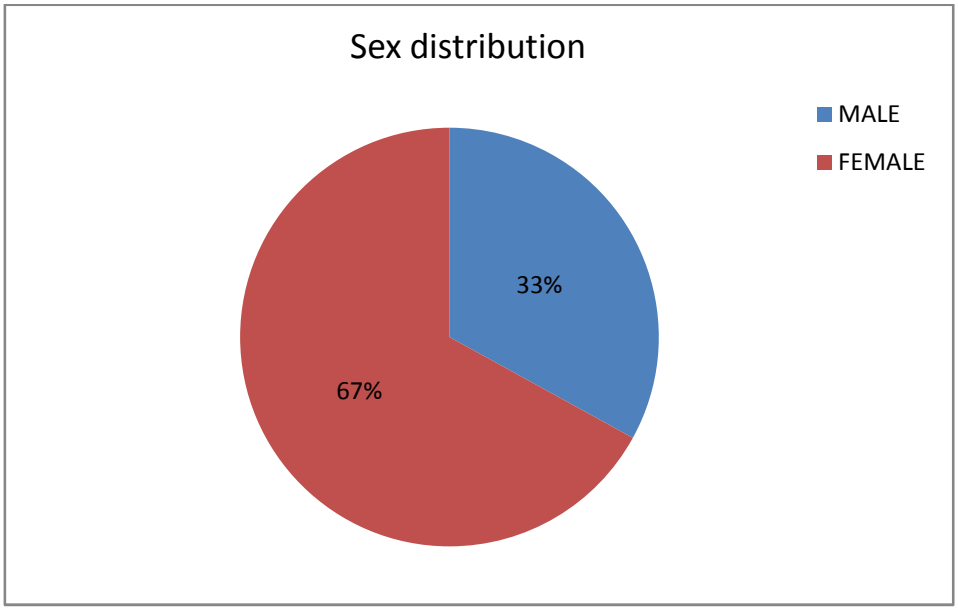
CONTROL GROUP

30 healthy adult volunteers were included in this group . None of the persons in the control group had diabetes mellitus, hypertension or history of smoking. Blood sugar , blood urea and Serum Creatinine were with normal limits. Hepatitis-C and HIV Serology were negative for all of them.

SEX DISTRIBUTION:

Gender		Frequency	Percent
Gender	Female	20	66.7
	Male	10	33.3
	Total	30	100.0

20 of them were females (67%) and 10 were males (33%)

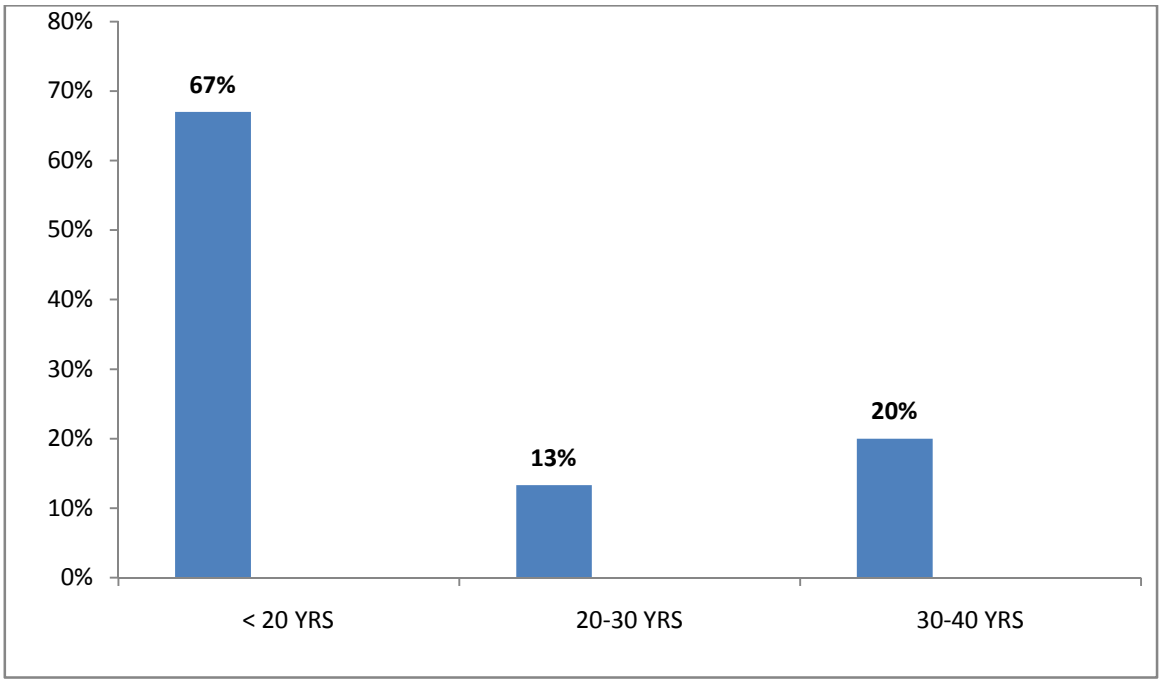


AGE DISTRIBUTION:

Age		Frequency	Percent
Age	**< 20**	20	66.7
	20-30	4	13.3%
	30-40	6	20%
	Total	30	100.0

Age varied from 18 to 40 years with mean of 23.33±6.9 and most of them were less than 20 years (67%)

AGE DISTRIBUTION

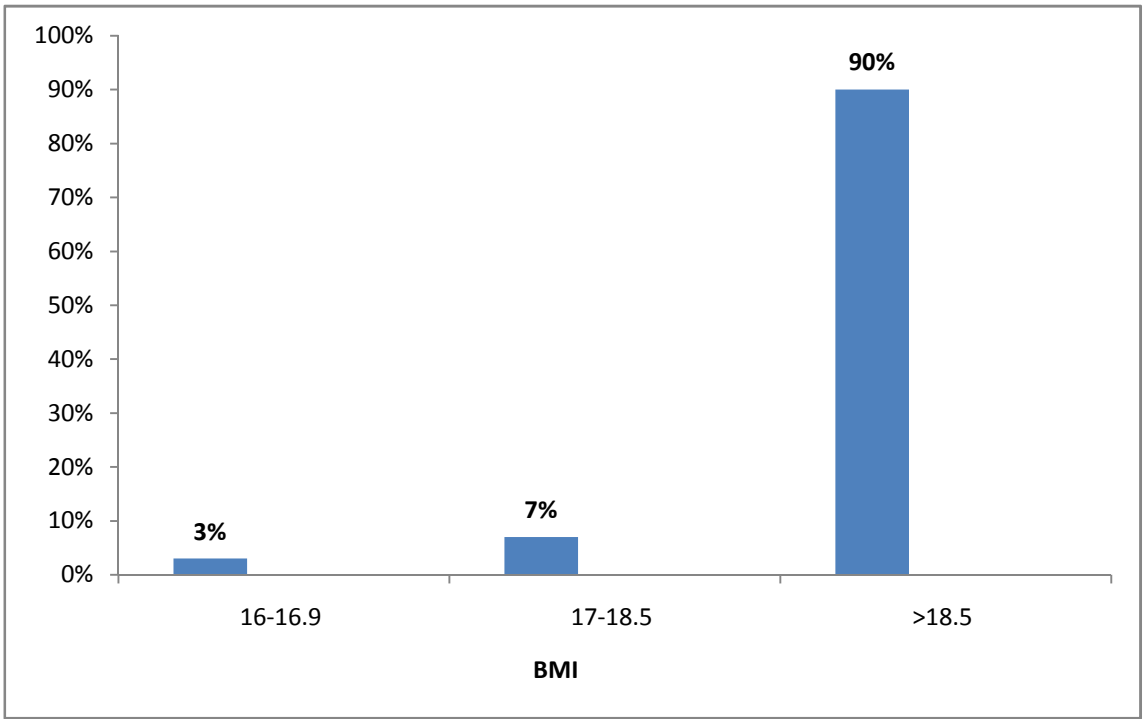


BODY MASS INDEX :

BMI		Frequency	Percent
Valid	**16 - 16.9**	1	3.3
	17 - 18.5	2	6.7
	> 18.5	27	90.0
	Total	30	100.0

BMI varied from 16.9 to 30.4 with mean of 22.74 ± 3.48. Most of them (90%) had BMI of more than 18.5 and only 10% had mild malnutrition.

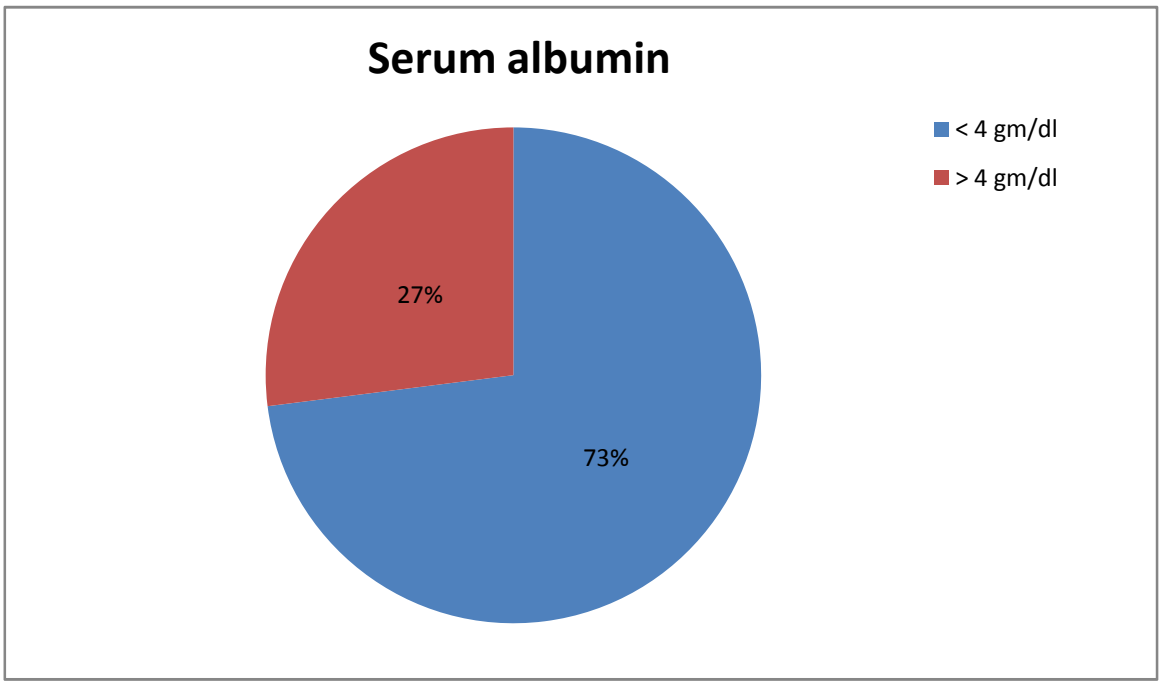
BODY MASS INDEX



SERUM ALBUMIN :

Serum albumin level (gm/dl)		Frequency	Percent
Valid	< 4	22	73.3
	> 4	8	26.7
	Total	30	100.0

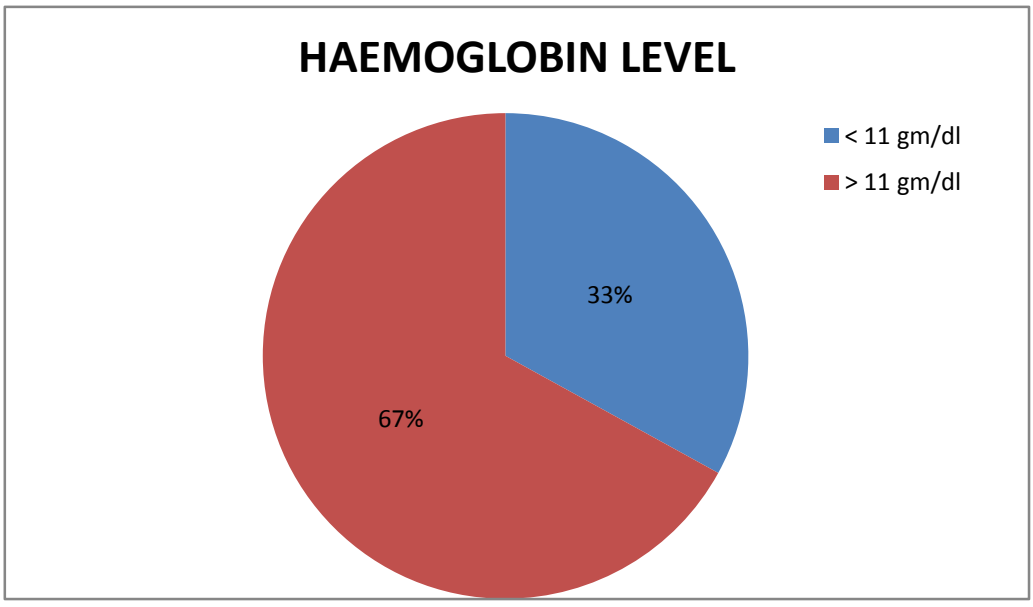
Serum Albumin level ranged from 3.1 to 4.3 wih mean of 3.63 ± 0.42. Most of them (73%) were hypoalbuminaemic.



BLOOD HAEMOGLOBIN

Blood Haemoglobin level		Frequency	percentage
Valid	< 11gm/dl	10	33
	>11gm/dl	20	67

Blood Haemoglobin level varied between 9 and 14.4 gm/dl with mean of 11.85 ± 1.7. 20 (67%) had haemoglobin level more than 11 gm/dl and 10 (33%) of them had less than 11 gm /dl.But all of them were immune responders.



SEROCONVERSION RATE

AntiHBs titer:

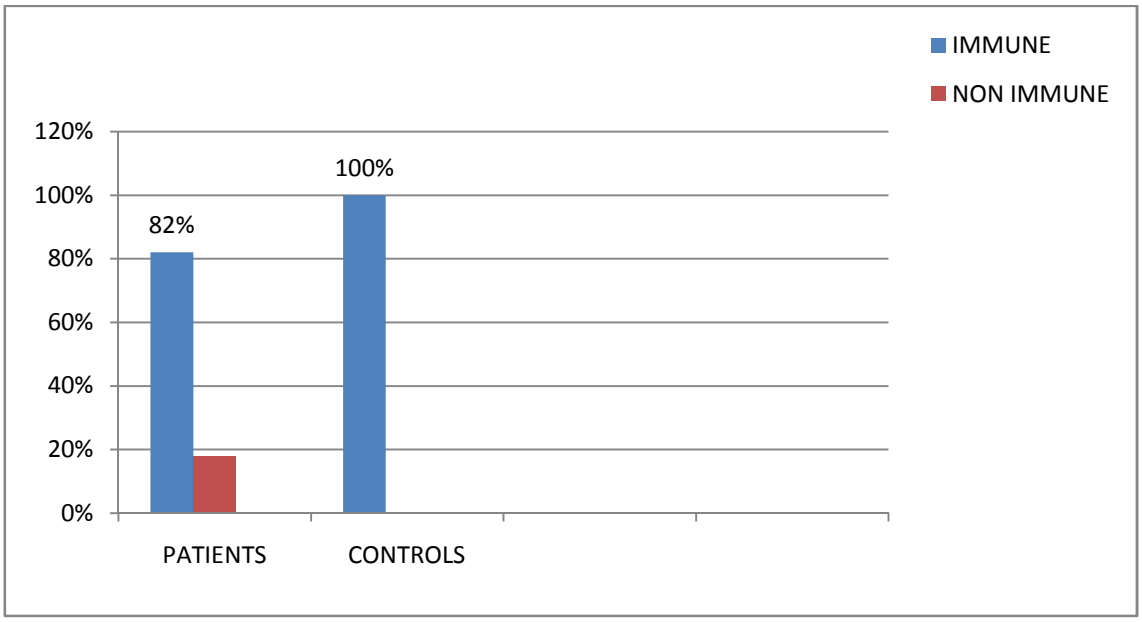
Group	N	Mean		Std. Deviation	Std. Error Mean
ANTI HBS	**Patients**	34	24.712	16.0995	2.7610
	Controls	30	31.870	20.4259	3.7292

P=0.123

AntiHBs titre varied from 10.1 to 76.4 mIU /ml with mean of 31.9±20.4. All of them had antiHbs titre more than 10mIU/ml(100%). Comparing the immune response in the study group and control group, in the control group the immune response was 100% and in the study group it was 82.4%.

PATIENTS AND CONTROLS

IMMUNE RESPONSE



Immune response was 100% in healthy adult control group and it was 82% in chronic kidney disease patients undergoing dialysis.

The mean antiHBs titer level of patients was 24.7 and in healthy adult control group it was 31.8.

DISCUSSION

Hepatitis B virus infection is an important health problem worldwide with nearly 2 billion people infected and about 350 million chronic carriers[1,2]. Though there has been a marked reduction in the incidence of hepatitis B virus infection in hemodialysis units compared to earlier data , probably due to screening of blood donors, decrease in blood transfusion requirements with more use of erythropoietin and the development of guidelines for infection control and vaccination, still there is a higher prevalence of HBV infection among hemodialysis patients than in the general population .It is because of increased exposure to blood products and shared hemodialysis equipment with impaired immune response in chronic kidney disease patients [3]. The prevalence of hepatitis B virus among Indian dialysis population varies from 20 to 45% but in Western countries it is < 1 %.[4,11]

The Centers for Disease Control and Prevention recommends that all dialysis patients should be vaccinated against HBV with double the standard dose (40µg) and intensified schedule (0, 1, 2, 6). But the response in CKD patients on haemodialysis even with intensified schedule as recommended by CDC guideline is low (50%-60%).[5,19] There are only very few Indian studies available regarding immune response following HBV vaccination and various factors determining it in dialysis patients.We studied the

immune response in healthy individuals, in dialysis patients and analyzed various factors that had an influence on immune response such as sex,age,duration of dialysis, diabetes, smoking, anaemia, body mass index, albumin level and adequacy of dialysis.

In this study we compared the sero conversion rate following 4 doses of 40µg of recombinant Hepatitis B virus vaccine (HBV vaccine) administered to chronic kidney disease Stage-V patients undergoing haemodialysis (N=34) to healthy adult volunteers with 3 doses of 20µg recombinant HBV vaccine (N=30).AntiHBs titer ≥ 10mIU/ml (immne responder) was present in 82% of patients undergoing haemodialysis and the titre of >10Miu/ml was present in all the healthy control volunteers (100%).

Shepard CW et al and Lai LL et al in their studies showed that seroprotection rate in normal healthy individuals following standard-dose vaccination strategy of 3 doses of 0, 1, 2 months with 20µg HBsAg with 0.5mg aluminium salt as adjuvant was around 95% [12,58].Almost similar to this one, the seroprotection rate following 3 doses of 20 µg of HBV vaccine to healthy adults in our study was 100%.

But the seroprotection rate following 4 doses of 40 µg of HBV vaccine was reported to be 60% by Tokar J I et al and Prabhat Singh et al

[12,15] in their studies. In our study the seroprotection rate was 82%.Some studies also observed the response rate of 80% in dialysis patients.[28]. Also dialysis patients develop lower peak antibody titer which persists for shorter duration than normal healthy population [17,18]. In our study the mean antiHBs titer in dialysis patients was 24.7mIU/ml compared to 31.8mIU/ml in healthy individuals.

Among 34 patients in this study group, 7 were females(20.6%) and 27 were males (79.4%).Thus men were the predominant group.86% of females and 82% of males were found to be immune responders and there was no statistically significant difference between them regarding immune response. In a study by Hans Kohler et al , 66% of female patients and 50% of male dialysis patients developed seroconversion following HBV vaccine.However he had found that the sex difference for seroconversion rate was not statistically significant.[40]

In our study age varied from 18 to 64 years with mean age of 35.88±12.13.Majority of them were more than 40 years old (41.2%) followed by 20-30 years age group(32.4%).There was no significant difference regarding immune response between different age groups.But Fabrizi et al and Fisman DN et al [41,38] in their meta-analysis of the effect of age on immune response to HBV vaccination in chronic kidney disease patients on haemodialysis found a decrease in serological response with

older age. Also Hans Kohler in his study shown that the mean age of patients who develop immune response was 39.8 years compared to patients with 52.8 years who did not develop immune response.

None of the native kidney disease was found to be significantly associated with immune response following HBV vaccine in this study group as in other studies [42 -44]. Duration of dialysis in our study population varied from 5 months to 23 months with mean duration of 11.8±7.07 and most of them had undergone 6- 8 months (70.5%) of dialysis.There was no significant association seen between duration of dialysis and immune response in our study population. According to Hans Kohler et al the mean length of the time on dialysis had no impact on immune response.[40]

Most of the patients in this study group were non-diabetic (82.4%) and only 17.6% were diabetic. Among 6 diabetic patients, diabetic nephropathy was the underlying cause for chronic kidney disease in 5 of them.85.7% of patients among non diabetics were immune responders compared to 66.7% among diabetics.Thus the immune response rate was better in non diabetics (85.7%) compared to the diabetics (66.7%). Sarah F. Schillie et al and Alavian et al [32,33] in their systematic reviews reported the sero protection rate after HBV vaccination in patients with diabetes varied from 34% to 80%. Most of the studies also confirmed this.But Lacson et al

[62] reported null association between diabetes and immune response in dialysis patients.

History of smoking was present in 5 patients (15%) and the remaining were non smokers (85%).Immune response rate was 83% and 82% between smokers and non smokers respectively and it was statistically not significant.Thus in our study smoking had no effect on vaccine response but other studies by Kara IH et al [17] also have shown the negative impact of smoking on immune response following vaccination.

Our study population's body mass index varied from 12.4 to 28.1 with mean of 20.1 ± 3.24. 8 patients(23.5%) were malnourished with 3 (8.8%) of them having severe malnutrition(BMI<16). Immune response was only 66.7% in severely malnourished patients compared to 80.8% in patients with BMI more than 18.5. Fernandez et al [36] in their study on effect of malnutrition on HBV vaccination response, morbidity and mortality in haemodialysis patients showed the negative influence of malnutrition on vaccine response.Nutritional status was assessed by serum albumin, prealbumin, anthropometric measurements,mid arm circumference and triceps skinfold thickness . Responders had significantly high levels of serum albumin, prealbumin and predialysis blood urea concentration compared to non-responders. Malnutrition reduces the ability to form antibodies and hence impaired response to HBV vaccination.

Malnutrition also increases the risk of morbidity and mortality in dialysis patients.Thus it is well clear that malnutrition and poor immune response are interconnected, but other factors also influence immune responsiveness.In this study it was shown that Serum albumin and predialysis serum urea levels were the strongest predictors of response to the HBV vaccination. Also Fabrizi F et al [37] in his meta analysis of seven studies on the impact of nutritional status on HBV vaccination response in CKD patients showed that the poor nutritional status as estimated by serum albumin levels was an independent and adverse factor on immune response after HBV vaccination in chronic kidney disease patients. In our study also the immune response was less compared to patients with good nutritional status.

In our study most of the patients (94%) were anemic with hemoglobin ranged from 4.7 to 11.6 gm/dl with mean of 7.28± 1.66. Only two patients (6%) had hemoglobin more than 11.gm/dl. Immune response was 100% in patients with hemoglobin more than 11.gm/dl.Anaemia has a negative impact on immune response [57,58]. Most of the patients (76.5%) in our study population were inadequately dialyzed with Urea reduction ratio (URR) of less than 65%. Immune response was 100% when URR was more than 65%. Khalid Al Saran et al showed that there no statistically significant association between adequacy of dialysis and immune response.But in our study the response rate was very good with adequate

dialysis but statistical significance was not present in view small sample size as by Fraser GM[39].85% of patients undergoing dialysis had hypoalbuminaemia(< 4gm/dl) and only 15% of patients had albumin level > 4gm/dl.However the immune response rate was almost similar between the groups.Fernandez et al [36] in their study reported the negative influence of nutritional status as assessed by serum albumin on immune response.But few studies also had shown that nutritional state had no effect on vaccination responsiveness.

Among our study group patients 6 were (17.6%) HCV positive. Majority of HCV negative Serology patients (87.5%) have developed protective HBV immunity (> 10mIU/ml) compared to 66.7% in HCV positive patients. Some studies reported the negative impact of coexisting HCV infection on immune response [28,63]. Our study also had shown poor response in HCV positive patients. Access Infection was present in 4 patients (11.8%). But all of them had antiHBs titre more than 10mIU/ml. This showed the negative correlation of the presence of infection and the response following vaccination.

In our study 30 healthy adult volunteers were included. None of them in the control group had diabetes mellitus, hypertension or history of smoking. Blood sugar , blood urea and Serum creatinine were within normal limits. Hepatitis-C and HIV Serology were negative for all of them. Hence

we did not compare both the groups regarding all these parameters.Most of them in control group were females and less than 20 years.Body mass index was normal in almost in all of them but serum albumin level was less than 4 gms/dl in many of them.None of the factors had an influence on immune response.

To summarize, our study had shown good immune response in healthy adult population (100%) than chronic kidney disease stage v patients undergoing dialysis (82%).

Limitation of the study:

Small sample size (N =34) of patients undergoing dialysis and healthy adult volunteers (N=30) is an important limitation of the study.

CONCLUSION

1. The seroconversion rate in chronic kidney disease Stage-V patients undergoing haemodialysis following 4 doses of 40µg of recombinant hepatitis B virus vaccine in our study was 82%.
2. The seroconversion rate in healthy adult volunteers with 3 doses of 20µg recombinant HBV vaccine in our study was 100%.
3. Female patients undergoing dialysis showed better immune response than males.
4. Non diabetic patients showed better immune response than diabetic patients.
5. Patients with higher body mass index had higher seroconversion rate than patients with malnutrition.
6. Patients with higher haemoglobin level had better response.
7. Adequately dialyzed patients were found to have better immune response.
8. Age,duration of dialysis and serum albumin had no impact on immune response following HBV vaccination.

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. E. Indhumathi,
Postgraduate,
Department of Nephrology
Madras Medical College &
Rajiv Gandhi Government General Hospital,
Chennai-3.

Dear **Dr. E. Indhumathi,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Seroconversion rate following Hepatitis B vaccination in Chronic Kidney Disease population with current immunization strategy"** No.22122012.

The following members of Ethics Committee were present in the meeting held on 11.12.2012 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, MMC, Ch-3	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BA, BL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

3/1/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

PROFORMA

NAME AGE

SEX

NC NO

NATIVE KIDNEY DISEASE

DIABETES ILLNESS

HYPERTENSION

CAD

H/O SMOKING AND H/O ALCOHOLISM

DURATION OF HAEMODIALYSIS

FEVER

EXAMINATION :

ANAEMIA HT WT

OEDEMA PR BP

SKIN CHANGES

CVS RS ABD CNS

INVESTIGATIONS

HB PCV

TC DC

PERIPHERAL SMEAR

ESR

URINE ROUTINE

BLOOD SUGAR

BLOOD UREA

SERUM CREATININE

SERUM ELECTROLYTES

SERUM CHOLESTEROL

SERUM BILIRUBIN

AST ALT

SERUM ALBUMIN

ANTI HBs TITER

HBsAg anti HCV HIV serology

ECG, X RAY CHEST , ECHOCARDIOGRAPHY

MASTER CHART - PATIENT

SL. NO	NAME	AGE	SEX	NC NO	BMI	NKD	DM	HT	SMOKING	DURATION	ACC. INFN	HB	PCV	ESR	wbc cou	SUGAR	UREA	CREAT	URR	AST	ALT	bilirubin	ALBUMIN	CHOLES	HCV	HIV	ANTI HBS
1	THIYAGARAJAN	41	M	172/12	20.2	DM	Y	Y	Y	18	N	8.4	23	15/30	9400	97	272	13.9	58.6	18	16	0.8	3.8	128	NEG	NEG	9.97
2	RAMU	53	M	2307/13	22.1	DN	Y	Y	N	6	N	7	19	40/90	7800	80	134	8.1	54.9	23	20	0.7	3.7	126	NEG	NEG	24.8
3	SIVASAKTHI	18	M	2522/13	16.4	NK	N	Y	N	6	N	4.7	13	55/120	8000	69	98	9.3	66.5	20	24	0.6	3.2	128	NEG	NEG	34.53
4	ACHUDHAN	47	M	603/89	19.1	HT/str ure	N	Y	N	5	N	7.3	21	40/90	5600	70	72	3.9	43.1	22	28	0.6	4.3	120	NEG	NEG	17.61
5	MANOHARAN	39	M	1131/11	25.5	AORTITIS	N	Y	N	8	N	8.4	16	50/105	5800	86	198	12.2	54.6	0.6	23	33	3	126	NEG	NEG	16.22
6	LAKSHMANAN	38	M	1983/13	20.3	ADPKD	N	Y	N	9	N	8.2	26	30/60	4500	106	177	8.5	68.42	18	22	0.6	4.1	118	NEG	NEG	15.06
7	SENTHILKUMAR	33	M	2004/13	18.5	CGN	N	Y	N	6	N	7	26	30/60	500	74	221	11	67.2	20	26	0.6	3	111	NEG	NEG	19
8	PARTHASARATHY	64	M	2697/13	23.1	DN	Y	Y	N	7	Y	10.5	34	15/30	8900	183	63	2.6	68.4	18	21	0.6	4	117	NEG	NEG	29.55
9	RAVI	42	M	813/12	20.58	CGN	N	Y	N	7	N	7	19	40/90	11600	110	164	8.5	72.5	24	20	0.5	3.2	120	NEG	NEG	19.12
10	JEYALAKSHMI	51	F	2624/13	20.8	LN	N	Y	N	7	N	7.4	21	25/40	10500	97	136	7	59.7	16	20	1	3.4	128	NEG	NEG	26.99
11	KRISHNAN.D	56	M	3939/13	20.8	DM	Y	Y	Y	24	N	11.6	26	30/70	6600	148	78	4.7	66.2	20	25	0.8	3	106	NEG	NEG	15.76
12	MUNUSAMY	53	M	3493/12	25.53	NK	Y	Y	Y	9	N	5.4	23	40/90	5100	82	79	4.4	63.4	16	18	0.8	3.1	120	NEG	NEG	16.57
13	JAMES	44	M	2050/13	24.43	CGN	N	Y	Y	7	N	7.8	23	35/70	6800	134	139	5.8	61.9	30	38	0.6	3.5	128	NEG	NEG	16.69
14	ARULSAMY	45	M	45/11	21.6	GRAF LOS	N	Y	N	7	N	6	22	40/90	7200	94	142	6.8	60.1	17	18	0.7	3.2	126	NEG	NEG	5.68
15	GOWTHAMI	23	F	43/13	19.5	IGA	N	Y	N	14	N	5.4	15	40/85	5000	87	219	12.1	71.1	15	19	0.7	3.7	128	NEG	NEG	79.37
16	SELVAKUMAR	22	M	731/13	18.4	CGN	N	Y	N	15	N	9.2	21	40/85	6000	86	168	10.4	56.8	20	25	0.8	3.7	110	NEG	NEG	32.79
17	TAMILNESAN	45	M	4684/12	20.6	CGN/CKD	N	Y	N	14	y	5.5	16	40/95	3800	94	158	8	58.9	34	28	0.6	2.2	120	NEG	NEG	23.52
18	KADARBASHA	29	M	3834/10	19.1	CGN/CKD	N	Y	N	14	N	11.4	35	20/40	4200	84	250	10.1	57.4	30	16	0.6	4.2	128	NEG	NEG	29.08
19	DAMODARAN	32	M	1603/13	28.09	GRAF LOS	N	Y	N	12	N	6	18	35/80	6200	84	117	6.4	75.1	16	19	0.8	3.6	130	NEG	NEG	19.7
20	RAMACHANDRAN	30	M	74/12	19	FSGS	N	Y	Y	24	N	5.8	16	50/105	4000	80	168	9.3	60.4	20	30	0.6	3.3	110	NEG	NEG	27.81
21	VEERAMANI	38	M	442/06	24.1	GRAF LOS	N	Y	N	6	N	6.4	21	30/60	6300	88	98	5.3	62.3	74	61	0.8	4.1	120	POSITIVE	NEG	8.23
22	JAIGANESH	26	M	2678/11	18.68	ALPORT	N	Y	N	30	N	6.4	19	40/95	2800	83	165	8.5	60.2	16	20	0.8	4.1	128	POSITIVE	NEG	19.7
23	SARAVANAN	33	M	2146/10	19.7	CGN	N	Y	N	7	N	6.4	23	40/90	3200	98	210	11	57.5	22	17	0.6	3.9	124	POSITIVE	NEG	43.92
24	MANI	19	M	1620/13	19.7	ALPORT	N	Y	N	13	N	4.8	13	50/105	4600	70	102	5.2	60.2	59	126	0.9	3.5	124	POSITIVE	NEG	7.18
25	NIROSHA	23	F	5334/10	15.2	GRAF LOS	N	Y	N	30	N	7.8	23	40/90	4200	90	106	5.6	59.9	30	62	0.5	3.8	130	POSITIVE	NEG	20.51
26	RAJAVEL	29	M	767/08	16.6	GRAF LOS	N	Y	N	14	N	7.2	16	40/90	3800	86	106	5.3	59.6	20	24	0.8	3.9	110	POSITIVE	NEG	10.2
27	PARTHIBAN	29	M	547/01	19.6	GRAF LOS	N	Y	N	8	N	7.8	18	35/85	5400	85	101	6	61.4	16	18	0.6	3.3	118	NEG	NEG	22.02
28	PONNIAH	43	M	1542/13	19.2	CGN/CKD	N	Y	N	7	N	7	15	30/60	8900	73	86	7.1	58.9	20	30	0.5	3.1	120	NEG	NEG	27.81
29	SHANMUGHAM	45	M	389/12	24.7	DN	Y	Y	N	8	N	8.1	21	40/90	6600	96	125	4.2	64.2	26	19	0.9	3.2	128	NEG	NEG	8.11
30	UDAYAKUMAR	42	M	790/12	18.9	CGN/CKD	N	Y	N	6	y	6.7	13	20/40	5200	103	87	7	57.3	18	16	0.6	2.9	118	NEG	NEG	48.09
31	THENMOZHI	26	F	1846/10	18.6	IGA	N	Y	N	24	N	7.8	23	40/85	4800	91	103	5.4	62.8	17	24	0.8	3.8	125	NEG	NEG	64.54
32	SRIMATHI	23	F	248/10	16.9	C3 GLO	N	N	N	12	N	8.1	25	30/60	7500	88	76	6.2	58.3	21	18	0.6	3.1	117	NEG	NEG	26.54
33	GAYATHRI	18	F	2814/09	16	GRAF LOS	N	Y	N	14	Y	7.9	24	40/90	6900	78	83	7.4	57.5	18	26	0.7	3	128	NEG	NEG	44.26
34	REVATHI	21	F	2614/08	12.4	IGA	N	Y	N	18	N	5.3	14	50/110	14000	69	58	6.3	54.1	18	32	0.7	2.6	126	NEG	NEG	9.27

MASTER CHART – CONTROL

SL.NO	NAME	AGE	SEX	NKD	DM	HT	SMOKING	DURATIO	BMI	HB	PCV	TC	BL.UREA	SR CREAT	BL.SUGAR	AST	ALT	BILIRUBI	SR ALBUM	CHOLES	HBSAG	ANTI HCV	ANTI HIV	HBV DOSEµ	HBV NO	ANTI HBs
1	ALAGUSAMY	20	M	NA	NO	NO	NO	NA	22.3	11.2	34	6800	18	0.8	93	24	18	1	4.1	117	NEG	NEG	NEG	20	3	10.31
2	BHAVANI	18	F	NA	NO	NO	NO	NA	30.4	12	35.8	7200	20	1	86	28	16	1.1	4.2	126	NEG	NEG	NEG	20	3	12.86
3	ARPUTHAVALLI	23	F	NA	NO	NO	NO	NA	21.7	10.1	31.2	5900	16	0.9	76	24	20	0.9	3.5	131	NEG	NEG	NEG	20	3	21.09
4	DEVI	19	F	NA	NO	NO	NO	NA	17.1	10	30.8	7000	19	0.8	80	25	24	1.2	3.2	126	NEG	NEG	NEG	20	3	56.89
5	DURGA	18	F	NA	NO	NO	NO	NA	28.6	9	28	8300	20	1	91	18	28	0.8	3.3	110	NEG	NEG	NEG	20	3	19.24
6	KANNAGI	18	F	NA	NO	NO	NO	NA	16.9	13	40.6	8700	18	0.8	75	20	23	0.8	3.9	127	NEG	NEG	NEG	20	3	39.86
7	KARPAGAM	20	F	NA	NO	NO	NO	NA	24.9	9.8	30.4	6900	21	1	88	21	17	0.9	3.2	120	NEG	NEG	NEG	20	3	59.56
8	INDUMATHI	18	F	NA	NO	NO	NO	NA	21.5	9	27	5800	20	1	93	23	20	1.1	3.5	128	NEG	NEG	NEG	20	3	33.26
9	NADIYA	19	F	NA	NO	NO	NO	NA	21.9	10.8	33.4	6100	18	1	101	28	19	0.9	3.3	126	NEG	NEG	NEG	20	3	28.74
10	NITHYA	18	F	NA	NO	NO	NO	NA	23.1	13.8	42	7000	19	0.8	87	32	16	1	3.2	130	NEG	NEG	NEG	20	3	58.98
11	MARY	20	F	NA	NO	NO	NO	NA	24.02	10.5	32.5	6600	19	0.9	93	16	22	0.8	3.1	118	NEG	NEG	NEG	20	3	14.72
12	PRAVEENA	19	F	NA	NO	NO	NO	NA	22.06	11.8	36	7200	17	0.9	101	18	26	0.9	4	120	NEG	NEG	NEG	20	3	12.75
13	RENUGA	18	F	NA	NO	NO	NO	NA	22.5	11.8	36	5600	16	0.8	79	28	24	0.7	3.7	123	NEG	NEG	NEG	20	3	12.28
14	PUSHPARAJ	28	M	NA	NO	NO	NO	NA	23.1	14	42.8	9000	17	1	97	20	18	1.1	4.2	130	NEG	NEG	NEG	20	3	32.68
15	SARANYA	19	F	NA	NO	NO	NO	NA	21.4	11.2	35.4	8800	19	0.8	103	19	16	1	3.2	129	NEG	NEG	NEG	20	3	36.96
16	SHARMILA	19	F	NA	NO	NO	NO	NA	20.2	12.6	36.8	7900	22	1	99	26	28	0.6	3.4	119	NEG	NEG	NEG	20	3	36.62
17	SABEENA	20	F	NA	NO	NO	NO	NA	19.6	9.3	27.4	5800	17	0.8	74	26	29	0.9	3.1	124	NEG	NEG	NEG	20	3	13.67
18	VELUPRIYA	19	F	NA	NO	NO	NO	NA	17.1	12	36.8	8100	19	0.9	78	18	20	0.5	3.1	131	NEG	NEG	NEG	20	3	17.61
19	SHAHIDA	19	F	NA	NO	NO	NO	NA	19	9.3	29.3	4800	20	1	80	16	16	0.8	3.4	128	NEG	NEG	NEG	20	3	11.24
20	CHITRA	19	F	NA	NO	NO	NO	NA	20.7	11.8	34	5900	18	0.8	92	19	21	0.9	3.3	118	NEG	NEG	NEG	20	3	15.99
21	DR ASHOK	31	M	NA	NO	NO	NO	NA	26.4	14.1	42	6100	16	1.1	88	21	24	1.1	4.2	118	NEG	NEG	NEG	20	3	35.92
22	DR KAMARAJ	32	M	NA	NO	NO	NO	NA	25.8	14.4	42.8	5700	18	1.2	93	20	19	0.7	4	118	NEG	NEG	NEG	20	3	76.43
23	DR NAGARAJAN	32	M	NA	NO	NO	NO	NA	24.7	14.2	42	5800	21	1.1	95	15	16	0.9	4.1	130	NEG	NEG	NEG	20	3	68.66
24	DR THIRUMURUGAN	32	M	NA	NO	NO	NO	NA	20.8	13.6	40.4	7200	17	1.1	101	18	22	0.8	3.9	127	NEG	NEG	NEG	20	3	23.29
25	VINOJA	40	F	NA	NO	NO	NO	NA	24.9	11.4	35	6500	22	1	98	19	20	0.6	3.6	134	NEG	NEG	NEG	20	3	54.8
26	AMAL RAJ	40	M	NA	NO	NO	NO	NA	27.8	13.8	41.2	7000	24	1.2	102	23	19	0.5	4.2	127	NEG	NEG	NEG	20	3	23.29
27	VINOTH	19	M	NA	NO	NO	NO	NA	18.6	13	40.4	8300	18	1	99	22	28	0.7	3.3	125	NEG	NEG	NEG	20	3	21.55
28	MENAGA	19	F	NA	NO	NO	NO	NA	21.4	10	31	7200	20	0.8	88	19	20	1	3.3	128	NEG	NEG	NEG	20	3	7.07
29	DR PAD KU	32		NA	NO	NO	NO	NA	26.2	13.8	41.2	8000	22	1.1	94	28	21	0.9	4.3	130	NEG	NEG	NEG	20	3	73.23
30	DR SUJIT	32		NA	NO	NO	NO	NA	27.6	14.2	42.6	8800	24	1	105	22	23	0.7	4.2	128	NEG	NEG	NEG	20	3	26.54

CONSENT FORM

TITLE OF PROJECT:

" A STUDY ON SEROCONVERSION RATE FOLLOWING HEPATITIS B VACCINATION IN CHRONIC KIDNEY DISEASE POPULATION WITH CURRENT IMMUNIZATION STRATEGY ."

Name of Researcher: Dr. E.INDHUMATHI

Please tick to confirm

- I confirm that I have read and understand the information provided to me for the above study.
- I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

I agree to take part in the above research study.

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature