5. DISCUSSION

The prevalence of hepatitis B infection varies in different parts of the world. Also this prevalence varies country to country from one region to another and from one group to another group in a country (Zali et al., 1996). Center for Diseases Control (CDC) advised that, by the year 2000, 90% of pregnant women should be screened for HBsAg prior to delivery (Mast et al., 1999). In our country, recent years, most of the pregnant women were screened in health centers. Hepatitis B virus infected pregnant women may result in chronic infection to the newborn.

5.1. Overall prevalence of HBsAg/HBeAg and HBeAb

In the present study, the overall prevalence of hepatitis B surface antigen in pregnant women attending hospital in Krishnagiri district, TamilNadu was 5.10%. The study reported an overall prevalence is on intermediate side, as per WHO criteria. The results are in accordance with Fisseha Walle et al., (2008), who stated that prevalence rate among pregnant women, was 5.0% in Addish (Ethiopia) 4.0%-6.0% in Jimma, 4.3% -4.6% in Nigeria, 6.5% in Zambia, 4.9% - 6.4% in Korea and 4.2% in Turkey. However, there are reports from other parts of the world showed low prevalence of 0.14% - 0.97% in USA, except Asian American, 1.65% in Mexico and 0.21% in the North Kerala state in south India. A higher prevalence (15.5%) was found among study population in Mali, 10.0% in Hong Kong, 12.0% in Taiwan, 7.05 in Oman and 18.5% in Brazil. Comparison of present study with other studies from different countries on pregnant women showed a variable result. There is a wide variation in the prevalence of HBsAg in different region of our country and the highest prevalence (9.5%) has been reported by Prakesh et al., (2011) in North India, Arunachal Pradesh.

The difference in demographic characteristics of the study population such as socioeconomic environment, tribal practice, traditional operation, sexual practice, medical
exposure and the difference in hepatitis epidemiology in these countries might have been the reasons for infecting pregnant women.

The overall prevalence of HBeAg in this study was 38.0%. Similar observation was reported by Arora et al., (2004) and by Tandon et al., (1996) from Madras and Chandigarh with a significant infection of 34.2% and 47.0% respectively. In India, the prevalence rate of HBsAg in pregnant women varies from 4.8% - 6.87%. In India only a single study has systematically looked at the issue of relative contribution of prenatal and horizontal transmission. A study from Delhi, North India, documented that HBsAg carrier rate in antenatal patients to be 3.7% and HBeAg carrier rate of 7.8% and vertical transmission rate of 18.6% (Nayak et al., 1984). In another study of HBsAg positive antenatal from Chandigarh, North India, prenatal transmission was 30.0% and HBeAg carrier rate among this mother’s was 30.0% (Biswas et al., 2007). A recent report from Bangladesh on the Eastern border of India documented 30.05 of antenatal mother to be HBeAg positive, indicating a higher risk for their babies to be persistently infected with HBV (Karim Rumi et al., 1998).

Besides the overall prevalence, HBeAg prevalence among carrier especially pregnant women is an important source of information for determining potential modes of HBV transmission in a population and underscores importance of giving a dose of hepatitis B vaccine soon after birth. HBeAg transmitted via placenta because it is smaller than HBsAg, so there may be the possibility of transmitting HBeAg from mother to fetus through placenta. Thus infant born to HBsAg positive mother should be immunized (Rosendahl et al., 1983). It appears that the HBeAg carrier in pregnant women population was similar to that of East Asia 30.0%- 50.0% (Soderstrom et al., 2003). Therefore the potential HBV transmission in Krishnagiri district is lower when compared to that of East Asia.
5.2. Hepatitis B antigen among different age group

This study reports epidemiological data of hepatitis B prevalence in pregnant women among different age group i.e., 15-45 years. The age specific exposure to HBsAg was highest (9.4%) in the teenage group (15-19years), which also recorded highest percentage for HBeAg (3.8%) and HBeAb (11.3%). However this difference, observed among different age group were not significant (P= 0.280). Motta Catro et al., (2003) and Slue et al.,(2011) they stated that age was not significantly associated with HBsAg seropositivity among pregnant women. The distribution of hepatitis B viral surface antigen in different age group could be due to anemia with increasing age.

Prevalence of HBsAg was lower in the age group 30-34 followed by 20-24 which recorded 3.8% and 4.2% respectively. Similar reports were found in other studies conducted in Turkey 3.5%, in West of Iran 3.0%, and in Mexico 3.9% (Mehmet et al., 2005). Mother to child transmission is an important transmission route for HBV; 30.0%-50.0% of chronic carriers of HBV in china can be attributed to maternal infantile transmission. Furthermore, Yu Zhang et al., (2013) pointed out that if the mother was HBsAg positive, plus HBeAg and HBVDNA positive, the risk for the neonate to be infected was about 90.0% and the child would become a chronic carrier in 80.0- 90.0% cases. The prevention and control of HBV infection among women of childbearing age should therefore, be a priority of public health intervention in order to reduce the transmission of HBV from mother to child.

Earlier studies on vertical transmission have noted higher HBeAg prevalence (30.0 % -40.0%) in children born to infected mother (Ludha et al., 2001). Teenagers were infected high due to risk factors of family contact with hepatitis B virus (Lacombe, et al., 2005). The limitation of the present study was that the information was limited to a population 0f 15 – 45 years. Information regarding the prevalence of infection in the pediatric age group would have given better insight regarding the mode of transmission in younger age group.
5.3. Hepatitis B antigen according to educational status

In this study HBsAg prevalence in relation to educational status of the patients showed higher (5.5%) in illiterate group compare with higher secondary (5.1%), primary (1.6%) and degree groups (0.1%), and this difference is not statistically significant association between educational status of patient and HBsAg positivity, this might be due to low level of public awareness on the carrier rates of HBV infection. Similar observation was made by Mehmet et al., (2005), that HBV seropositivity was higher in illiterate women in urban areas. Ezegbudo et al., (2004) earlier reported that prevalence rate of infection such as HIV, HBV and HIV/HBV co-infection were inversely associated with education status. This indicates the influence of education on the prevalence. The literate woman has the advantages of being able to read about the viral infection and methods of preventing it. A similar observation was reported from study in Minna by Ndams et al., (2008) and Rabiu et al., (2010) advised that screening pregnant women for HBV on the basis of risk factors may not be effective.

5.4. Prevalence of HBsAg in relation to occupation

The important parameter in this study was occupational exposure. Prevalence of HBsAg in relation to occupation revealed that the highest prevalence in student (7.4%) followed by house wife and employee. The difference observed among the occupational group was not significant. Further work is required to identify the reasons.

5.5 Distribution of HBsAg /HBeAg /HBeAb among pregnant women with reference to number of pregnancy

As regard to number of previous pregnancy, the prevalence HBs Ag was high (6.4%) in first and third pregnancy and the second pregnancy recorded 3.6%. The results are in accordance with Khakhkhar et al., (2012) who stated that the prevalence rate among pregnant
women was high in first pregnancy followed by third and second. Maheswari et al., (1985) stated that prevalence increases from 38.5% to 67.0% if infection occurs in third pregnancy.

5.6. Prevalence of Hepatitis B surface antigen in relation to associated risk factors

5.6.1. Immunization status

The prevalence rate associated with previous history of immunization revealed that one patient shows positive for HBs Ag among six patient tested this was similar to that of reports from Northern and central European countries (Gay et al., 1999) and (Lindh et al., 1993) whereas quite lower prevalence recorded in other Mediterranean countries or region such as France (0.65%) Croatia (0.75%) Italy (1.0%) Slicily (1.1%) and Greece (1.5%) (Llucis Salleras et al., 2009).

The study of Al-Mazrou et al., (2004) shown that 79.9% of the pregnant women have a non immune status making them liable to HBV infection. This suggests that the full impact of the HBV vaccination programme has not yet reached all women during the maternity period.

Jurema et al., (2001) have shown that hepatitis B immunization in the postpartum period is feasible and effective. The availability of a safe and effective vaccine encourages us to accelerate viral elimination. Thus hepatitis B immunization can be recommended giving the first dose immediately on the first postpartum day before the mother gets discharged from the hospital and the second dose to coincide with her child vaccination dose at the age of 2 months, and third dose to be given to the mother when her child gets vaccinated at the age of 6 months.

In order to prevent chromic HBV and hepatocellular carcinoma the WHO has recommended universal immunization. It is known that the lack of administration of
protective antibodies to newborn babies results in the development and progression of the chronic disease. In Tanzania, vaccination against HBV infection was introduced in 2002 and has been continuing since then. The vaccine is provided together with DPT to babies as three doses given at 4, 8 and 12 weeks of age (Metodi et al., 2010).

Although WHO recommended that hepatitis B vaccine be incorporated into routine infant and childhood immunization programme for all countries by 1997, only 130 of 216 countries introduced hepatitis immunization into national infant and childhood immunization programme by beginning of 2001. The major hurdle to universal hepatitis B immunization is cost of HBV vaccine especially in developing countries. Even though the price of HBV vaccines for developing countries has dropped from about $3.00 per dose in 1990 to as low as $0.30 per dose in 2001, the cost of these doses of HBV vaccine remain higher than the cost of other vaccine include in routine infant immunization programme (Mohammed Awole et al., 2005).

5.6.2. History of blood transfusion

Prevalence of HBsAg among the patient with history of blood transfusion revealed that one out 7 (14.2%) have positive, that the history of blood transfusion was not significant with HBsAg seropositivity. Significantly higher prevalence among this group might be due to exposure to unscreened blood or blood products (or) other practices such as tattooing, ear piercings, face marking (Tribalmarkes). This finding is in agreement with several epidemiological studies (Maddawa et al., 2002). In another study conducted in Anyigba, Kogi state by Slue et al., (2011) in which pregnant women with history of blood transfusion had significantly higher HBsAg and they reported that blood transfusion is very significant route of HBV transmission. The result in this study are similar with study of Pennap et al.,(2011) who reported prevalence was 10.46% They demonstrated that the unsafe injection
from unqualified medical personnel using HBV contaminated needle and syringe, transfusion of blood and blood products and sociocultural practices such as tribal marks, circumcision and scarification were important routes of HBV transmission.

5.6.3. Previous history of Jaundice

As regard with previous history of jaundice, the pregnant women in our study 2 out of 15 mothers who had history of jaundice (13.3%) were HBsAg positive. The history of Jaundice found highest in the risk factor, next to the immunization status. These results are similar to that of Khakhkharvipul et al., 2012), and they concluded that screening pregnant women for HBsAg is necessary in order to identify those neonates at risk of transmission. The Study demonstrated history of jaundice was associated with hepatitis B infection. The significant association between HBsAg positivity is comparable with the findings of other studies (Al-waleedi and Khader, 2012).

In the region of Amazon out of 258 contacts 97 cases with HBV infection 51.6% had serological markers of past infection, the author stated that the important of intrafamilial transmission of HBV infection (Brasil et al., 2003). This prevalence rate was high when compare to our study (13.3%). There are many other reports showing evidence of familial transmission of HBV and chronically complicated to HBV infection (Zampino et al., 2002). Evidence showing familial transmission of HBV was significant effect of familial jaundice on to the HBV positivity and HBsAg positivity. Risk factors of positive familial jaundice had an important effect in rural area. In rural families are more crowded this may be increasing familial transmission (Dursun Mehmet et al., 2005).

5.6.4. Previous history of surgery and dental therapy

In the present study patient undergone dental therapy showed 7.1% prevalence of HBs Ag. This finding is similar to the situation in Brazil and some other developing countries
(Pereira et al., 2009) and it could be explained by the improvement in sterilization and hygienic practice in dental clinics. Dental therapy is a major risk factors associated with HBV infection. Our findings are contradicted with the studies associating with use of dental therapy (4.9%) in Pakistan with HBV seropositivity. This unsafe risk factor may lead to transmission of blood born pathogen. The result of our study is quite low (7.1%) as compared to a study from Karachi which showed 53.0% (Ghulam Mujtabab siddiqi et al., 2012). The prevalence of previous history of surgery 2 out of 26 (7.6%) were infected for HBsAg .The significant association between HBs Ag positivity is accordance (6.96%) with the finding of other studies (Al-waleedi and khader, 2012 and Ibrahim Bani et al., 2012).

Risk factors including use of dental therapy and surgery were associated with HBV infection in pregnant women (Awole et al., 2005). Jefferson et al., (2000) stated that number of risk factors analyses studies confirmed that HBV infection had statistically significant association with absence of vaccination and other risk factors such as needle stick injury, sharp needles, surgery and blood transfusion.

In conclusion, results from this study have shown that HBV prevalence in pregnant women is of intermediate endemicity and use of surgical instruments in dental procedure and surgery were associated with hepatitis b infection in the study area. Therefore awareness of transmission of hepatitis infection through prediction practice is needed.

5.7. Virological markers (HBVDNA) among pregnant women

Serological markers are indispensable in the diagnosis of HBV infection. HBsAg, inspite of being a common diagnostic marker of HBV infection does not provide information about active virus replication. Also the critical issue is that the sensitivity of current serological test for HBsAg (1 ng/ml) is lower than the minimum infection dose of the virus (1pg/ml). On the contrary, PCR technique with reported sensitivity in the range of 0.01-1
Fig DNA has the potential of filling in this gap (Rodrigues et al., 2001). Moreover the presence of serum HBVDNA in chronic patients indicates the active virus replication.

In this study, the sera of patients positive for HBsAg, HBeAg and HBeAb were analyzed for the presence of HBV DNA by PCR technique. The result indicated 21/39 (53.8%) patient tested were positive for HBVDNA markers, which shows as active viral replication. This results suggest that seroconversion from HBeAg to anti-HBe can be accompanied by a decrease, but not a total disappearance of virus particles in the serum. This finding supports the hypothesis that HBV antiviral replicated in the liver of HBsAg positive patients after seroconversion to anti-HBe.

In this study all the patient positive with HBeAg was positive for HBVDNA by amplification. This is in accordance with Rodrigues et al., (2001). In another study by Lieberman et al.,(1983) the sera of 14 chronic hepatitis patients positive for HBeAg were assayed for the presence of HBV DNA by PCR analysis showed less prevalence rate (64.0%). Col Chopra et al.,(2004) also reported less prevalence in his analysis for HBV DNA by real time PCR from positive HBeAg cases, reported 26%(24/91) from hepatitis B group, 14.6% (6/41) from immunocompromised group and 59.2% (29/49) from chronic hepatitis group which is less when compare to our study.

In our study 28 out of 39 (72.0%) patients were infected for HBeAb. Among these 28, only 6 were positive for HBVDNA (21.4%). This finding is high when compare with the study conducted by Shuichi Kaneko et al., (1989) who reported only 22.0%. The important issue is that seroconversion from HBeAg to anti-HBe has been recognized and this may be due to mutation in the pre C region that prohibits the synthesis and secretion of HBeAg (Carman et al., 1990). In the present study these who negative for HBVDNA individual could be due to low viremia (non-replicative) HBeAg and HBeAb (OR) HBV disease are due
to the presence of a pre-core/core stop codon mutation leading HBeAg/HBeAb negative for HBVDNA (Horikita et al., 1994). However 11 patients positive for HBeAb and the samples does not contain detectable serum HBV DNA. This may due to virus replication is occurring in tissue but the titer of virus in the serum is low and the amount of HBV DNA present may be below our detection limit. Virus replication may not be occurring with HBsAg expressed from HBV genomes that are integrated into the host genome, analogous to HBV DNA-containing hepatoma cell liver (Shuichi Kaneko et al., 1989).

Seroconversion from HBeAg to anti-HBe during the course of acute hepatitis is usually accompanied by the resolution of clinical and biochemical evidence of liver disease and the low detectable serum and liver HBVDNA. On the other hand, HBVDNA is often detected in the liver of patients with chronic hepatitis (Liberman et al., 1983). Similar study by Brechot et al., (1981), indicated 9/10 chronic carriers were positive for serum HBVDNA, and 4/9 were positive for HBVDNA in the liver. Thus, it is likely that circulating virions were present in patients. Therefore the presence of anti-HBe in the serum of chronic hepatitis patient is non accurate marker for virus replication in the host. Measurement of serum HBVDNA concentrations in Hepatitis B patients facilitates prediction of hepatic inflammatory activity in hepatitis B and it is clear that the PCR technique represents a significant advance in analysis of serum sample. The lack of serological markers in many infected persons adds a further complexity for detecting carriers, since only PCR can detect the infection such cases. This technique should revolutionize the diagnostic assay for detecting virus DNA and yield valuable information on biology of HBV.

5.8. Genotyping of hepatitis B virus

Genotypes are considered as useful tools in understanding the epidemiology of HBV infection. Further, the possibility of their association with severity of liver diseases has been
actively pursued in recent years. The important issue is whether a particular genotype is associated with certain clinical presentations of the diseases (Selcuk Kaya et al., 2007). Six genotypes of HBV denoted A-F have been described. Genotype A and D are significantly represented in the Western European population where they account for 90.0% for HBV infection (Lindh et al., 1997). The remaining 10% consist of genotype B and C predominant in Asia. The prevalence of E and F is close to Zero (Mayerat et al., 1999).

HBV genotype A and D have been well documented from different parts of mainland in India (Thakur et al., 2002; Gandhe et al., 2003; Banerjee et al., 2006; Chattopadhayay et al., 2006). In two different studies from North India reported genotype D and A found to be equally distributed (kumar et al., 2005). However in another study from North India reported genotype D to be predominant with a low frequency of genotype A,(Chattopadhayay et al., 2006) which was comparable to our study in Krishnagiri district. These studies suggest that the history of migration in different parts of India may lead to the distribution of different types of genotypes (Sibnarayan Datta, 2008).

In the present study among 21 HBVDNA positive samples, 20(95.23%) samples were detected as genotype D which is the most prevalent genotype and genotype A with 4.76% was detected with low prevalence rate. Genotype D was found to be most prevalent among pregnant women in Krishnagiri district. This findings is similar to the study by Arankalle et al., (2003) with (91.4%), Gandhe et al., (2003) with 98.29%, Muhammad Hanif et al., (2013) with 58.5% and Leila Mohammadnejad et al., (2012) with 100%.

This result however differs from that of two earlier studies on chronic liver diseases where genotype A and D were found to be prevalent in equal proportions (Thakur et al., 2002; kumar et al., 2005). In his study mixed infection of genotype A and D of HBV were found in 2% CRB cases, 3% CHB and 7.65 HCCB cases. This is in accordance with what has
been reported earlier (Kumar et al., 2005). In our study, genotype A was found only in the minority of the patients categories.

The initial studies on HBV genotyping revealed that genotype B and C are the most prevalent genotype in Asian region. It was because of the fact that all such studies were reported from Japan and China where genotype B and C are the most prevalent types. Later on it was found that all the seven HBV genotypes can be found in Asia (Toan et al., 2006). For instance, the predominant genotype in India is genotype A and D (Thakur et al., 2002). The predominant HBV genotype in Afghanistan was found to be genotype D (Amini Bavil Olyaee et al., 2006). Similar reports from Pakistan revealed predominant with genotype D (Abbas et al., 2006).

The patients with chronic HBV infection had predominantly genotype A and D in almost equal proportion (Ashish Kumar et al., 2005). Till date only three studies have been published from India on the frequency of various HBV genotypes (Thakur et al., 2002; Gandhe et al., 2002; Arankalle et al., 2003). Thakur et al., 2002 found nearly equal proportion of genotype A and D. The other two studies Gandhe et al., (2002), Arankalle et al., (2003) found genotype D to be the predominant genotype with genotype A being responsible for 5-8% of patients. The above study was close similar to our report. The variation in relative frequency of HBV genotype in studies from India may reflect geographical variations. The study by Thakur et al., (2002) from northern India and our current study from southern India have found high proportions of genotype D. In comparison the other two studies one from western India and other from Andaman and Nicobar Island have reported predominance of genotype D. This raises the possibility that the Indian population had HBV genotype D which has been partially replaced by genotype A,
Distribution of genotype A is less when compared to D and the reasons may be lower antigenicity of HBV genotype A than genotype D. HBV genotype A could inducing its immune clearance and would be associated with high risk of chronic hepatitis. HBV genotype may induce more severe hepatocytic lesions than HBV genotype D. genotype A could be more silent than genotype D more frequently escapes diagnosis and thus be under represented in a selection of patient with acute hepatitis (Mayerat et al., 1999).

Determining the genotype could be helpful for the outcome of antiviral therapy in patients with chronic hepatitis B. HBV influence the severity of liver diseases and response to interferon and antiviral therapy. Patient infected with HBV of certain genotype can be directed to the other therapeutic option to spare the cost and burden of treatment (Muhammad Masoor et al., 2007). Therefore, the appropriate and definite knowledge of all the HBV genotype prevalent in a certain region is of immune importance for the proper and effective management of HBV patients.

In conclusion, HBV genotype D is the most prevalent genotype among pregnant women attending primary health centers in Krishnagiri district and genotype A appears to be the minor genotype. Therefore it is worth importance consideration for clinicians adopt better strategies for appropriate prevention and cure of infection. Further studies are important for epidemiological reasons as well as developing effective therapeutic management against such infection.