6. DISCUSSION:

6.1. Epidemiological pattern of HBV infection in the adult population of Tamil Nadu

Although India is classified under intermediate prevalence zone, variations in the HBV infection rates among general population in different states of India as evidenced mostly by screening of voluntary blood donors is observed. In the present study although the HBsAg positivity rate was 5.7 % (95 % CI 4.65 - 6.75), considerable variation is observed even among the districts in the state of Tamil Nadu. The reason for this variation could be due to the better health awareness within the state. India being, a country with diverse cultural & religious practices, within its individual states, and this factor could also contribute to the variation observed in the present study. Elavia and Banker (1991) observed a relatively high positivity rate of HBsAg among the jain community. Although studies on epidemiological pattern of HBsAg among different communities within India are beyond the scope of this study, considerable variation could be expected among many communities within Tamil Nadu itself. In a study by Singh et al., (2000) in Rajamundhry town of Andhra pradesh, 41.3 % of the community acquired jaundice infection was due to hepatitis B. This observation further substantiates our study where HBsAg positivity was 5.7 % which is slightly above the national
average of 3.34%. In a rare study by Chandrasekaran et al., (2000) in blood donors of Madurai, a 4% HBsAg positivity was seen. Even though the three districts where the present study was done were around the same geographical region as that of Chandrasekaran et al., (2000) variation of 3.7% in Thanjavur, 3.6% in Dindigul and 6.8% in Ramnad was observed.

In studies done by Roy choudhary and Bhattacharya (1989) in Calcutta, socio economic status seemed to have an important bearing on the persistence of the carrier state for HBV. Although our study covered different strata of socio economic status, significant difference among various socio economic levels was not observed. A review by Tandon, et al., (1996) suggested that in India HBV infection is established in early childhood and horizontal means of transmission maintained the carrier pool. The present study also emphasised the fact that horizontal means of transmission is prevalent in our adult population.

Innumerable studies have been carried out in different geographical regions across the globe on the prevalence of chronic hepatitis B virus infection in various types of populations. But only a limited number of these studies focus on the magnitude of HBV infection in different communities and in the adult general population at large. The present community based study was done in the three districts of Tamil Nadu using the sampling method of proportionate to population
based community cluster survey size technique is representative of the state of Tamilnadu.

Behaviour, lifestyle or occupational exposures seem to be the reason for most of the reported community acquired cases of hepatitis B virus infection in the United States. Acute infections of about 1-3 % which occur in children under five years of age account for 20-30 % of all chronic infections in adult hood (Alter, 1993). As evidenced in our study, the prevalence of HBsAg is uniform in all the age groups between 15-45 yrs thereby suggesting acquisition of HBV chronicity by adulthood in our population. Heterosexual contacts seemed to be the mode of transmission in the study by Rosenblum et al., (1990) in Belle Glade Florida as suggested by the fact that persons having two are more life time partners (OR=3:2, 95 CL 1.6-6.4) have a higher prevalence of HBsAg. Although prevalence of HBsAg was not significant in persons having heterosexual promiscuity in our study population, the potential risk of acquiring hepatitis B through this mode of transmission is always present. The changing epidemiology of chronic hepatitis B in the Canadian North has been observed by Minuk et al., (1985). Chronic hepatitis B has become relatively uncommon in the inhabitants of this community born during the past 20-30 years. Although follow-up of the present study with a view to analyse the change in the epidemiological pattern is being envisaged, this aspect is to be carried out in the near future.
In a community-based serosurvey in Sweden by Struve et al., (1992) only 3 % of the young Swedes had encountered hepatitis B virus. As Sweden is a land where immigration is high, the factors influencing presence of hepatitis B virus markers was origin from a country with high HBV endemicity and increasing age. In our study samples were distributed from ninety clusters from the length and breadth of Tamil Nadu. Although many of the risk factors were statistically not significant, the overall prevalence in the adult community of Tamil Nadu was found to be 5.7 % (95 % CI 4.65 - 6.75). Moreover the study population is of the native Indian population and does not include immigrants as seen in the study of Struve J et al., (1992).

Almog et al., (1999) while studying the hepatitis B viral markers among immigrants of the former USSR who arrived in Israel in 1990-91, found that HBV markers were higher in males. Moreover prevalence of anti HBs increased with age among males and that of HBsAg and anti HCV increased with age overall. HBV seropositivity was significantly associated with mother’s education and republic of origin. In our present study, no male preponderance for HBsAg was observed. The HBsAg positivity was 6.4 % in male subjects and 5.2 % in female subjects. Studies in Italy by Petrosillo et al., (1995) on 5813 health care workers revealed a 23.3 % (95 %CI 22.3-24.4) HBV seropositivity and needle stick injury
seemed to be a predisposing factor for HBV. The present study also showed that non-usage of disposable needles was a predisposing factor for acquiring hepatitis B (p<0.03). The other risk factor which were statistically significant in the present study was history a family contact with HBV (p<0.01). The Ethiopian refugee community in Israel was screened for HBV markers by Chemtob et al., (1991) and the study revealed a 19 % HBsAg positivity rate. The percentage of subjects with no HBV marker decreased sharply with age, and similarly there was an age-linked increase in anti HBs, indicating importance of horizontal transmission. Although such a observation was not seen in the present study, a high prevalence of 7.2 % observed in the age group of 15 - 20 yrs suggest that HBV in Tamil Nadu establishes itself by the early years of adulthood and stabilises itself in the succeeding years.

In one of the first reports by Garcia-Fulgueiras et al., (1996) the prevalence of hepatitis B in the general adult population of Murcia region (Spain) revealed a 0.9 % prevalence for HBsAg. In this case, Murcia region was considered an area of low intermediate endemicity for hepatitis B virus infection. The risk factors for hepatitis B virus infection analysed by Gonzalez et al., (1994) in a University student community in Spain revealed 9.1 % to be sexually active, 16.7 % receiving blood transfusion and 8.2 % having a previous history of jaundice.
King et al., (1991) are of the opinion that unless appropriate health education dispels the widespread misconceptions and fears associated with diagnosis of chronic viral hepatitis, prevention of hepatitis B will of limited use in non European communities in the UK. Studies on the prevalence of chronic hepatitis B in the developed countries like Australia by Campbell et al., (1989) have proved that the aboriginal population are at high risk compared with the non-aboriginal population (low risk). Although such a comparison is not valid in prevalence studies in a developing country like India, HBV positivity seems to be more in the rural areas where the education standards are low compared with the urban population. Moreover the question of immigration and prevalence among refugee population are few and scanty in our country. A survey of the general population by Jelic et al.,(1994) in the municipality of Slavenski Brod in Croatia, revealed a prevalence of 1.8 % HBsAg positivity and there was no difference in the prevalence of HBV infection in males, 15.7 % (177/1130) and females 11.6 % (117/1012). The prevalence of anti HBs and anti HBe gradually increased with increasing age, which started at 3.2 % in the youngest, and reaching 15.5 % in the adults over 50 years of age. Our study in the three districts of Tamil Nadu revealed no variation in anti HBs positivity in the different age groups as observed by Jelic et al., (1994). It was 24.8 % in the age group of 15-21 years and ranged between 22.5- 19.0 % between the age group of 21-45 years.
Olsen et al., (1989) while studying the prevalence of HBV infection in a local community in Greenland suggests that heterosexual transmission as the main mode of transmission. The authors also suggest that increased use of condoms and reduction in the number of casual sexual contacts could reduce the risk of transmission of HBV. Contraception as a method employed in the prevention of HBV could go a long way in prevention of increased spread of HBV in our population. Even in the present study non adherence to contraceptive methods in women (p<0.02) was a significant risk factor.

In a hyperendemic New Zealand community. Milne et al., (1987) associated prevalence of anti HBs with amateur tattooing. Spooner et al., (1990) while studying healthy antenatal patients attending the Gurkha Hospital in Papua New Guinea found that there is no relationship existing between tattooing practices and prevalence of hepatitis B as infections occur in early childhood in their population. Although only 19 % of our study group had a history of tattoos, the prevalence of HBsAg in both the groups were not significant. This suggests that although the practice of tattooing is potentially risky, it is not important in the transmission of hepatitis B virus infection, in our population.

In an sero-epidemiological community based cross sectional study in Zimbabwe by Tswana et al., (1986) the overall prevalence of HBsAg was 15.4 %,
which is very high. In the Zimbabwe study, the difference between sexes was consistent in all age groups and was statistically significant. In our present study, a high prevalence of 7.2% was observed in the 15-21 year age group, and only 3.2% prevalence in the age group between 21-25 years. There is a consistent prevalence of 6.3%–6.0% in the age group between 25-40 years. The reason for the moderate prevalence in the 21-25 year age group is not very clear. The prevalence of any HBV marker namely, HBsAg and anti HBc was 66% in males and 61% in females in the Zimbabwe study, whereas in our study the overall prevalence was 28.2% in males and 25.5% in females.

In a study done in Somalia by Sebastiani et al., (1985) 331 village subjects aged between one to 83 years were screened for HBsAg, anti HBs and anti HBc and 12.08% of them were HBsAg positive. 34.7% of them were HBeAg positive as against 23.2% observed in our present study. In Somalia where the present study was done, perinatal infection plays an important role in determining the reservoir of virus carriers. In another East African study done at Maragua between June 1986 and Nov. 1987 by Okoth et al., (1990) the HBsAg carrier rate observed was 3% in the community and horizontal transmission did not play an important role. The present study has stressed the importance of the role of horizontal transmission in the Tamil Nadu population.
Yamanaka et al., (1991) in their study of hepatitis B virus markers in Kenya observed increased HBsAg and anti HBc Ab prevalence two to three times more in family members of carriers as observed in our study, thereby suggesting the role of vertical and horizontal transmission of chronic HBV infection. Prevalence of HBeAg was 23.1 % in our study in comparison with 10 % in the adult carrier population in Kenya. Nearly 1/4th of our HBsAg infected population are replicating carriers as HBeAg is a marker of infectivity and subsequently more number of newer infections are likely in our population. Studies in South Africa by Abdool Karim et al., (1989) have stressed the need to assess the role of risk factors like scarification and pierced ear lobes in the transmission of HBV, while such a observation was beyond the scope of this study the practice of piercing earlobes and use of nose rings is a very common feature among the women folk in our community.

In a community based study in the Philippines by West et al., (1986) the risk of infection seems to be on the increase if one or both of the parents are infected, compared with both parents susceptible (odds ratio: 3.3 and 7.6). The present study also showed evidence of familial clustering of chronic HBV in our population. Song et al., (1988) while studying HBV marker pattern in South African Chinese are of the opinion that because of improved hygienic and socioeconomic circumstances, the overall infection rate increased progressively
with increasing age, although such an observation was not seen in our community based study. Arevalo and Arevalo (1989) found that risk factors like previous exposure to sexually transmitted disease and past history were predictive in their study population, however such an observation was not seen in the present study. In a point prevalence study done in a randomly selected village of Rangpur district, Bangladesh from June 94 to May 95 by Rahman et al., (1997) revealed that age, sex, religion, income, occupation, education and martial status did not show any relationship with HBsAg status and 25 % of the positive cases revealed no risk factors. 75 % of them had one or more exposure to known risk factors (p<0.05). This observation is similar to our study population also.

The seroprevalence of hepatitis B virus infection in the Gurkha community of Singapore by Goh et al., (1993) revealed 2.8 % prevalence for HBsAg. The HBsAg prevalence was high in males (3.6 %) when compared with females (1.9 %) with highest rate of prevalence in the 15-19 year age group. The interesting observation seen in this population was that the seropositivity of one spouse significantly correlated with the seropositivity of the other (p<0.05) thereby suggesting a role for heterosexual transmission of HBV infection. In our study there was no difference observed in the prevalence of HBsAg among persons who had one sexual partner compared with persons who had more than one sexual
partner. This could be because of the increasing awareness of the observance of safer sexual practices seen in our population.

Community studies are very rare in India and the only published community study is by Chowdhury et al., (1999) from West Bengal. A 5.3 % positivity HBsAg rate was observed, very similar to the 5.7 % positivity rate observed in our study. The replicative status of HBV in the chronically infected community population as observed by Chaudhary et al., (1999) was 3.9 %, while our present study revealed a 23.4 % HBeAg positivity in Tamil Nadu which coincides with the national pattern of geographic variation as seen in the general population studies based on blood donor data. The present community based study has confirmed that:

a) Chronic HBV infection is a major public health problem in Tamil Nadu.

b) Tamil Nadu belongs to the intermediate endemicity zone of prevalence.

c) Increased role of horizontal mode of transmission in our population.
6.2. Epidemiological pattern of Hepatitis C in the adult community.

Hepatitis C virus (HCV) represents as a major cause of post transfusion non-A non-B hepatitis and is an important cause of chronic liver disease in India. Very few studies have been carried out in the general population and the community at large in this part of the country. The age at which the infections can occur plays an important role in understanding the epidemiological scenario of HCV infection. In the present study done, the age group, which was selected, was between 15-45 years and the anti HCV positivity was 1.08 % (95 %CI 0.55-1.45). In a study done by Arankalle et al., (1995) in apparently healthy population from Pune, HCV was not a common viral infection among the urban children. Even a large number of rural children were also found to be anti HCV negative.

Singh et al., (1991) in the only study from North India, found that none were positive out of 206 tested in the adult healthy population. Prevalence data in the adult general population in India is based on screening of volunteer blood donors all over the country. In this aspect, this study is the first of its kind where such a large number of samples in the adult general population age group has been studied. Based on the few available published reports the anti HCV prevalence in the general population of India ranges between 0.2 % - 4.0 % (Arankalle et al., 1998). Surprisingly, a study done by Gosavi et al., (1997) in voluntary blood
donors from Mumbai showed anti HCV positivity of 15.9 %, which needs further investigation.

In one of the rare studies done in Madurai, Tamil Nadu by Chandrasekaran et al., (2000) in blood donors, the anti HCV positivity was 0.75 % (27/3754). Anti HCV was present in 4 % of males and 2 % of females and more than 93.5 % anti HCV positivity was observed in the age group of above 15 years. The present study also showed that nearly 72.2 % of the anti HCV positive cases in the age group of 25-40 years which suggests that HCV infection is established in the later years of adult life in this population. Moreover, the study done in the three districts of Tamil Nadu, showed that, Thanjavur district had the least prevalence of 0.87 % (95 % CI 0.32-1.92) while Ramnad and Dindigul had an anti HCV positivity of 1.08 % (95 % CI 0.47 - 2.13) and 0.95 % (95 % CI 0.38 - 1.95) very near the national average of 1.2 %. This observation exhibited geographical variation among different districts of Tamil Nadu. Whether these variations are due to the differential levels of public health awareness among the three districts is to be further assessed.

Globally many studies have been carried out particularly in different communities. It has been estimated that there are around 170 million HCV carriers in the world, and in a country like India, a substantial carrier pool is being
maintained. Abdel-aziz et al., (2000) observed that married individuals in the
community were more likely to be seropositive for anti HCV and this view does
not hold good in our present study. The 1.08% positivity rate of anti HCV
observed in this particular study ensures that there is a greater risk of chronic liver
disease in future for this population. Although the over all prevalence of anti HCV
in the three districts of Tamil Nadu is only 1.08 %, as India is fast attaining the
highest population status in the world, the number of persons with silent HCV
infection will definitely increase in future.

The prevalence rate of 1.08 % in the general population can be considered
as a region of low prevalence as far as HCV is concerned. Our study correlates
with the study of Coppola et al., (2000) in which majority of HCV positive
persons among the general population ran a sub clinical course of infection. In our
study too, all the HCV positive persons were clinically asymptomatic at the time
of specimen collection.

Studies on the prevalence of hepatitis C virus infection in the adult
community population attains major importance as there is no vaccine against
hepatitis C. The main goal of current efforts should be prevention until such time
low cost effective therapies become available. Murphy et al., (2000) in their study
on the risk factors for acquiring HCV in the United states have listed intravenous
drug use, blood transfusion and sex with an IDU being the major risk factors,
while other minor risk factors like incarceration, religious scarification, being stuck or cut with a bloody object, pierced ears or body parts and immunoglobulin injection should be interpreted with caution. Although in the present study many of the risk factors analysed like tattooing, non usage of disposable needles, blood transfusion, multiple sex partners etc did not have statistical significance, other inapparent parenteral modes of transmission could have resulted in the transmission of HCV infection.

Similarity with the observations made in the present study is also echoed in the study of Vardas et al., (1999) done in asymptomatic first time blood donors in Namibia. The overall prevalence was 0.9 % (95 %CI 0.5-1.5 %). There was no correlation associated between HCV serostatus and gender, region of residence or previous exposure to HBsAg. The only significant association in a logistic regression model was increased positivity with increasing age. However in the present study there was no increase in the prevalence rate among adults. However, anti HCV positivity was observed only in the elderly children above the age of eight years.

In the United States, 10 % of the infections have no recognized source of infection, although most persons in this category belong to the low socio economic level (Alter, 1999). In the present study no statistical significance could be
associated to the various risk factors analysed but majority of the HCV positive subjects belonged to the low socio economic levels in the society. Studies in India have proved that increased HCV serorprevalence in the young adult age groups preferably among the intravenous drug users. As blood transfusion is one of major risk factors for transmission of hepatitis C virus infection in the United states (Williams, 1999) routine screening for hepatitis C virus brought down the annual incidence of new infections to approximately 36,000 new infections in 1996. Similar strategies in the Indian subcontinent would prevent newer HCV infections in future. Surveillance and evaluation activities also are important to determine the effectiveness of these programs in reducing the incidence of disease, thereby identifying persons infected with HCV and promoting healthy life styles and behaviours.

According to the WHO report on the burden of HCV infection in the general population of south east Asian countries and based on the present study, nearly 12 millions in India would be infected with HCV. Therefore a need for a successful national public programme to prevent HCV infection and HCV related chronic liver disease in the general population is immediately warranted. In a developing country like India, the primary objective of HCV related public health services should be prevention of new HCV infections and identification of HCV infected persons to control HCV related chronic liver disease. The present study
makes it imperative that HCV infection levels and HCV disease burden are significant enough on the public health status of our country to warrant immediate remedial measures.


HBsAg positivity rate among the school children in Chennai was observed to be 1.86% which is very similar to studies conducted by Thapa et al., (1995) who found the prevalence rate of HBsAg in 334 healthy urban school children to be 1%. This low level of HBsAg positivity observed could be due to the increased awareness among the urban city population about hepatitis B virus infection. Moreover, the low prevalence rates observed could be because the study population consisted mainly of urban middle class who have higher literacy rates and better access to public health education. Susan Wheeley et al., (1997) studied 15 children who were positive for HBsAg and found that fourteen of them continued to be carriers. Girls seem to develop antibody to HBeAg more than the boys. Although in our study we observed males had a slightly higher HBsAg prevalence than the females, it was not statistically significant (p>0.05).

In the present study, the age group studied was between 2-18 years. In a study conducted in Taiwan, by Chiu et al., (1991) there was a age dependent increase of HBsAg prevalence among school children. The percentage positivity
increased from 15.4 % (Grade I) to 23.7 % (Grade IX). Although in the present study 50 % of the HBsAg positive children were in the age group between 10-13 years, the observation cannot be considered conclusive as we had more samples in this particular age group. Williamson et al., (1985) observed a 11% HBsAg seropositivity among children aged between 1-9 years. Here HBV infection is established very early in childhood and the author calls for mass vaccination of all new born babies. Although the prevalence of HBsAg among the school children studied was low, because of the risk of horizontal transmission, vaccination of school children should be considered imminent.

Antibody to hepatitis B surface antigen was observed in 6.86 % of our cases and this goes to prove that the exposure to hepatitis B in this population is considerably low. Hong Yuan- Hsu et al., (1984) in a study conducted in Taipei demonstrated HBeAg positivity in 83.3 % of the 120 HBsAg positive children. As HBeAg is a marker of infectivity, these HBeAg positive children are a source of extrafamilial transmission. We observed 37.5 % of the children to be positive for HBeAg and this percentage positivity is higher than that reported from other parts of the country. However, it coincides with the earlier reports made from Chennai and South India (Thyagarajan et al., 1996).

Tanaka (2000) observed that majority of childhood infections in Japan was due to mother to child transmission. Studies done elsewhere and in our studies
done at Chennai have proved that there is nearly 90-100 % chance of vertical transmission from the infected mother to her offspring if the mother was HBeAg positive. However, in our study only 31.25 % of mothers of the HBsAg positive children were HBeAg positive. This observation indicates besides vertical and intrafamilial transmission observed, horizontal transmission plays an important role in the spread of HBV among school children in Chennai.

Tandon et al., (1991) have observed that India's carrier pool is established in early childhood predominantly by horizontal spread due to crowded living conditions, close person to person contact and poor hygiene. But as evidenced in the present study, both vertical and horizontal modes of transmission seem to be present in our population. In a classical classroom setting study by James Oleske et al., (1980) there was spread of the same subtype of hepatitis B virus between two classmates. Although such an observation of same HBsAg subtype among students of the same class was not studied, the possibility of play injuries among children logically emerging as one of the horizontal modes of transmission is substantiated.

Alison Martin et al., (1996) has observed a 24.3 % HBsAg positivity rate among household members of HBsAg positive school children. Previous studies conducted at our institute have revealed a four-fold increase in HBsAg positivity in families with a known index carrier when compared with controls. Even though
we did not study the families of these HBsAg positive children, higher rates of prevalence could be expected in these cases. Hayashi et al., (1987) observed 5.6 % positivity for HBsAg among nursery school children (Mean age 2.9±1.4 years). In the present study, 3/85 (3.52 %) in the nursery age group was found positive for HBsAg. Although person to person transmission of HBV through articles contaminated with oral secretions from toothbrushes, baby toys and chewing gum are reported, the exact routes of transmission in the nursery age group could not be determined.

Chiaramonte et al., (1991) observed higher HBsAg positivity among suburban school children when compared with central town school children in Cameroon. This does not hold good in our study because all the schools which participated in the study belonged to the main city of Chennai. Decline in HBV prevalence over the years was observed by D'Argenio et al., (1989) in Naples, Italy and this could be due to decreased family size and lower prevalence of HBeAg among carriers. Smaller family size could also be considered as a factor for the low prevalence observed in our population.

Anti HCV seroprevalence studies among healthy school children in our country are very rare. Studies by Arankalle et al., (1995) and Singh et al., (1991) have found that none of the healthy children tested were positive for anti HCV. This is one of the first report from India where HCV seroprevalence has been
shown in healthy school children. In our study the prevalence rate for anti HCV was 0.93 %, which demonstrates a low-level seroprevalence of HCV seen among our school children. Moreover, anti HCV positivity was observed among children who belonged to the 8-16 year age groups. 37.5 % of those infected for HCV gave a previous history of jaundice. The observation by Chang, (1995) that HCV in children occurs in high risk groups, such as those who have received blood products, those who have used unsterile needle injections etc. does hold good in our study population. Twenty five percent of our anti HCV positive children gave a history of surgery and blood transfusion while one child gave a positive history of play injuries. While horizontal modes of transmission is common in case of HBV, the same cannot be conclusively said for HCV infection. Many studies involving larger sample size including familial clustering studies of HCV are to be carried out to analyse other horizontal modes of transmission of HCV. Sokal and Vorlotti (1999) observed that acquiring HCV during childhood is through true vertical transmission. The risk of acquiring hepatitis C is related to the presence and amount of HCV RNA in mothers at the time of birth. Dynamics of vertical transmission of HCV is currently being carried out separately to see whether HCV viraemia could lead to HCV transmission from mother to children.
6.4. Characterisation of asymptomatic chronic Hepatitis B in Chennai.

Chronic hepatitis B continues to be a major public health problem in the world today. It is estimated that there are around 300 million carriers of hepatitis B virus (Mahoney, 1999). In India alone, 42.5 million HBV carriers are present (Tandon et al., 1996). Majority of these persons carry the virus without any major clinical symptoms and are asymptomatic. It is also known that the risk of chronic clinical sequelae in these asymptomatic HBV infection cases is two hundred times higher than the normal population. With this background information, the present study was undertaken to analyse and characterise the various demographic, risk factors, serological, biochemical and molecular markers in asymptomatic chronic hepatitis B virus cases coming to the Department of Microbiology, Dr. ALM. PGIBMS, Taramani, Chennai. The present study was done in asymptomatic chronic hepatitis cases, which were referred from various blood banks, Govt. Hospitals, Private practitioners & Gastroenterologists within Chennai and from other parts of India.

Shouval et al., (1981) in their study on 90 hepatitis B surface antigen carriers in Israel, confirmed the predominance of males among HBsAg carriers, although the association with sex did not achieve statistical significance with ethnic origin. Even in the present study, there was a male preponderance and the
male: female ratio was 4.4:1. The prevalence of HBeAg was moderate (17.3%) in our study, in comparison to 62.9% HBeAg prevalence in Taiwan (Ding-Shinn Chen et al., 1986). The high prevalence of HBeAg in Taiwan was related to the younger age of the HBsAg carriers. HBeAg in HBsAg carriers has also been found frequently in the Japanese (Miyakawa and Mayumi, 1978), Vietnamese (Aldershvile et al., 1980), Mainland Chinese (Xu and Xie, 1983), Koreans (Chung et al., 1985) and the Alaskan Yupik Eskimos (Alward et al., 1985) but not in the Caucasian population in Denmark (Aldershvile et al., 1980), the United States, and Canada (Mushawar et al., 1978). Based on the fact that most HBV infections in Asians occur early in childhood, recent acquisition of HBV infection as the cause of high HBeAg prevalence in adults seems unlikely. The reasons for variations in the prevalence of HBeAg and anti HBe in HBsAg carriers remain unclear.

According to a study by Barr et al., (1979) factors such as age of acquisition of infection, prevalence of various subtypes, varying transmission routes and possible differences in life expectancy for those carriers who are HBeAg positive could conceivably influence HBeAg / anti HBe prevalence data. The authors are unable to conclude that seroconversion from HBeAg to anti HBe over a number of years commonly occurs in the natural history of the carrier state. We did observe more number of HBeAg carriers in the younger age group, and HBeAg seroconversion does seem to occur more frequently in the older age
groups (31-40 years) as there was a moderate prevalence of HBeAg in the older age group.

Analysis of the risk factors in our study population revealed 30% of carriers with a previous history of jaundice, while 17% gave a history of surgery. In 25% of the cases, there was no known risk factor and majority of them could have acquired the infection through horizontal modes of transmission. This finding is comparable to that of Shouval et al., (1981) where 24% of HBsAg positive carriers had a history of contact with jaundiced persons in the six months preceding the detection of the carrier state. In contrast, in a study done on 471 HBV carriers by Bar-Shany et al., (1994) only 12% gave a positive history of jaundice in the past and 5.2% had contact with a jaundiced member at one time. Only one percent of the population gave a history of blood transfusion.

Serological profiles of hepatitis B carrier patients in Singapore by Wang et al., (1996) revealed 21% of 1132 carriers tested to have both HBsAg and anti HBs. While our study revealed only 1.6% positivity of both HBsAg and anti HBs, the possibility of surface antigen escape mutants as seen in the Singapore study has to be worked out. The clinical significance of presence of HBsAg and anti HBs due to the presence of low levels of heterotypic antibody (directed against HBsAg sub-determinants not present in the serum of the individual) can also be
thought off. In some cases, the coexistence may be associated with immune complex renal disease.

The presence of HBV DNA in persons with chronic hepatitis B has been demonstrated by many workers (Hess et al., 1977; Ding-Shinn Chen et al., 1986; Lieberman et al., 1983; Ran Tur Kaspa et al., 1984; Wing Kam et al., 1982; Barnet Berris et al., 1987). In studies by Ran Tur Kaspa et al., (1984) on HBeAg negative individuals, 10 out of 153 were positive for HBV DNA.

The reasons for observation of HBV DNA in the anti HBe positive sera as seen in 18.4 % of our cases, could be due to:

(a) Ongoing viral replication might be inapparent and could be masked by immune complex formation.

(b) Occurrence of core/precord mutants

(c) Catch point of the case at the period of ongoing HBeAg seroconversion.

The presence of high titers of HBsAg seen in HBeAg positive carriers in our study is similar to the findings by Luis A Viola et al., (1981) where 38 out of the 39 patients had a high titer of more than 1:4096 and were HBeAg positive. Although studies by Nath et al., (1980) suggest that samples with high titers of HBsAg are HBeAg positive, our study was in contrast to this observation. Anti HBe was positive in 79.6 % of the cases which had high titers of HBsAg. As seen
in our study anti HBe positive HBsAg carriers could also have high titers of HBsAg. We observed high HBsAg titres (100 μgm/ml) among subjects with asymptomatic chronic hepatitis with a longer duration of HBsAg positivity, and it was statistically significant (p<0.01).

6.5. Familial clustering of Hepatitis B virus infection in carrier and non-carrier families of South India.

Bancroft et al., (1972) reported for the first time the occurrence of clustering of HBV with in the family of 5½ year old, giant cell hepatitis child, wherein 3 of the four family members were found to be asymptomatic carriers of hepatitis B surface antigen (HBsAg). The first family study to characterize intrafamilial spread of HBV was reported by Reeves et al., (1975) conducted in 255 Panamanian Guaymi Indians representing 48 families and 32 living units. It was observed that infection rate in antigenaemic index families was 0.64 in contrast to 0.19 in infected families without antigen carriers (P < 0.001). Their results indicated that families with HBV carriers had higher HBV infection rate than families without carriers.

Subsequently, studies from USA (Peters et al., 1976; Scott Mazzur and Norman Jones, 1977) European countries (Hess 1979; Pastore et al., 1981), Australia (Judith Barrett, 1976) New Zealand (Powell et al., 1985) Africa (Abdool
Karim *et al.*, 1991) and Asian countries (Sung *et al.*, 1978; Myron Tong, 1979) including India (Sanjay Dhorje, 1985) have proved in unequivocal terms:

a) Intrafamilial as a possible means of HBV transmission (Tan, 1989).

b) The increased risk of acquiring chronic HBV infection due to long-term living with a carrier (Perrillo *et al.*, 1979).

c) Prevalence of HBV markers to be significantly higher among the contacts of more than one HBsAg carrier (75.9 \%) than among those with only one (26.0 \%) (P < 0.001) (Juan Carlos Porres *et al.*, 1989).

d) Horizontal, non-parenteral transmission of HBV among siblings played a major role in the household of HBsAg carriers (Antonio Craxi *et al.*, 1991).

e) The family clustering was striking in all populations where the genealogies were known (Judith Barrett, 1976).

f) Viral markers were detected more frequently in blood relatives than in non-blood relatives of index HBsAg carriers (Myron Tong *et al.*, 1979).

g) The subjects in the group without clustering had a higher rate of HBeAg negativity than the group with clustering. Of the original HBeAg negative subjects, the group without clustering had less damage to the liver than the groups with clustering (Habu *et al.*, 1991).

h) In an epidemiological study by Ala Toukan *et al.*, (1990) among family members in the middle east, a history of contact with a jaundiced person and socio-economic status were independent risk factors for HBsAg positive status, while contact with a jaundiced person, rural background and age were
independently related to HBV infection. There was a trend towards an association of HBsAg positive children with HBsAg positive mothers. Postnatal early childhood transmission through contact among children of poorer and larger families probably accounted for the high endemicity of HBV in that region.

i) Wang (1993) adopting HBV subtyping in a family clustering study showed that familial transmission was classifiable into six types, namely generational, horizontal, recessive, intra and extra familial, non familial and undetermined ones.

j) Yao (1996) suggested that in China, horizontal transmission is an important route of HBV infection during early childhood, and the proportion of chronic HBsAg carriage attributable to perinatal transmission has been estimated at only 13-20%.

k) An African study by Abdool Karim et al., (1991) hypothesized that low socio-economic status, living in crowded conditions with an average of seven people in a house with one or two bedrooms, might provide an appropriate milieu for the spread of HBV infection.

Indian studies on intrafamilial transmission of HBV are scanty. The only study report available is that of Dhorje et al., (1985). They had conducted a study on household contacts of HBsAg positive patients in comparison with those of HBsAg negative patients, in Pune, India. The risk of HBV infection was
significantly higher in the former group with 80 % positivity for HBV markers than in the latter group, which had only 48.1 % HBV marker positivity. The other report available is that of Thyagarajan et al., (1996 b).

In all these studies conducted across the globe, the possibility of family clustering and intrafamilial HBV transmission has been authenticated. The actual mechanism of contact associated HBV transmission in these households is not always known. However, sexual transmission or inapparent percutaneous exposure thro' shared razors, tooth brushes etc are frequently suspected. The impact of replicative HBV status in determining HBV transmission rate within families gives credence to the hypothesis, that a consistent, cumulative exposure of family members to one or more HBeAg/HBV-DNA positive persons, blood contaminated body fluids especially saliva has to be thought of as the principal source of horizontal transmission (p< 0.0004).

Interestingly (Tables 5.7.1, 5.7.2 and 5.7.3) family clustering data from the present study and from our previous study in Chennai suggest that the rate of intrafamilial transmission of HBV is on the decrease in this geographic region. This could most probably due to (i) the awareness created among the public with regard to the infectivity of HBV within families and (ii) primarily due to the increasing practice of vaccinating HBV negative family members of known HBsAg positive carriers. It is to be strongly recommended that as a mandatory rule
all HBsAg negative family members of known HBV infected individuals shall have to be routinely administered with hepatitis B vaccine.

6.6. Experimental Purification of Hepatitis B surface antigen from Hepatitis B virus carrier plasma and evaluation of its immunogenicity in animal models.

This study was conducted to evaluate two published methods for purification of HBsAg and to characterize the purified preparations by SDS-PAGE and also to test the immunogenicity of the purified products in animal models namely Rabbits and Goats. This study was undertaken to establish the most suitable method of purification and to check whether the purified products had the potential to be used as a candidate vaccine. To eliminate any differences due to the starting material, high titered HBsAg positive serum was used. Although many established procedures have been published in scientific literature, it is very difficult to compare them because of the differences of not only in the starting material but also in the quantitation of both total protein and HBsAg. The concept of specific activity was applied to the purified preparation and the total protein was quantitated using the method of Lowry et al., (1951), which is universally done in all the laboratories.

The two methods which were evaluated were the methods of Mishiro et al., (1980) and Govender et al., (1985). The method of Govender et al., (1985) yielded
the highest specific activity with a purification index of 64. The method of Mishiro et al., (1980) gave a specific activity of $2.84 \times 10^6 \text{U/mg}$ but a purification index of 251. This higher purification index observed could be due to the use of the zonal rotor. The method of Mishiro et al., (1980) employs two isopycnic separations and one rate-zonal separation, while the method of Govender et al., (1985) uses one polyethylene glycol precipitation and a single isopycnic ultracentrifugation step in which the gradient is formed in situ. Although this method works out well with both the zonal as well as the swing out rotor, in this case, swing out rotor was employed. The method of Govender et al., (1985) is rapid and simple in comparison with the procedure of Mishiro et al., (1980). Even smaller volumes of plasma can be purified using the method of Govender et al., (1985). Urea (8M) may be used in place of sucrose and because of its dissociating effect it would probably improve the purity of the final product.

SDS page analysis of the two methodologies revealed the following: In the method of Mishiro et al., (1980) bands corresponding to p49, gp32 and p39 were visualised. The major polypeptide bands gp 27 and p24 were visualised. While the method of Govender et al., (1985) yielded an additional band of 66200, the major polypeptide bands of mol.wt 22,700-24,400 and 26,600-28,500 were visualised along with three more additional bands in the mol wt range of 41,300-54,600. Although the higher SA values should yield lesser contaminating bands, we do not know the reason for additional bands visualised in the method of Govender et al.,
This could possibly be because of only a single isopycnic ultracentrifugation step is employed in this procedure. The slightly lesser SA value observed in the method of Mishiro et al., (1980) could also be because of lower HBsAg titer of initial starting material when compared with the method of Govender et al., (1985).

Although evaluation of purified products for their immunogenicity have been done most in Balb/c mice (Howard Fields et al., 1988) and guinea pigs (Zdenka Valinger et al., 1990) very few studies have been done in rabbits or in larger animals like goats. It is with this idea and to produce larger quantities of antiserum, we carried out the immunogenicity of the purified product by the method of Govender et al., (1985) and with plasma derived vaccine and recombinant vaccine controls. The purified product of Mishiro et al., (1980) was not made available due to certain project constraints. In the study done by Howard Fields et al., (1988) the purified products with higher SA values gave a better response in comparison with purified products with lower SA values. In the rabbit immunogenic model, although the purified product elicited a good immunogenic response after the fourth week of immunisation (114 mIU/ml), the Recombinant vaccine control gave a slightly better anti HBs response (152 mIU/ml). This could be because of the purity of the recombinant antigen used for immunisation. In studies conducted by Zdenka Valinger et al., (1990) in guinea
pigs plasma derived HBsAg gave a better response in comparison with recombinant antigen. The major factor affecting the immunogenicity is the structure and source of the antigen used.

HBsAg, which is the surface component of the hepatitis B virus is a glycosylated protein and is composed of the pre-S and the S gene encoded regions. In the process of isolation and purification from plasma, the structure of the native HBsAg could be altered or affected, because various inactivation procedures lower the immunogenicity. This could be one of the reasons as to why we observed a lower immunogenic response with our plasma derived preparation. The immunogenic studies revealed that the plasma derived purified product was well tolerated and as it elicited an good immunogenic response in rabbit model, it exhibits the potential of being used as a candidate vaccine and for further evaluatory studies.

The Goat immunogenic model also yielded a moderate response of 80 mIU/ml by Elisa. The method of immunisation was as per the procedure of Dreesman et al., (1972). The purpose of using goats was to obtain large quantities of antiserum. Production of high titer anti HBs serum in goats also avoids the high levels of antibody seen against human alpha globulin in rabbit antiserum. Immunogenic studies in goats although did not elicit a very high antiHBs titer the
sensitivity and specificity of the antiserum has to be evaluated for its use in various diagnostic and evaluatory procedures.

6.7. Reproducibility efficacy of *Phyllanthus amarus* an Indian medicinal plant in the treatment of Chronic Hepatitis B virus infection.

Even though clinical uses of *P. niruri* and other species viz. *P. amarus* cited for over a century in the Ayurvedha and Siddha literatures, scientific evaluatory studies have been attempted only during the last 50 years for its efficacy in the treatment of jaundice/viral hepatitis. A logical approach towards identification of the active principles of *P. amarus* is to fractionate the plant extracts and identify biologically active compounds and to chemically characterise them.

In the light of these studies in other countries, it has become necessary to explain the reasons for non-producibility of the clinical efficacy of *P. amarus* on one hand and to conduct further clinical trials independently in different places using the *P. amarus* preparation of Thyagarajan. Accordingly clinical trials were conducted on a total of 153 chronic HBV carriers (3 in Chennai (Madras), 1 at Vellore and 1 at Glasgow, UK).

Thyagarajan *et al.*, (1982) were the first to demonstrate that the extracts of the plant *Phyllanthus amarus* produced consistent inhibition of the hepatitis B
virus in invitro studies. It was also found that the extracts was found to be capable of eliminating the virus from the serum of woodchucks. Studies done by Unander and Blumberg (1991); Jayaram et al., (1996); Yanagi et al., (1989); Mehrotra et al., (1990); Ogata et al., (1992); Shead et al., (1990) have confirmed that *Phyllanthus amarus* is able to inhibit HBV virus replication as well as reverse transcriptase and DNA polymerase. These findings suggest that *Phyllanthus amarus* has a beneficial effect on HBV, possibly through inhibition of HBV DNA polymerase activity, mRNA transcription and replication.

Although Niu et al., (1990) was unable to demonstrate any significant reduction of circulating viral DNA in ducks congenitally infected with HBV, such discrepant findings can be attributed to a combination of many factors, like variations in plant material or animal species, period and place where the botanical samples were collected, age of the plants and also the part of the plant that was used.

The mechanism by which *Phyllanthus amarus* acts in the treatment of chronic HBV carriers has been worked out by Ott et al., (1997). Recently, the authors showed that *P. amarus* extract is able to suppress HBV by a specific mechanism of interrupting the interaction between HBV enhancer I and cellular transcription factors. The same authors give further support to the antiviral mechanism of action for *P. amarus* extract by demonstrating anti hepatitis B
properties of active principles present in the extract of *P. amarus* are associated with disruption by the compound (s) of HBV polymerase activity, and mRNA transcription and replication. Although the chemical constituent present in the plants belonging to the genus *Phyllanthus* responsible for anti HBV action is not properly defined, it is believed that the hydrolysed tannins mainly ellagic acid might account for the beneficial effects of *Phyllanthus* plants against HBV (Unander *et al.*, 1995). Moreover in another study by Polya *et al.*, (1995) hydrolysable tannins isolated from *P. amarus* are potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalytic subunit, with IC 50 values ranging from 0.2 to 1.7 um.

Although it has been well established that *Phyllanthus amarus* shows activity against hepatitis B virus both in invitro and in invivo systems, there is an element of controversy involved with respect to the reproducibility of the efficacy of the plant in different studies done mostly in clinical trials done outside India. After Thyagarajan *et al.*, (1988) reported 59 % HBsAg seroconversion after one month treatment in Chronic HBV carriers, studies done by Leelarasamee *et al.*, (1990); Milne *et al.*, (1994); Thamilukkutil *et al.*, (1991) and Wang *et al.*, (1991) have failed to reproduce the same HBsAg seroconversion rate observed in HBV chronic carriers. A single clinical trial study done by Doshi *et al.*, (1994) in India also failed to reproduce the same beneficial effect on Chronic carriers of HBV.
The present study showed a HBeAg seroconversion rate of 54.28 % among the HBeAg positive treated group. Similar observations have been made in a recent study by Wang, (2000) who observed 45 % HBeAg seroconversion in the Phyllanthus treated chronic hepatitis B patients. Moreover the present study also showed a 28.5 % complete viral clearance, with loss of HBsAg consistently in these treated patients. HBsAg clearance was higher in the HBeAg positive group (28.6 %) as against the HBeAg negative group (17.6 %). In a very recent review on Chinese medicinal herbs, by Liu et al., (2001) the activity of Phyllanthus amarus on HBeAg seroconversion has been confirmed, which further substantiates the efficacy of Phyllanthus amarus in the treatment of chronic hepatitis B virus infection.

There can be many explanations that can be provided for such discrepant results as observed by different workers in various parts of the world. Phyllanthus amarus, which belongs to the Family Euphorbiaceae, consists of nearly 400 species. In all the trials conducted earlier the authors have not clarified if the chemo-biologically bio-typed variety of Phyllanthus species was used. As evidenced in the subsequent open trial conducted in Glasgow by Eric Walker et al., (1992, Personnel communication) and in open clinical trial conducted at our center (Results unpublished) a increased and better response is observed when treatment is given for a longer duration of time (6 months).
It has also been observed that there is intra species and inter geographical variation in biological properties of *Phyllanthus* species. Therefore, selection of the right variety and to chemo biologically fingerprint the biotype of *Phyllanthus* species is of utmost importance in obtaining the enhanced clinical response. The other point which is to be clarified in the trials conducted earlier is the dosage given to the patients with chronic hepatitis B. Although, Leelarasamee et al., (1990) have provided with a higher dose of 1,200 mg to the patients, the duration of treatment was only for a period of thirty days. Till date, no assay to quantify the half-life of the plant drug has been reported. Inview of the above variables, it has to be summarily accepted that a multicentric clinical trial using the chemo-biologically standardised biovariety of *P. amarus* has to be conducted in future. This might lead us into better understanding of the therapeutical potentials of this plant.