Discussion
6.1 DETERMINATION OF BASELINE MEASLES IgG AB TITRE

The lifelong immunity that follows natural measles infection or vaccination is usually effective. Although secondary cases occur they are extremely rare. Following natural infection, with the appearance of rash, both IgG and IgM antibodies become detectable. The IgG antibody titres reach a peak at about 30 days, fall two or four fold and then remain very nearly stationary for life. Hence measles virus IgG antibody should be detectable in normal healthy population, who have been exposed to natural infection or have been vaccinated. The assessment of immunization campaigns and the age distribution of measles susceptibility can be determined by one of the three methods: assessment of the age distribution of the cases, analysis of vaccine coverage profile over time, or by laboratory assessment of population immunity by looking at protective levels of antibody in representative subsets of the population (David Featherstone et al, 2003). Since IgG antibody levels are known to be greatly elevated in SSPE patients, which is a definite diagnostic criteria, it is necessary to determine the base line measles IgG antibody levels in the representative subsets of population.

In this first part of our study 2 groups of subjects were screened, which included 146 apparently healthy school going children of the age group 5-15 (Group I), and 44 healthy adults of the age group 16 – 40 (Group II). Of these 135 (92.4%) of Group I, and 42 (95.4%) of Group II were positive for
measles IgG antibody. The mean IgG antibody titre in these subjects was found to be 1:40. In total, 190 subjects were screened, of which 177 (93.2%) were positive for measles IgG, and the mean IgG antibody titre was found to be 1:40, which was determined as the baseline titre in the study population (Table 5.1.2).

A common principle to all measles elimination strategies currently being implemented is the need to maintain the number of susceptible individuals in the population below a certain critical number required to sustain transmission of virus (Manual for the laboratory diagnosis of measles infection, 1999). Hence it was necessary to determine the vaccination status and history of past infections in the study groups.

173 (97.7%) of the 177 IgG positive subjects had a definite history of vaccination, whereas the vaccination status could not be ascertained in the remaining 4 (2.3%) subjects. Of the 13 IgG negative subjects, 8 (61.5%) had no history of vaccination or natural infection. In the remaining 5 (38.5%) IgG negative subjects the vaccination status or history of infection could not be ascertained (Table 5.1.3).

Although the available measles vaccine is effective in inducing protective antibody levels, the percentage of children being vaccinated for measles in affected countries remains at around 73%, slightly below the recommended 80% (Measles Deaths Drop Worldwide, Still Big Killer, United nations agencies, Reuters Health Information 2004. © 2004 Reuters Ltd.). In India the reported coverage levels with measles vaccine has increased
considerably from 1.3% in 1985-86 to 87.3% in 1998-99 (CD Alert, 2000). However the present study highlights the need for a more effective measles vaccine implementation programme, as still a considerable amount of the population are unaware of the need for the vaccine, or remain without access to the vaccine.

6.1.1 Virological and serological studies on acute measles

The reported incidence of measles cases in Tamilnadu was 512 in 2002 and 514 in 2003. In the present study, 28 patients with clinical presentation of acute measles in and around Chennai, Tamilnadu were selected. The patients were between the age group of 2-11 years (mean age 6.9). The median age of infection is 1-2 years in developing countries and 6-7 years in developed countries. A review of Indian data on age distribution of measles in pre-immunization era had revealed that in metropolitan areas the median age was about 24 months and virtually all cases were recorded in 5 children under 5 years of age. In contrast, median age in most of the rural areas was 4-5 years and all the persons were not affected until 10 years of age and the situation was in between in other areas (Singh et al, 1997). In our study, the majority of the cases (82%) hailed from rural areas and the mean age was 6.9.

All the 28 patients were screened for IgM antibody, and all were positive in single serum samples (Table 5.2.1). The presence of IgM is generally accepted as evidence of primary measles infection (by wild virus or vaccine). However absence of IgM, does not exclude infection, particularly in samples drawn within 3 days of rash onset as sensitivity of some of the IgM
assays may be low (Manual for the laboratory diagnosis of measles infection, 1999).

An increased IgM antibody titre of 1:80 was seen in 16 patients (57.1%), whereas in 10 patients (35.7%) an IgM antibody titre of 1:40 was seen and in 2 patients it was 1:20 (Table 1.5). All the 16 samples with elevated IgM antibody level of 1:80 were collected between 4-12 days after the onset of symptoms (Table 5.2.2). In patients with 1:40 IgM antibody titre, the rise in antibody titre could have been observed with paired sera. IgM ELISA test are more sensitive between 4-28 days after rash onset, however a single serum obtained at the first contact with the healthcare system, regardless of which day following the rash onset the sample is collected, is considered adequate for measles surveillance.

Measles virus isolation is most successful on specimens collected most soon after the rash onset as possible and at least within 5 days of rash onset. Virus isolation was attempted in all the 28 patients by collection and processing of throat swabs and urine samples (Table 5.2.4). Of the 28 urine sample screened, one isolate of measles virus was obtained. Of the 26 throat swabs screened, isolation was positive in 5 samples. The poor isolation rate in our study is probably due to collection of samples 5 days after the onset of symptoms in most cases. However, the isolation rate and other results of the study correlated well with a similar study by Wairagkar et al, 2001 in Chandigarh (4 isolates from 28 specimens tested).

Since 1990, immunization coverage surveys carried out in different parts of the country show that actual coverage levels are much below
the reported coverage levels. In addition, there has been a wide variation in vaccine coverage between different states and also among different districts of a state. As a consequence, widespread outbreaks of measles continue to occur in many states, especially in tribal and remote areas (CD Alert, 2000). In Tamilnadu, although the annual measles incidence reports are relatively lower than in other states, occasional if not widespread outbreaks still occur. The study highlights the lacunae in surveillance and control of the disease in some areas and the challenge posed by measles in those areas.

6.1.2 Correlation with Canine distemper virus

Similarities between the clinical and pathological features of human measles and canine distemper virus has been recognized for many years. Canine distemper is a severe disease of canines, with many similarities to measles but direct infection of the CNS and neurologic disease is more common. Hence an attempt was made to study the antigenic protein profile of measles virus isolated and canine distemper virus vaccine strain. The study revealed a similar banding pattern between the antigenic protein of both the viruses in at-least one region (47kd) revealing a possible sharing of antigen between the two viruses, although its relevance in pathogenesis is not clear.

6.2 SERO DIAGNOSIS OF SSPE

Although SSPE is believed to be widely prevalent in India, there is a paucity of SSPE incidence data, except for occasional case reports and clinical investigations. Around 200,000 cases of measles infection were
reported out of which 8000 turned out as SSPE cases in the country during 1990's (Ravi V, 1992).

In the present study, two groups of patients were screened, SSPE group – which included patients with clinical and EEG findings suggestive of SSPE (n=215, 115 males and 100 females) and the control group – which included patients with neurological symptoms, but clinical and EEG findings not suggestive of SSPE (n=12, 7 males and 5 females) (Table 5.3.1).

The age group of the patients was between 1-20. The majority of the patients 107 (49.7%) belonged to the age group of 10-15. All the patients belonged to the middle or lower middle class and marginal classes of the society, with poor or no knowledge and access to primary health care. The majority of clinically suspected SSPE patients were from West Bengal, Bihar and other north eastern states of India where primary health care and vaccine coverage is poor. Only 12 (5.5%) patients of the SSPE group had a definite history of measles vaccination. 160 (74.4%) patients had no history of vaccination and in 43 (20%) patients the vaccination status could not be ascertained (Table 5.3.2). The study highlights the gross difference in vaccine coverage levels in different parts of the country.

Dyken (1985) had laid down definite criteria to diagnose SSPE which included, classical presentation and clinical findings like intellectual deterioration and myoclonus, EEG findings that show characteristically periodic slow bizarre complexes, elevated CSF immunoglobulin levels, Elevated CSF measles specific antibody and classical histopathological findings.
In the present study, clinical history was recorded in all SSPE and control group patients. Myoclonus 90.2%, mental regression 84.6%, decrease in scholastic performance 82.7%, and maculopathy 13.1% were the major clinical symptoms observed. Typical periodic complexes in EEG were observed in 77.6% patients (Table 5.3.3), thus fulfilling Dyken’s criteria. Our observations were similar to the previous observations made on clinical symptoms of SSPE in India by Thakare et al, (1989) who reported myoclonus in 95.3% of patients and mental regression in 83.3%; and Rao et al, (1994) who reported similar clinical symptoms in 2 patients.

CSF and serum samples were collected from these patients and processed. Laboratory findings of the CSF showed an elevated protein level of 40mg/dl in 89.7%. In majority of the patients 68.8% the CSF glucose level remained in the normal range. The CSF picture was similar to the reports of Rao et al, 1994.

Abnormally high levels of serum and CSF IgG characterize SSPE, and is also one of the criteria laid down by Dyken (1985) for the diagnosis of SSPE. In our study an in house ELISA to detect measles IgG Ab was standardised and performed on all the serum/CSF pairs of the SSPE group (n=215) and serum/CSF pairs of the control group (n=12). In the SSPE group 89.3% serum samples were positive for measles IgG antibody, and 78.1% CSF samples were positive. Thus the serum/CSF pair positivity by ELISA was 78.1%. None of the control group patients were positive in CSF for measles IgG (Table 5.3.5). Thus the specificity of the assay was high
Lakshmi et al, 1994, reported 100% sensitivity and 93.3% specificity in sero diagnosis of SSPE by ELISA.

Of the 215 samples analysed, 104 samples from (48.3%) 62 male and 42 female, showed a measles IgG antibody titre of $\geq 1: 160$ in the serum samples (Table 5.3.6). Samples from 56 (26.1%) patients, comprising of 33 male and 23 female showed an antibody titre of 1:80. In the CSF, 101 (46.9%) patients, comprising of 61 males and 40 females showed a high measles IgG antibody titre of $\geq 1: 8$. The IgG level findings in the present study correlated well with previous serological studies on SSPE by ELISA in India (Thakare J.P, et al, 1987 who reported elevated CSF IgG in 87.5% samples).

The serum/CSF sample pairs of the same group of patients was also screened by IIF technique, for the presence of measles IgG antibody. In the SSPE group, 192 (89.3%) serum samples were positive for measles IgG antibody, and 164 (76.2%) CSF samples were positive. Thus the serum/CSF pair positivity by IIF was 164 (76.2%). None of the control group patients were positive in CSF for measles IgG (Table 5.3.8). A similar study by DJ Manayani et al, 2002 reported measles Ab in clinically suspected cases of SSPE by IIF technique. In their study antibody to measles was detectable in 48% of the serum-CSF pairs tested and their ratio ranged between 5:1 to 80:1.

Of the 215 samples, 103 (47.9%) comprising of 62 male and 41 female, showed a measles IgG antibody titre of $1: 160$ in the serum samples (Table 5.3.9). Samples from 57 (26.5%) patients comprising of 33 male and 24 female showed an antibody titre of 1:80. In the CSF, 96 (44.6%) patients
comprising of 58 males and 38 females showed a high measles IgG antibody titre of 1:8 (Table 5.3.10).

Thus 164 patients clinically suspected of SSPE were found to be serologically positive for measles IgG antibody by IIF technique and 168 were seropositive by ELISA. The agreement rate (κ) between these two techniques was found to be 98.13%. The in-house ELISA technique was found to be slightly superior to the IIF technique (Table 5.3.11). Overall, 168 cases were clinically and serologically confirmed as SSPE.

The age and sex distribution of clinically confirmed and seropositive SSPE patients by both IIF and ELISA techniques (n=168) (Fig 5), showed the age group distribution between 2-10 years. The mean age was 11.5. Reports on the mean age of occurrence of SSPE, has been variable among various parts of the world, however many studies indicate 11-15 yrs as the average age group for occurrence of SSPE.

All previous reports have indicated a definite preponderance of male over female in the incidence of SSPE. However the degree of male:female ratio has been variable. In our study of the 168 positive patients, 101(60.1%) were males and 67(39.9%) were females. The male, female ratio was 1.5, which is consistent with some of the previous studies.

The study brings to light the continuing high incidence of SSPE in India, and the challenges posed by the disease. The present study was conducted between 1999 and 2004, nearly fifteen years after the introduction of measles vaccination in India. This relatively high incidence may be due to
the fact that protective effect of vaccination bringing a decline in the SSPE cases will take some time to manifest, given the usual long incubation period between primary measles and SSPE. (Incubation period ranging 7-10 years) (Manayani DJ et al, 2002a).

In our study of the 168 SSPE cases, 12 cases had a definite history of measles vaccination. This could be due to poor seroconversion or vaccine failure, as there are no reported cases of vaccine associated SSPE. As recent reports suggest, this could be due to poor quality of vaccine (due to improper cold chain) or administration of measles vaccine at an earlier than recommended age of 12-15 months or may be due to variants of measles virus (Katayam Y et al, 1997). In our country, the measles vaccine is recommended at 9 months of age, or earlier, if there is an increased incidence of measles at that point of time. If measles vaccine administration starts at 12-15 months of age or later, majority of infants would have contracted measles infection before the new recommended age of vaccination and may later succumb to SSPE (Paul Y, 2002).

Molecular epidemiology of SSPE

Molecular epidemiology studies of measles virus have made significant contributions to measles control programs by providing a means to identify the source and transmission pathways of the virus. Although there are relevant data on genetic characterisation of wild type measles viruses from India, there has been no well-documented reports of genotypic study of SSPE in India.
In the present study, of the 168 clinically and serologically confirmed cases of SSPE, 28 relatively fresh CSF samples were chosen for the study. All the 28 CSF samples were subjected to RT-PCR for measles virus RNA detection. A 169 bp product specific for measles virus NP genome was detected in 16 CSF samples. 2 CSF samples of JE virus and an attenuated CD virus vaccine were used as control group, which were negative. Although to the best of the authors knowledge there have been no such studies on molecular epidemiology of SSPE in India, there have been several reports of similar studies from various parts of the world. T. Nakayama et al., in 1995 reported detection of MV genome by RT-PCR in acute measles, measles encephalitis and SSPE patients in Japan. PCR products of the N and H regions were sequenced and the presence of measles virus genome was confirmed.

Kenji Miki et al., 2002, reported sequence analysis of hyper variable region of the N gene from 13 MV genomes including 2 amplified from clinical specimens of SSPE patients.

Of the 14 PCR positive products, 2 were purified and sequenced, using the forward and reverse primers NPB1, and NPB2 respectively.

Sequences from two specimens were submitted for genotype analysis. The sequence from the reverse primer was reverse complemented and aligned with the forward sequence. The aligned sequences were 171 nucleotides in length and were identical to each other.

The sequence is most closely related to measles viruses of genotype A. However, there were only 130 nt in common between the WHO
reference sequences and the sample SSPE sequence analysed. A window this small does not permit a statistically valid phylogenetic analysis (Paul Rota, personal communication). In addition, there may be some errors in the sequence because the T at position 1 and the A at position 171 did not match any other sequences in the measles database. These sequences should be checked.

Genotype A contains both vaccine and wild-type sequences (for example, the original Edmonston strain). Since the sequence window is so small, it is possible that the sequence could have been obtained from a wild-type genotype A virus or a vaccine strain (Paul Rota, personal communication). However Nitin Waigrekar et al, in 2002 have reported the prevalence of genotype A in acute measles patients from Pune, India. It is also important to note here that in countries like India, SSPE continues to occur presumably due to late introduction of vaccination in 1986 or poor quality of vaccine used (Saha et al, 1990). Given the usual long incubation period between primary measles and SSPE, the isolated genotype could be a wild type prevalent at the time of primary infection.