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Low Incidence of Congenital CMV Infection in North India: A Prospective Study Using Pp65 Antigenemia Assay

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ABSTRACT

Introduction: Neonates, especially pre-term infants are a high risk sub-group prone to develop CMV infection because of their underdeveloped immune system. A total of 105 neonates, 60 pre-term and 45 term symptomatic (fever, respiratory distress, cyanosis, bicytopenia, sepsis, congenital anomaly etc.), reporting to the Neonatal Intensive Care Unit were sampled within 1 to 4 weeks of their birth. PP65 Antigenemia assay by indirect immunofluoroscence technique was carried out in all the cases. None of the cases showed positivity for the CMV antigen, though appropriately run control samples were all positive and the test is well established and standardized in our laboratory. This prospective study is first of its kind to be carried out in neonates in India wherein an antigen detection method was used to detect congenital CMV infection/disease and it concludes that the incidence of active CMV disease in neonatal population of Northern India is <1%.

KEYWORDS : Antigenemia assay, congenital, cytomegalovirus, neonatal, pre-term

INTRODUCTION

Congenital cytomegalovirus infection continues to be a serious problem world wide and is one of the leading infectious causes of mental retardation and congenital deafness in children. Majority of the congenital CMV infections are usually asymptomatic (90%), however 5-10% of these are associated with sensory neural hearing loss. Symptomatic congenital CMV disease is known to have severe adverse outcomes as mental retardation, chorio-retinitis and microcephaly. CMV infection can be diagnosed either by serological tests or by demonstration of viral antigen in various fluids or tissue specimens by antigenemia assay, PCR, in situ hybridization or viral culture techniques. PP65 Antigenemia assay has emerged as a rapid, sensitive and specific assay to detect active CMV disease before onset of symptoms. The test is based on detection of an early CMV antigen (pp65 phosphoprotein) in the peripheral blood neutrophil nuclei by either immunofluoroscence or enzyme studies. India has a high prevalence of CMV infection as 90% of adults show seroconversion (presence of anti IgG antibodies), infection being acquired in early life. The problem of congenital CMV infection has not been seriously addressed in India, except for a study by Gandhoke et al[1], in which 96 symptomatic infants from Delhi and surrounding areas were tested for congenital CMV infection by a serological assay. No study has been carried out in our country in this high risk group using the more sensitive and specific antigenemia based assay.

In this article, we report the results of a prospective study carried out over a period of two months in pre-term infants and sick neonates. The aim of the study was to detect CMV infection/disease in preterm and term sick neonates by PP_{65} Antigenemia assay, to quantify the CMV infection load by counting the number of peripheral blood leucocytes showing antigen positivity and to correlate the antigenemic levels with symptoms of CMV disease.

MATERIAL AND METHODS

Over a period of two months, 105 neonates admitted in the NICU and PICU unit of Post Graduate Institute of Medical Education and Research, Chandigarh, were enrolled in a prospective study to note the incidence of congenital CMV infection. The study was approved by the Institutes Ethics committee.

Study population: Of the 105 cases screened, 45 were symptomatic preterm infants, 45 symptomatic term infants and 15 preterm asymptomatic infants. There were a total of 68 male babies and 37 female babies. The mean gestational age of babies ranged from 23 weeks to 40 weeks, with a mean of 34.29 weeks. Out of 105 cases, 60 were in age group 23 weeks to 36 weeks and 45 were in age group 37 weeks to 40 weeks. Neonates with 2 or more of the following symptoms were included in our study-fever, respiratory distress/ pneumonia, cyanosis, bicytopenia, blood picture proven sepsis and or associated congenital anomaly.

Collection of specimens: About 1ml of EDTA sample was taken by venipuncture after informed consent from the parents of the babies. Thereafter the sample was processed and pp65 antigenemia assay was carried out on all the samples. This assay had already been standardized in our Department of Immunopathology for detecting CMV disease in post renal transplant patients [2]. The procedure employed was as follows:-

1ml of EDTA blood obtained was kept in a test tube at 37c for 30 minutes. The neutrophil rich plasma was removed and centrifuged at 800 rpm for 10 minutes in a cold centrifuge. Twice washings were done with PBS (phosphate buffered saline). Cytospin spots with 100ul of diluted neutrophil suspension were made at 500 rpm for 5 minutes in a Shandon cytospin. After fixation of spots in 2% sucrose and 5% formalin for fifteen minutes, Indirect Immunoflourescence assay was carried out by layering 1/20 dilution of specific monoclonal antibody pp65Ag (Novacastra) on cytospin smears of patients neutrophils. The slides were then incubated at 37^0C for ½ hour in a humidified chamber. After incubation washings with PBS was carried out for 5 mts and then FITC labeled anti-mouse antibody (DAKO/Sigma) 1:20 dilution was layered for ½ hour at 37c. Again washings with PBS were given and the slide after drying was mounted in glycerin buffer mountant and examined under a fluorescence microscope for fluorescence within polymorph nuclei. A quantitate cut off of >5signals/50,000 polymorph nuclei was taken as positive. Appropriate positive and negative controls were run along with the samples in batches.

STATISTICAL METHODS

Comparisons were evaluated by chi-square analysis or the student's t test, where appropriate. In order to make a preliminary assessment of which risk factors were significantly associated with congenital CMV infection, variables were evaluated separately by chi-square analysis.

RESULTS:

The mean data regarding gestational age and sex of 105 babies is de-

picted in table 1. The age range of patients in various groups was from 23 weeks to 40 weeks, with a mean of 34.29 weeks. Out of 105 cases, 60 were in age group 23 weeks to 36 weeks and 45 were in age group 37 weeks to 40 weeks. The total numbers of male babies were 68(64.76%) and female babies were 37(35.24%).

Most of the symptomatic infants (term and pre-term) had fever (98%) and respiratory distress (90%) as the main symptomatology, followed by bicytopenia. Sepsis was more commonly noted in term symptomatic infants 14/45 (31.1%) and cyanosis in pre term symptomatic infants 18/45 (40.0%).

Congenital anomaly in form of syndactaly and microcephaly was noted in 3 and 1 case respectively in preterm symptomatic infants.

The results of PP65 Antigenemia assay were evaluated by examining the peripheral blood neutrophil nuclei for immunofluoroscence under high power of fluorescent microscope and the same are depicted in table 2. All the 105 cases in which PP65 antigenemia assay was performed were negative for CMV antigen expression.

DISCUSSION

Many studies in past have been carried out regarding incidence of congenital CMV infection from different parts of the globe and an average incidence noted is in the range of 0.3% to 1%. [3-7]

None of the 105 cases screened in our study showed evidence of CMV infection by the pp65 Antigenemia assay, implying that the incidence of congenital CMV infection is less than 1%. The sample size in our study was small as we had planned a pilot study to generate preliminary data on incidence of congenital CMV infection in our region. Moreover, problems pertaining to the collection of blood sample and amount required were also limitations to the small sample size. The results of our study are in accordance with studies by Griffith et al,[7] MacDonald et al[4], Anderson et al[5], and Ahlfors et al[6]. In a study by Allison S lastas[8] and the national congenital cytomegalovirus disease registry, 285 cases of congenital CMV infection were reported from different parts of the United States over a period of 4 years. The detection of CMV in their study was done by either tissue culture technique or by histopathological examination of available tissue specimens. The mean gestational age in their study was 36 weeks, mean birth weight of 2224 gms, length 45 cms, and head circumference of 30 cms. Sixty-eight (68) percent had CNS involvement. Their study concluded that the estimated frequency of congenital CMV disease is 100 cases per 100,000 live births. In another study by Casteels et al[9], the incidence of congenital CMV infection was noted to be about 0.49 %(20/3075). They detected CMV by urine culture of each live-born child within 7 days after birth.

Studies on incidence and prevalence of congenital CMV have been reported from some other Asian nations too, having a high seroendemic population. In a study by Yamamoto et al[10], the frequency of congenital CMV infection in preterm infants was 2.1 %(6/289 cases).and 1.8 %(3/163 cases) in term infants from a population with high seroprevalence rate. The CMV infection in these cases was diagnosed by viral isolation from urine samples by tissue culture and viral DNA detection by multiplex PCR. In a study by Sohn et al[11] and team from Korea with a high endemicity, 514 newborn urine samples for CMV DNA by PCR were evaluated and an incidence of 1.2% (6/514) was noted. In India, studies by Sakhuja et al[12] and Minz et al[2] had employed pp 65 Antigenemia assay to detect CMV infection in renal transplant patients. Their studies showed that the assay had a high specificity to detect active CMV disease (95%). In present study, the assay has been preferred over PCR based methods to detect incidence of CMV disease in symptomatic infants. India also has a high seroprevalence rate of CMV[1, 13] but in our study the prevalence of congenital CMV was less than 1%.

The reason for low prevalence of congenital CMV infection in our study could be due to screening of infants with a broad symptomatology and not only the ones having symptoms more suggestive of an intrauterine infection. The symptoms which we screened in our study were- respiratory distress/pneumonia, fever, cyanosis, bicytopenia, sepsis and any congenital anomaly. But Gaylant et al[14] and Allison et al[8] screened neonates with following symptoms: hepatomegaly, spleenomegaly, chorioretinitis, thrombocytopenia, pneumonia, cerebral calcification and any congenital anomaly. None of the preterm infants or sick neonates admitted during our study period had a severe symptomatology in form of chorioretinitis and cerebral calcifications in NICU of PGIMER, Chandigarh. This data concludes that neonates in our region, presenting with fever, respiratory distress, cyanosis or sepsis usually have an etiology other than cytomegalovirus infection.

The presence of high CMV seropositive levels in mothers, in our population could also be responsible for low incidence of active CMV disease in neonates. In a study by Fowler K B et al[15], who studied relationship between maternal immunity and incidence of congenital CMV infection, 18 out of 604 newborns (3.0%) born to initially seronegative mothers developed congenital CMV infection, while 19 out of 2850 newborns (1%) born to immune mothers developed congenital CMV infection. Thus their study concluded that naturally acquired immunity results in 69% reduction in risk of congenital CMV infection in future pregnancies.

CMV infection can also be diagnosed by detecting CMV DNA in various body fluids by PCR based molecular amplification assay. These newer tests like quantitative PCR and Hybrid capture assay provide comparable results as Antigenemia assay, in terms of sensitivity and specificity and the same has been addressed in various studies[16,17]. However, PCR based assays do not differentiate between latent infection or active disease and being a costly method they are not routinely recommended as a first line test to detect active CMV infection. Rather cases with seropositivity for CMV can be confirmed by doing a PCR assay. A study by Barbi et al[18] found that CMV was detected in peripheral blood leucocytes mainly in the most severely affected children with active CMV infection. They recommended monitoring by Antigenemia and viraemia in CMV infected infants to demonstrate persistent systemic infection and to evaluate results of treatment.

CONCLUSION

So our study concludes that there is low incidence (<1%) of active CMV disease in our neonatal population as detected by the specific PP65 antigenemia assay. Antigenemia assay being a rapid and less costly test, still remains as a standard assay to detect active CMV disease, as well as to monitor and evaluate the response to treatment. The present pilot study to detect active CMV infection/disease in neonates by an antigen based assay is one of the first of its kind in Indian population and points to a low evidence of active CMV disease in this subgroup. However larger prospective epidemiological studies designed to target broader neonatal population risk group is needed to determine the actual incidence and prevalence of congenital CMV infection.

TABLES

Table 1: The spectrum of cases studied and the mean age and sex distribution in each study group (Results-Para1, Line 1)

Groups	Number of cases	Mean age	Mean birth weight	Males	Females
1	45	31.19	1421.47	31(68.8%)	14(31.11%)
2	45	37.43	2650.31	29(64.44%)	16(35.56%)
3	15	34.14	2414.00	8(53.33%)	7(46.67%)

Group 1 - Preterm symptomatic infants
Group 2 - Term symptomatic infants
Group 3 - Preterm asymptomatic infants

Table 2: Results of PP65 Antigenemia assay (Results- Para 4, Line 3)

Groups	Number of cases	Antigenemia assay	Number of positive cases	Number of negative cases
Group 1	45	45	0	45
Group 2	45	45	0	45
Group 3	45	45	0	45

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