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PATIENT PROFILE AND BURDEN OF HERPES SIMPLEX ENCEPHALITIS AMONG PATIENTS PRESENTING WITH AES IN WEST BENGAL:

Microbiology

Dr.Parama Ghosh* (MD)Senior Resident,Department Of Microbiology,Midnapore Medical College and Hospital, Paschim Medinipur. Pin:721101 *Corresponding Author

Dr.Nemai Bhattacharya (MD)Professor and Unit InCharge(Retired), Department Of Microbiology (VirologyUnit), School Of Tropical Medicine , Kolkata-700073.

ABSTRACT

Context: Acute Encephalitis Syndrome(AES) caused by viruses is a matter of concern.Japanese Encephalitis(JE) is considered to be the major cause of AES in India but non JE viruses such as Herpes Simplex Virus(HSV) contribute significantly towards the causation of AES.Studies on HSE in West Bengal is limited.

Aim: The present study is designed to find out the patient profile and burden of HSV encephalitis among patients suffering from AES in Kolkata and surrounding districts. **Study Design:** The present study is a descriptive observational study. **Study Settings:** The present study was undertaken at Virology Unit,Microbiology Department,School Of Tropical Medicine,Kolkata from March 2017-March 2018.**Subjects and Methods:** Cerebrospinal fluid (CSF) was collected from 120 suspected AES patients and was tested for HSV-1 DNA by RealTime RT PCR using specific primers and probes for Herpes Simplex Virus -1.**Results:**Out of the 120 suspected cases 6(5%) were found to be positive for HSV-1 DNA in their CSF.The Male:Female ratio of infection was 1:1.A bimodal age distribution was noted and positive cases were found mostly from December to June.All the positive cases had a duration of illness of 5 to 15 days. **Conclusion:** The burden of HSV-1 infection among patients of AES is 5%.Real Time PCR for HSV-1 DNA in CSF becomes positive within 2 weeks of onset of AES.Therefore, testing of HSV-1 DNA in CSF of suspected patients should be done preferably within 2 weeks of onset of symptoms.

KEYWORDS

Acute Encephalitis Syndrome(AES), Herpes Simplex Virus Encephalitis(HSE),Real Time RT PCR,RNase P -Ribonuclease protein gene-internal control.

INTRODUCTION:

The WHO(World Health Organization) has defined AES(Acute Encephalitis Syndrome) as "clinically,any person of any age, at anytime of the year presenting with acute onset of fever and a change in mental status(including signs and symptoms such confusion, disorientation, coma or inability to talk and or new onset seizures, excluding simple febrile seizures"[1] In India, it has been estimated that a population of 375 million people residing in 171 districts of 17 states are at risk of acquiring AES[2].

For several decades, based on confirmed outbreak reports JE(Japanese encephalitis) has traditionally been considered as the major cause of AES in India.However, several recent outbreak investigation and surveillance studies have increasingly reported non JE etiologies in AES[3].New viral agents such as chandipura and Nipah virus and other viruses such as HSV,VZV,Dengue,Enteroviruses,West Nile virus are also responsible for the causation of [AES3,4].HSV is the most common cause of severe focal encephalitis in man[5].HSV causes progressive cerebral necrosis and edema with upto 70% mortality if untreated[6]. Antiviral drugs against HSV are available, but they are most effective when given early[7].Researches on HSV encephalitis and its epidemiology has not been done abundantly in India.

So, we have designed a study to find out the patient profile and the burden of HSV encephalitis among patients suffering from acute encephalitis syndrome in Kolkata and the surrounding districts using RealTime PCR of CSF of patients presenting with AES.

MATERIALS AND METHODS:

A study was undertaken at the virology Unit, microbiology department, School of Tropical Medicine , Kolkata , from March 2017 to March 2018.

Inclusion criteria was , a person of any age , at anytime of the year with acute onset of fever with a change of mental status and /or new onset seizure.

CSF samples were taken from 120 suspected acute encephalitis patients admitted in different medical college hospitals and health care facilities in Kolkata and surrounding districts.

Patients were included in the study after taking informed consent following which, CSF samples were collected from suspected AES cases.CSF was tested for HSV-1 DNA by RealTime RT PCR and the results were analyzed.

DNA was extracted from 200 microlitre of CSF using QIAmp DNA minikit(Qiagen).It is designed for rapid purification of DNA.After optimal lysis, the sample was loaded into QIAmp mini spin column and the DNA that was adsorbed into the QIAmp silica membrane was washed for the removal of any residual contaminants.Purified DNA was eluted in a concentrated form in buffer AE for direct use in PCR.

Amplification was carried out in an Applied Biosystem detector 7500 machine programmed for a 3 step protocol that is 30 sec incubation at 50^0C, 15 min at 95^0C for Taq activation and 50 cycles of PCR amplification(45 sec at 94^0C for denaturation and 1 min 15 sec at 60^0C for annealing, extension and data collecting).Fluorescence data was collected during the 60^0C incubation step.

Primers used:
HSV FP (HSV-IF TTC TCG TTC CTC ACT GCC TCC C)
HSV RP(HSV-IR GCA GGC ACA CGT AAC GCA CGC T)
RNAase P-F (AGA TTT GGA CCT GCG AGC G)
RNAase P-R(GAG CGG CTG TCT CCA CAA GT)

Probes used:
HSV-1 P 6-Fam- CGT CTG GAC CAA CCG CCA CAC AGG T-BHQ-1)
RNAase P 6-Fam TTC TGA CCT GAA GGC TCT GCG CG-BHQ-1)

Positive samples for HSV 1 was defined as those with cycle threshold (Ct Values) less than or equal to 40.

Negative samples for HSV 1 was defined as those with cycle threshold more than 40.

The test was taken a valid , if NTC(Non Template Control) and (Extracted Water Negative Control) did not show any fluorescence,if positive control(HSV-1 and RP)showed Ct<=40 and if all CSF samples tested are positive for RNAase P(Ct<=40) irrespective of whether they are positive or negative for the pathogen tested.

RESULTS:

Out of the 120 cases,6 were found to be positive for HSV 1 DNA in their CSF which comes to a percentage of 5% overall
(Figure 1).

The number of males affected with the disease was equal to that of the females giving a Male:Female ratio of 1:1 (Table 1).3 out of the 6 positive patients were below 20 years of age and 2 out of 6 were above 40 years.One patient was aged 28 years among all 6(Table 2).

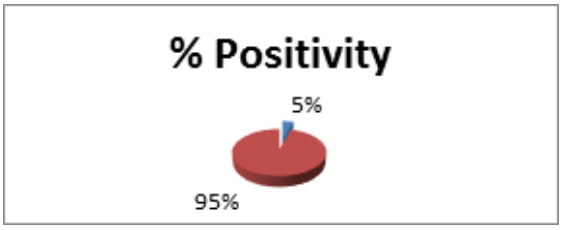


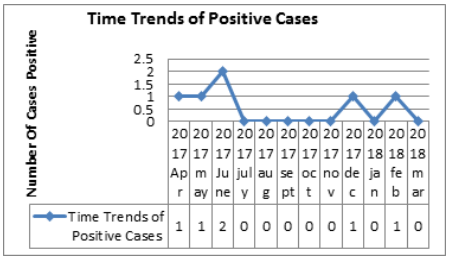
Figure 1:

Table: 2

Age	Number Positive
< 20 Yrs	3
20 – 40 years	1
>40 Yrs	2

Positive cases were mostly found during the month of December, February,April, May and June(Figure 2).

Figure 2: Cases were positive from Kolkata(South Bengal) Coochbehar and Alipurduar(North Bengal) and Birbhum district.



All the positive cases had a duration of illness of 5 to 15 days.Total number CSF tested from AES cases of who had a duration of illness of <6 days were 45 and the number that tested positive was only 1 giving a percentage positivity of less than 6 days of illness of 2.2%.The total number of CSF samples from confirmed AES cases who had a duration of illness of 6 -15 days was 66 and the number that tested positive was 5 giving a percentage positivity in this timeframe of 7.57% and the total number of samples tested who had a duration of illness of > 15 days was 9 and none of the samples were positive.

DISCUSSION:

In our study, HSV 1 etiology in acute encephalitis patients was detected in 5% of cases by molecular method.

Studies on HSE in India is limited[8].However, among the few studies available one older study states that HSV 1 encephalitis constitutes a very low proportion(1.1%) of acute viral encephalitis cases seen in eastern Uttar Pradesh, India[9].In another study describing the etiology and clinicoepidemiological profile of acute viral encephalitis in children in Western Uttar Pradesh,India, it was found that about 10.5% of viral etiology was contributed by Herpes Simplex Virus[10].In one more recent study in Uttar Pradesh,India it was found that the most common causative agent of AES was JE virus(16.2%),followed by dengue virus(10.8%),HSV(9.3%)[11].In a very recent study at Rajasthan, NorthWest India,looking into the Aetiological study of viruses causing acute encephalitis syndrome,HSV was the most common virus(8.45%) followed by EBV,VZV,CMV,EV,MPV,MV and RV.[12]

Age distribution of the positive patients was somewhat bimodal.3 out of 6 patients were below 20 years of age and 2 out of 6 were above 40 years.Previous studies have shown a bimodal age distribution of HSE with one third of the cases occurring in those less than 20 years and two third of the cases were aged 50 years or more[13,14].In a study done by Whitley et al. it was found that the disease was more common in patients aged < 20 years and >40 years[15].

Other studies have also shown that there is a bimodal age distribution in HSV encephalitis patients with a peak incidence in very young(upto 3 years of age) and again in adults aged>50years,with both sexes equally effected[16,17-18].

Males and females were affected equally in our study with the male: Female ratio being 1:1.Different studies have different perspective on the gender distribution of HSV encephalitis patients.Some state that both the genders are equally effected whereas others say that males are more likely to have the disease as compared to females.

Positive cases were found from North Bengal(2 from Coochbehar and 1 from Alipurduar).2 patients were from Kolkata and 1 out of the 6 positive patients was from Birbhum district of West Bengal.No specific hotspot zone of infection could be pinpointed out.

While following the time trends of the disease, it was noted that no positive cases were found during the months from July to November.Positive cases were found during the months of December,February, april,may and june which means that the disease is more likely to flare up during the winter and early summer months.

HSV DNA detection by PCR of cerebrospinal fluid samples is a very efficient method for diagnosis of HSE[19,20].Although, PCR of CSF is the diagnostic method of choice for HSE, negative results need to be interpreted in the context of patient's clinical presentation and timing of CSF sampling[21].Herpes Simplex encephalitis is one of the very few neurological infections in which a direct comparison has been made between the frequency of detection of CSF PCR and brain biopsy.Thus,HSV DNA was detected by PCR in CSF of 53(98%) of 54 patients with brain biopsy proven HSE and a positive PCR was detected in 3/47(6%) of patients whose brain tissue was culture negative[22].Overall, the sensitivity of PCR of CSF was 98%,indicating a false negative rate of 2%.The specificity of CSF PCR was 94% indicating a false positive rate of 6%.These figures have largely been confirmed in subsequent studies. A detailed study of issue using a decision model analysis estimated that the sensitivity of CSF PCR is 96% and specificity is 99%[23].Based on such data, CSF PCR can be recommended as a highly reliable method of diagnosing HSE without the need for brain biopsy.

The timing of CSF sample used for PCR is an important factor influencing the sensitivity of the method.The CSF PCR for HSE can be negative during the early stage of infection with documented failures to detect HSV -1 DNA during first 3 days after the onset of illness[24,25].Re examination of the same negative CSF a few days later may yield positive PCR result[25].The CSF PCR may also be negative if the sample is taken too late during the infection[26].

In our study, all positive cases had a duration of illness ranging from 5-15 days,which is during the late first week and second week of infection. However, out of the 120 AES cases 34 had a duration of illness of <5days.So, these patients might turn out to be positive if the CSF PCR test could be repeated after 3-4 days of the first test but, unfortunately these patients could not be followed up.

CONCLUSION:

This study conducted from March 2017 -March 2018 revealed that HSV-1 positivity amongst patients presenting with AES was 5%.The disease followed a bimodal age distribution with a predisposition for ages <20 years and >40 years.Males and females were equally affected by the disease with the infection rate being more during the late winter and early summer months.No specific area distribution could be mapped from the study.The chances of getting a positive PCR result was more if the CSF was tested in the late first week and early second week.Chances of CSF PCR positivity was very less if samples were tested after the second week of illness.

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