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Clinicopathologic study of Acute Viral Encephalitis Syndrome in Greater Gwalior region-A one year study

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Abstract

Background-*Viral Encephalitis has existed in world from time immemorial and has concurrently inflicted hazardous and lethal diseases to human beings. The most challenging aspect of viral encephalitis infection is its definite diagnosis.*

Objectives-*The present study was carried out in tertiary centre to find out the incidence, the various diagnostic modalities-conventional CSF and hematological analysis as well as the newer molecular techniques PCR and RTPCR and Clinicopathologic correlation of Acute Viral Encephalitis Syndrome.*

Material & Method-*The study was a one year prospective study carried out in JA Group of Hospitals in greater Gwalior region. It included collection of blood and CSF samples of 120cases and 120 controls and determining the etiological agents of dreadful illness by conventional and newer molecular techniques RTPCR and Taqman Real Time PCR.*

Results-*The incidence of Acute Viral Encephalitis in our study was found to be 5.8%. Males were more commonly affected and the incidence was found to be more in children less then 10 years of age. Enterovirus(EV) and Measles was identified as the most common etiological agents in Greater Gwalior region. Out of 7 positive cases 5 were detected in CSF and 2 were in serum indicating specificity of CSF more than serum. CSF Pleocytosis was seen in 61 cases out of 120.*

Conclusion – *Newer molecular techniques used for specific diagnosis can prove a big asset to society as they facilitate the early diagnosis of Acute viral encephalitis and reduce the morbidity and mortality. Diagnosis of Acute Viral Encephalitis has improved remarkably with application of Rapid molecular techniques eg RTPCR (Reverse Transcription)and Real time RTPCR for early detection & identification of virus in clinically suspected samples.*

Key words: *CSF, Viral Encephalitis, encephalopathy, Agarose gel electrophoresis, PCR, RTPCR.*

Introduction

During the last few decades of 20 th century, many viral diseases like Dengue, Viral hepatitis, AIDS, Viral encephalitis have been emerged with pronounced virulence[(1)]. Encephalitis literally means an inflammation of Brain .Among abnormal infections, viral hemorrhagic fever and encephalitis syndrome caused by Dengue, JE, Westnile and Chikengunya virus are extremely prevalent in India and South EastAsia. Viral

encephalitis ,a major global emerging public health problem is responsible for thousands of deaths every year in India .Many outbreaks have been reported since 1955 [(2)]. The first epidemic of viral encephalitis occured in UP in 1978 and in 2005 epidemic of JE has been reported from Gorakhpur and adjoining area [(3)]UP was effected with 5737JE cases and 1334 deaths(CFR 23.3%) & Bihar experienced 360 Cases with64 deaths(CFR`17.8%(WHO Report).

Hundreds of viruses causes central nervous system diseases including meningoencephalitis and postinfectious encephalomyelitis. Encepahlitis means an inflammation of brain parenchyma present as diffuse and or focal neuropsychological dysfunction. The commonest cause in US is Herpes Simplex Virus (HSV) whereas in India and South East Asia it is Japanese Encephalitis [(4)]

In Uttar Pradesh the commonest agent was found to be Enterovirus 42.6% followed by Measles 21%,VZV 15.8%,HSV-110.5% in year2008-09. Acute Viral encephalitis syndrome is characterized by fever, headache, altered mental state combined with focal neurological deficits depending upon area involved.[(5)] The final goal in case of encephalitis is to identify the specific agent so as target a specific treatment eg Herpes simplex encephalitis[(6)].The common viral agents causing AVE syndrome in South East Asia region are - Enterovirus, HerpesSimplex Virus, Japanese Encephalitis, Measles, Varicella Zoster Virus Mumps, Epstein Barr Virus, Vericellazoster Virus ,HIV.

Material &Method

The present prospective study was conducted in department of pathology GR Medical College and JA group of in association with Virology division of Defence Lab, Gwalior. Total of 120patientswho were clinically diagnosed as acute viral encephalitis were selected and similar number of controls who were also diagnosed for neurological disorder other then encephalitis syndrome, were selected.CSF and blood samples from cases and controls were screened for etiological agents by newer molecular techniques.

Inclusion Criteria

All those patients were included who presented with acute onset of fever and change in mental status -confusion, disorientation, coma inability to talk and or onset of seizures,

All patients with CSF pleocytosis ie WBC count more than 5 cumm with or without parenchyma lesions.

Substantial meningeal enhancement as identified by brain CT/MRI seen

Presence of definite neurological dysfunction without CSF pleocytosis, it includes aphasia, ataxia, UMN, LMN weakness, involuntary movements, cranial nerve deficits.

Exclusion

Patients with simple partial seizures are excluded. In all cases as well as controls CSF examination and hematological tests were carried out. Complete blood counts by Medonic CA 530,(Hb , Hct and ESR was noted in all cases and controls).Blood sugar estimation was done.

With strict aseptic precautions, CSF and blood samples were collected within 6days of onset as viral load is maximum during this period.2-5 ml of CSF and 5ml blood was collected.

CSF was withdrawn from Lumbar puncture and immediately stored in freeze at 4'c to 20'c. **Routine CSF examination** consist of physical & chemical examination –protein and sugar estimation and total WBC count (By Neubaur chamber)was done. **CSF culture** was done by Pour Plate method on Mackonkey and choclate agar. CSF sample was immediately transferred to defence lab for viral detection by RTPCR and Real time RTPCR.

For molecular diagnosis Total nucleic acid(RNA /DNA) was done by using Quigen Ultrasense viral total nucleic acid extraction kit.

RTPCR was carried out on all 120 RNA/DNA by a one step RTPCR protocol using Access quick RTPCR assay (Promega USA).The PCR

amplification was carried out in a final volume of 25 microlitre using Viral RNA as a template Primer sequence used for amplification of different viruses(pg 59)

Agarose gel electophoresis- was used to confirm size and quantity of the PCR amplicon. 1% Agarose gel electrophoresis was used and amplicon were then visualized in a gel documentation system (Bio Rad, USA) and then photographed.

Reverse Transcription Polymerase Chain Reaction (Real Time RTPCR)- All samples were utilized for Real Time RTPCR which has many advantages over conventional RTPCR including rapidity, quantitative measurement ,lower contamination rate, linear sensitivity higher specificity early standardization.

RTPCR –Taqman probe based one step real time quantitative RTPCR amplification was performed in the Mx3005P Quantitative PCR system (Stralagene, USA), according to the instructions of the manufacturer. Amplified products were further confirmed during initial standardization by the size assessment of the RealTime RTPCR amplicons by 1% Agarose gel elcetrophoresis.(6)

Results-

Table 1-Incidence of Acute Viral Encephalitis

Total no of cases	Cases identified by RTPCR/Real Time PCR	Cases not identified by RTPCR/Real Time RTPCR
120 (cases)	7(5.8%)	113(94.2%)
120(controls)	0(00%)	120(100%)

Table 2 Positive cases of Specific Virus diagnosed by RTPCR/RealTimeRTPCR

	EV	Measles	JE	CMV	HSV1	HSV2	VZV
120(cases)	4(3.33%)	3(2.5%)	-	-	-	-	-
120(control)	-	-	-	-	-	-	-

Table 3 Comparison of RTPCR and Real Time RTPCR

	RTPCR	Real Time PCR
Total Cases (120)	4(3.33%)	7(5.8%)
EV (4)	3(75%)	4(100%)
Measles(3)	1(33.33%)	3(100%)

Table 4-Comparision of Positivity in CSF and Serum samples in all cases.

	CSF Sample	Serum Sample
Total sample	120(100%)	120(100%)
Positive Cases	05(4.1%)	02(1.6%)

Table 5-Age & Sex distribution in cases & controls

Age group	No of Cases (Males)	No of Controls (Males)	No of Cases (Females)	No of Controls (Females)	Total Cases	Total controls
<1 yr	12(66.66%)	06(60%)	06(33.33%)	04(40%)	18(15%)	10(8.33%)
1-10yr	32(61.5%)	42(70%)	20(38.4%)	18(30%)	52(43.33%)	60(50%)
11-60yr	14(50%)	13(50%)	14(50%)	13(50%)	28(23.3%)	26(21.66%)
>60 yr	16(72.7%)	14(58.88%)	06(27.2%)	10(41.66%)	22(18.33%)	24(20%)
Total	74(61.66%)	75(62.5%)	46(38.5%)	45(37.5)	120(100%)	120(100%)

Table 6- CSF Microscopic Examination in all cases

Total no of CSF samples	CSF cell count <5/cumm	>5/cumm	>100/cumm	Lymphocytosis	Neutrophilia
120 cases	18(15%)	94(78.33%)	08(6.66%)	104(86.66%)	16(13.33%)
120 controls	86(71.66%)	30(25%)	04(3.33%)	28(23.33%)	02(1.66%)
07 Positive	00(0%)	06(85.7%)	01(14.28%)	07(100%)	00(0%)

Table 7- Table of Abnormal hematological parameters in our study

	Hb <10 gm%	Hb 10-12 gm%	Hb >12 gm%	TLC >11000	Lympho cytosis (Absolute)	Neutro philia (Absolute)	Plt <1.5 Lakh/ Cumm	Bld Sugar Reduced	ESR Raised
Total Cases (120)	*34 (28. 33%)*	42 (35%)	44 (36.66%)	47 (39.16%)	22 (18.33%)	06 (5%)	27 (22.5%)	18 (15%)	19 (15.8%)
Total controls (120)	09 (7.5%)	73 (60.8%)	38 (31%)	18 (15%)	13 (10.8%)	05 (4.1%)	14 (11.6%)	08 (6.66%)	07 (5.8%)
Positive cases (7)	2 (28.5%)	4 (57.14%)	1 (14.28%)	1 (14.28%)	1 (14.28%)	0 (0%)	1 (14.28%)	0 (0%)	1 (14.28%)
EV (4)	1 (5%)	2(50%)	1 (25%)	1(25%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Measles (4)	1 (33.33)	2 (66.66%)	0 (0%)	0(0%)	0 (0%)	0 (0%)	1 (33.33%)	0 (0%)	1 (33.33%)

Table 8- Comparative Chart of clinical features among Positive cases

Features	No (%)of EV Positive Patients	No (%) of measles Positive Patients
Fever	4(100%)	3(100%)
Altered sensorium	4(100%)	3(100%)
Hepatomegaly	1(25%)	1(33.33%)
Splenomegaly	1(25%)	1(33.33%)

Brisk DTR	2(50%)	1(33.33%)
Meningeal sign	2(50%)	2(66.66%)
Total No of Patients	4(100%)	3(100%)

Table 9- Correlation of clinical features, Viral Load with relation to specific viral Agent in Acute Viral Encepahlitis.

S.No	Viral Load	Viral Agent	Fever	Altered Sensorium	Meningeal Sign	DTR	Hepato Megaly	Spleno megaly
1	$1X10^4$	EV	+	+	+	+	-	-
2	$1x10^4$	EV	+	+	+	+	+	-
3	$3x10^4$	EV	+	+	-	+	-	+
4	$1x10^4$	EV	+	+	-	-	-	-
5	$8x10^4$	Measles	+	+	+	-	-	+
6	$6x10^4$	Measles	+	+	+	+	-	-
7	$2x10^4$	Measles	+	+	-	-	+	-

Discussion

Acute Viral encephalitis syndrome is characterized by fever, headache altered mental state combined with focal neurological deficits depending upon area involved. (Harrison Medicine.) Acute Viral encephalitis is an inflammation of the brain parenchyma, most commonly caused by Viruses and associated with substantial morbidity and mortality. It presents as diffuse and or focal neuropsychological dysfunction. Mortality was quite high in our study, 30% died during hospitalization with about half deaths during 3-7days after onset of illness and half affecting children. Morbidity was found in 25% of survivors. Worldwide reported mortality ranges 0-11%.LeVan Tan et al reported Mortality 33%and morbidity 25% respectively in their one year study over viral etiology of Encephalitis in children in South Vietnam[(7).] Radhakrishnan et al reported fatality rate 20% in 1987 study[(8).] Seriousness of Quantum of problem is well understood by the outbreaks of AES in UP, Gorakhpur, Lucknow were hundreds of deaths have occurred. The cause of encephalitis varies between geographical regions. JEV was the most common cause of encephalitis in children in Combodia, Enterovirus and Tickborn Encephalitis virus were the two most common virus found in young patients in US and Sweden respectively. The most common etiological agent found was Entero virus (EV) and measles in Greater Gwalior region in our study accounting for 3.33% and 2.5% respectively. Similar study shown EV as commonest etiological agent in study done by Ganjan Sakpal et al ((2006), S karmakar (2008) and L. kupila et al (2003[)(9)(10)(11).] Male to female ratio in our study is 1.6:1.Majority of our patients and positive cases as well fall in age group <10 yrs of age which coincides with similar study by Beig FK, Malik et al (2010)[(12)]which show M:F 1.27:1and the most common etiological agent was found to be Entero Virus. Study by Fidan Jmor et al shows(2008)that AES is more common in younger age group (<15 yrs)[(13).]Conventional CSF findings were also

significant -86.66% show lymphocytosis, CSF protein was raised in 70% of cases. Whereas out of 7 positive cases, 6 had pleocytosis and raised CSF protein. Out of 120 cases 74(61.66%)were anaemic with 47(39.16%)having increased total WBC count and 18 (22.5%) having low platelet count.

Conclusion

This evaluation and comparision of new molecular techniques RTPCR and Real Time RTPCR indicate that these tests are highly specific in identifying etiological agents in Acute Viral Encephalitis. Real Time RTPCR has several advantages over conventional PCR-it is more rapid and sensitive test as compared to conventional RTPCR. Also it produces less false positive results by contamination during sample preparation .There are currently five main chemistries for the detection of PCR product during real time PCR[(14)(15)]among these the most widely used format is the 5'-3' nuclease oligoprobe (Taqman assay)due to its commercial utility. In this study EV primer and measles primer from highly conserved region showed reliable specificity for detection of EV and measles virus in real time RT-PCR. Taqman based one step Real Time RTPCR assay described here for detection and quantitation of Enterovirus and measles virus has been shown to be simple, sensitive, specific ,rapid and economic approach to surveillance and epidemiological studies. These features make it an excellent tool for laboratory detection of Enterovirus and measles virus in clinical samples. Thus these molecular techniques can prove a big asset to society as important tool in reducing mortality and morbidity by early diagnosing Viral Encephalitis.

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