ENCEPHALITIS WITH JE, DENGUE AND MALARIA IN A TERTIARY CARE HOSPITAL OF UPPER ASSAM

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ABSTRACT

North Lakhimpur civil Hospital is a multispecialty hospital, located at North Lakhimpur town of Upper Assam which was established by government of Assam to treat the disease patients. In this tertiary care hospital every year lots of encephalitis patients admitted from various area of the district and from some other districts too. Some of them fully recover, some of them recover with side effect and some of them expired. To control the burden of this disease source of infection must be known by the treating physician. That is why our study tried to check the vector born encephalitis by testing the encephalitis patients with Japanese Encephalitis (JE), Dengue and Malaria reported to the hospital. In our study it was found that Japanese Encephalitis Virus (JEV) was the causative agent in 24% encephalitic affected patients, dengue virus was the causative agent in 2% encephalitic patients and Malaria parasite (Pf) was the causative agent in 1% encephalitic patients. Again, 14% patients were found JE Equivocal by JE IgM ELISA. Another important Point was 27% patients were found negative by JE, Dengue and Malaria test. Age wised distribution showed that 46% affected patients were within 0-10 Years i.e. maximum affected patients were children. From our study it was confirmed that government disease control strategies and identification of cause of disease can minimize the disease burden.

KEYWORDS: Encephalitis, JEV, Dengue, Malaria, Pf, IgM ELISA, Equivocal.
INTRODUCTION

North Lakhimpur civil hospital is an important tertiary care Hospital of Upper Assam or north side of Assam. This was established in 1987 by government of Assam to treat the disease condition peoples of the district. This is a 200 bedded Civil Hospital officially, but due to heavy patients load the hospital serves more than the capacity. The hospital not only serves the patients of Lakhimpur district, patients from Dhemaji district, Jorhat district, Sonitpur district and different districts of Arunachal Pradesh also reported the hospital for their treatment. As the hospital is facilitate with the specialist physicians as well as located at the city (North Lakhimpur) of the Lakhimpur district, patients prefers first for their treatment. There are different disease conditions patients reported to the hospital on daily basis either to the OPD (Out Patient Department) or IPD (Indoor Patient Department). It has been noticed that from the month of June the numbers of encephalitis patients as well as death due to encephalitis increases in the hospital. That is why to check the etiology of the disease we have chosen the disease encephalitis as our study subject. We have selected three common diseases, JE (Japanese encephalitis), Dengue and Malaria to check the etiology of encephalitis, as the diseases were found prevalence in the district.\cite{1,2,3} Again to vector control it is important to check the encephalitis patients as all these three diseases are vector borne.

Encephalitis is the disease condition of patients which may be caused by bacteria, virus, parasite, fungi and others.\cite{4} The disease includes the symptoms of fever, headache, confusion, changing mental status, neck rigidity, disorientation, seizure, confusion, semiconscious, unconscious etc.\cite{5} There are many outbreak of JE, dengue or malaria reported by different districts of Assam time to time, including Lakhimpur district. In the year 1989 in between July and August there was a major outbreak of JE reported from Lakhimpur district of Assam. In the year 2006 an outbreak with 200 deaths reported due to Malaria in the Lakhimpur district. Again, in 1992 Regional Medical Research centre (RMRC), Dibrugarh confirmed that the dengue is prevalence in the Lakhimpur, Dibrugarh, Golaghat and Dhemaji district of Assam. In a study by Regional Medical Research Centre (RMRC), Dibrugarh during the year 2008-2010 found JE and dengue in encephalitic patients, they have tested/studied 550 numbers of encephalitic patient’s samples where it was proved by them that Japanese encephalitis virus was the encephalitic causative agent in 259 numbers of patients and Dengue virus was the encephalitic causative agent in 28 numbers of patients.\cite{6} This proved that etiology of the negative samples is very very important for the study and encephalitis causative agent would be JEV is not necessary.
MATERIALS AND METHODS

Study area
The study area of the research includes all the patients reported to the North Lakhimpur Civil Hospital of different districts. The districts observed during study were Lakhimpur, Dhemaji, Jorhat districts of Assam and Naharlagun of Arunachal Pradesh. If we check the community of each districts Dhemaji and Naharlagunhaving maximum numbers of tribal peoples in comparison with other districts.

Study Design and Sample collection
After getting the approval to work from the Joint Director of Health Services (JDHS) the study was started. Before the starting of the sample collection the patients or the guardian of the patients (in cases if patient was minor or in unconscious/semiconscious/changing mental status in condition) were informed regarding the study. After getting consent from the patient or guardian the samples were collected. One of the important points was that all the consented attendance or guardians of the patients were blood relative of the patient.

The patients were clinically confirmed as encephalitis by the specialist doctors of the hospital. None of the healthy or asymptomatic peoples were included in our study. The duration of the study was January 2015 to September 2015.

Laboratory Methods

Blood Sample collection
2 ml blood Samples were collected in sterile plain vacutainer (red coloured) from the patients by the trained Laboratory Technician by using disposable syringe, spirit swab, tourniquet. Before collection the site of the collection of hand were sterile by using spirit swab. The blood samples were collect after drying of the site of swab used. After drawing of the sample in disposable syringe the samples were transferred to the plain vacutainer and syringe needles were destroyed in needle destroyer. The blood samples were allowed to clot in plain vacutainer for 15-30 minutes. Each vacutainers were labeled before the sample collection.

Blood smear preparation
After completion of blood collection both thick and thin smears were prepared in clean sterile glass slide before clotting the blood in plain vacutainer with the help of another clean glass slide. The slides were pulled in $45^0$ angles to prepared the thin film in the labeled glass slide.
where the film prepared. Thick films were spread in 1 cm circle. Both the smear were allowed to dry and fixed with fixative.

**Malaria smear staining and Microscopy**
JSB staining was done for Malaria microscopy. The fixed smears were dipped in JSB II contained coupling Jar for 2-3 times and than dipped in coupling Jar contained tape water. The smears were transferred from tape water contained coupling Jar to JSB I contained coupling Jar and dipped till 40-60 seconds. Again, smears transferred to tape water contained coupling Jar and dipped 2-3 times. Stained smears were allowed to dry for microscopy observation. Magnus binocular electronic compound microscope was used for microscopic observation. The smears were focused in high power and than observed under oil immersion filed (100x) with the help of microscopic oil.

**JE IgM ELISA**
The tested JE MAC ELISA kit was prepared by National Institute of virology (NIV), Pune, India. The sensitivity and specificity of the kits were very high. Robonik ELISA reader and washer used in the test. Before the test the samples were bring to room temperature from the deep freezer (-20°C) and diluted with sample diluent. Washing buffer was prepared before the test start for washing. At the final step the developed colour intensity or optical density was measured against 450nm.

**Dengue NS1 ELISA**
Based on the date of onset the JE negative samples were selected for either Dengue NS1 ELISA or Dengue IgM ELISA. The NS1 ELISA test was done till 7 days from the date of onset though the kit informed till 9 days. The panbio Dengue NS1 ELISA kit was used for the test. All samples were bring at room temperature before performing the test and diluted with the diluent. Negative control, Positive control and Calibrators were used in the test as per the kit instruction. The test results were calculated by Robonik ELISA reader against 450 nm.

**Dengue IgM ELISA**
National Institute of virology (NIV), Pune, India manufactured kit was used for Dengue IgM ELISA. The JE negative samples whose onset were more than 7 days were selected for the test. Micropipettes & microtips (2-10µl size, 10-100µl size, 100-1000µl size, 50-100 multiple µl size), Incubator, ultra distilled water, Incubator, Robonik ELISA washer & reader were
used for the all ELISA tests. The test results were calculated by Robonik ELISA reader against 450nm as per the kit instruction.

RESULTS AND DISCUSSION
In our study it was observed that maximum numbers of patients were within 0-10 Years which were 46% and male children (within 10 years) were more affected in comparison with female children, the ratio of affected 0-10 Years male and female were 11:9 Figure 4. The distribution of the three vectors borne diseased showed that 24% encephalitic affected patients’ were due to Japanese encephalitis virus, 2% encephalitic patient’s cause were due to dengue virus and 1% encephalitic patient’s cause were Plasmodium falciparum Malaria parasite Figure 1. Again JE IgM ELISA, Malaria microscopy and Dengue NS1 & IgM ELISA showed that encephalitis causative agent of 27% patients were unknown. 14% patients were JE equivocal by JE IgM ELISA.

The death rate of the patients showed that 8.04% encephalitis patients expired within the hospital during treatment Figure 2. It was noticed that no malaria and dengue positive patients were died within the hospital due to encephalitis during treatment. 1.14% encephalitic patients expired due to Japanese encephalitis and 7% patients death causative agents were unknown (negative for JE, Dengue and malaria).

If we check district wise which was showed in Figure 3 that 83.90% encephalitic patients were reported to the hospital from Lakhimpur district (Assam), 11.49% encephalitic patients reported to the hospital from Dhemaji district (Assam) and 2.29% patients were reported from both Jorhat (Assam) and Naharlagun/Papumpare (Arunachal Pradesh) district. Lakhimpur district reported 21.83% confirmed JE and 10.34 % Equivocal JE and 1.14% were both dengue and malaria from the hospital reported encephalitis patients. Dhemaji district reported 5.74% confirmed JE, 2.29% Equivocal JE and 1.14% Dengue, no encephalitic malaria reported from Dhemaji district. No confirmed JE reported from Jorhat and Naharlagun/Papumpare district but 1.14% Equivocal JE reported from Jorhat district. Again 1.14% dengue reported from Naharlagun/Papumpare district of Arunachal Pradesh. No positive malaria reported from Jorhat and Naharlagun/Papumpare too Figure 5.
Figure 1: Distribution of JE, Dengue and Malaria in encephalitis patients reported to the hospital.

Figure 2: Total encephalitis patients reported to the hospital with death ratio within the hospital.

Figure 3: Patients encephalitis from different districts reported to the study hospital.
Figure 4: Age and sex wise distribution of encephalitis patients reported to the hospital.

Figure 5: District wise encephalitic JE, Dengue and MP reported to the hospital.

Image 1: Author during Malaria Microscopy.
CONCLUSION
From our study it was confirmed that etiology of the encephalitis patients is very important to control the diseases as well as for treatment of the patients, as the all encephalitis patients were neither JE nor dengue and malaria, where 27% patients were negative for all three vector borne diseases. Again children were mostly affected which may be due to their living behavior, lack of knowledge of human health/disease transmission and less immunity. Where government and guardian of children must take initiative to reduce transmission, awarethem, improve their living behavior and immunize them (if available). Another important point was maximum encephalitis patients were confirmed JE and Equivocal JE. As per some of the studies Equivocal JE may be confirmed as JE by RT-PCR. So, it is clear that maximum encephalitis patients were JE and to reduce this disease burden government must plan or improve the control strategies of Japanese encephalitis. To identify the causative agent, government must introduce the different encephalitis identification tests with microbiology laboratory in each districts of the state.

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