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Sero-epidemiological Study of Japanese Encephalitis in Some Selected Hospitals of Nepal

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Abstract

Japanese encephalitis is a major public health problem in Nepal, about 1000-3000 cases and 200-300 deaths reported annually, mainly in endemic areas and sporadic cases have also been reported from non-endemic areas such as Kathmandu. The diagnosis of JE is based on clinical signs and symptoms. This study was conducted in some hospitals of Nepal to find the epidemiological trend of the disease. Serological surveys have revealed that about 10 % of people living in JE endemic areas are infected with the virus, most of whom are infected before age 15. This type of study is essential to be carried out in our country to know the seroepidemiology of the JE virus. A descriptive cross-sectional study was conducted, and 267 serum samples from suspected Acute Encephalitis Syndrome and viral fever cases were collected from three different hospitals in Nepal. The results were confirmed by an IgM capture enzyme-linked immunosorbent assay. Of 267 serum samples, 242(90.6%) were clinical suspects of Acute Encephalitis Syndrome, and 25(9.4%) were clinical suspects of viral fever. Of 267 cases, 84(34.7%) Acute Encephalitis Syndrome cases and nine (36.0%) viral fever cases were positive for anti-Japanese encephalitis virus IgM antibodies. The seropositivity was higher in males 60(39.9%) than in females 33 (29.2%). Out of the total positive cases, the highest seropositivity (40.2%) was observed in the age group 15-50, and the least (25.0%) was

observed in the age group above 50. Tribhuvan University Teaching Hospital accounts for highest seropositivity (36.6%) and the least (32.2%) in Bheri Zonal Hospital. The overall seropositivity rate was found to be 34.8% which was higher than the national data (21.6%). This study reports that the seropositivity was higher than national figure and the seroepidemiology pattern of JE has changed, indicating a need for urgent intervention. Diagnostic facilities should be expanded in every hospital in the endemic region of Japanese Encephalitis for better surveillance.

Keywords: Acute encephalitis syndrome, IgM capture ELISA, Japanese encephalitis, japanese Encephalitis Virus (JEV)

Introduction

Japanese Encephalitis (JE) is an infection transmitted by mosquitoes and caused by the Japanese Encephalitis Virus (JEV). The virus belongs to the Flavivirus family and is transmitted between Culex mosquitoes and vertebrate hosts, mainly pigs and wading birds, in an ongoing cycle (Solomon et al., 2000). The main carrier of the virus is the Culex tritaeniorhynchus mosquito. Humans can become infected through mosquito bites when they interrupt this cycle. However, individuals infected with JEV do not have high levels of the virus in their blood and are considered to be dead-end hosts (Solomon, 2003). Seizures, altered mental state, and fever are signs of acute encephalitis syndrome (AES). several microorganisms cause AES, but the Japanese encephalitis virus is most common (Kumar, 2020). JE is main causes of viral encephalitis worldwide, with an estimated 50,000 cases and 15,000 deaths annually (Solomon et al., 2000).About one in 250 individuals infected with JEV develop clinical symptoms but most of the infections with JEV in human are asymptomatic, and (CDC, 2008). Despite the availability of vaccines worldwide, an estimated 67900 cases of JE occur annually, resulting in approximately 13600 to 20400 deaths. The overall incidence rate is 1.8 per 100,000 in 24 countries at risk for JE (Campbell et al., 2011).

JE cases in Nepal mainly occur from April to May, peak from late August to early September, and decrease in October. The plain regions of Nepal (Terai and inner Terai) are always endemic for JE. Around 12.8 million people are at risk of the disease in 24 districts in the Terai and inner Terai regions (EDCD, 2008). The first confirmed JE outbreak in Nepal was in 1978 in the Rupandehi district of Western Nepal (Joshi, 1983), and since then, seasonal JE outbreaks have been reported yearly. Between 1978 and 2006, a total of 32,481 cases of Japanese Encephalitis (JE) were identified nationwide in Nepal, with 5,902 reported deaths, resulting in a case fatality rate (CFR) of 18.17% (EDCD, 2008; Joshi et al., 2005). Beside the 24 endemic districts, sporadic cases have also been reported in non-endemic districts, including the Kathmandu Valley (Zimmerman et al., 1997; Partridge et al., 2007). In Nepal between 1000-3000 cases and 200-400 deaths

occur annually (EDCD, 2008), which reveals that Japanese Encephalitis is a significant public health concern in Nepal. In Nepal 1,823 cases of Japanese Encephalitis (JE) were reported between 2007 and 2015, with an average incidence of 0.735 per 100,000 people (Kumar Pant, et al., 2017). In 2021, 413 AES JE cases and 25 laboratory confirmed cases of JE were reported. Before JE vaccination, about 50% of Acute Encephalitis Syndrome (AES) cases tested positive for JE. Lumbini Province had the highest number of confirmed JE cases (9 out of 25; 36%) (DoHS, 2021).

Vaccination is the only effective way to control Japanese encephalitis (JE) in humans. Japan, Korea, Taiwan, China, and Thailand have successfully used vaccination to control JE. The World Health Organization (WHO) has recommended the use of JE vaccines in places where they are affordable (WHO, 2006). In line with the guidelines from the Government of Nepal, the JE/AES vaccine is approved for administration at 12 months of age in Nepal (DoHS, 2021). As a focused effort to control JE, mass vaccination campaigns were carried out in phases starting in 2006 and were completed in 31 high-risk districts by 2011. In 2012 the JE vaccine was gradually integrated into the routine immunization programs in these 31 districts, resulted in a significant decrease in Japanese Encephalitis cases in Nepal. However, over the years of surveillance data showed that JE cases have also been reported from other districts in Nepal. Therefore, a mass vaccination campaign was carried out and the JE vaccine was subsequently integrated into the routine immunization programs of all 44 remaining districts in July 2016. As a result of these efforts, the incidence of JE in Nepal was significantly decreased by 2019 compared to the early years of surveillance (DoHS, 2021).

This study was carried out in three hospitals of Nepal and was focused to determine the epidemiological status of Japanese encephalitis within selected hospitals in Nepal, representing the areas where JE is endemic and sporadic. The findings from this study could play an important role in policy making, improving Japanese encephalitis virus (JEV) surveillance, intervention, prevention, and control efforts in Nepal.

Research Methodology

Study Design

A laboratory-based descriptive cross-sectional study was carried out from July 2007 to August 2008 at Everest International Clinic and Research Centre, Kathmandu, Nepal, to find out the epidemiological status of Japanese encephalitis in some selected hospitals in Nepal.

Inclusion and Exclusion Criteria

The patients clinically diagnosed with AES and viral fever were included and the patients with other febrile cases were excluded in the study. Purposive sampling method was adopted; therefore, samples were taken only from AES and viral fever cases.

Sample Collection

Before collecting blood specimens informed consent was taken from the patients guardians, ensuring the ethical conduct of the study. Serum samples were collected from 267 patients with acute encephalitis syndrome (AES) clinical features and viral fever infections admitted to Tribhuvan University Teaching Hospital (TUTH) in Kathmandu, Lumbini Zonal Hospital (LZH) in Rupandehi, and Bheri Zonal Hospital (BZH) in Banke districts. The samples were transported to Everest International Clinic and Research Center (EICRC), Kathmandu, maintaining the reverse cold chain and stored at -20°C before assay.

IgM Capture ELISA (MAC-ELISA)

The immunoglobulin M (IgM) antibody capture ELISA (MAC ELISA) for serum was performed according to the manufacturer's protocol. During the testing procedure, the protocol provided by Panbio Diagnostics was strictly followed to achieve high accuracy (PanBio Diagnostics).

Japanese Encephalitis-Dengue IgM Combo ELISA Test

The PanBio JE IgM capture ELISA was performed according to the manufacturer's instruction. A diluted serum sample (1:100) was added to the assay plate. Serum antibodies of the IgM class, when present combine with anti-human IgM antibody attached to the surface of the wells of the assay plate and incubated at 37^{o}C for 60 minutes. Concurrently, peroxidase-labelled anti-flavivirus monoclonal antibody conjugate was added to the vials containing lyophilized inactivated JEV, which resuspended the antigen and allowed formation of antigen-antibody complexes. After the residual serum was removed from the assay plate by washing, antigen-antibody complexes were transferred from the antigen vials to the assay plate. After a further 60 minutes incubation at 37^{o}C, the assay plate was washed and tetramethylbenzidine (TMB) substrate was added. After 10 minutes, the reaction was stopped by the addition of 1 M phosphoric acid, the TMB becomes yellow, development of color was the indicative of the presence of anti-JEV IgM antibodies and the absorbance was read at 450nm. A sample was defined as positive if JE PanBio units were >11 and as negative if JE PanBio units were <9.

Statistical Analysis

The collected data was entered in excel sheet and was analyzed using WIN PEPI software (version 7.9, November 24, 2008). The chi-square value and p-value were determined to determine whether the findings were statistically significant. P-values lower than 0.05 were regarded as statistically significant. The variables used were age, sex, serology, microscopy, Japanese encephalitis infection, hospitals.

Results and Discussion

Results

Out of 267 samples, 242(90.6%) were clinical suspects of AES, and 25(9.4%) were clinical suspects of viral fever. Of 267 cases, 154 (57.7%) were males, and 113 (42.3%) were females. The highest number of cases was observed in TUTH (142), which constituted 53.2%, and the least in LZH (35), which constituted 13.1% of total cases (Table 1).

Of the 267 clinical cases with serum, 93(34.8%) were positive for the presence of anti-JEV IgM antibody and were confirmed as JE. Of 267 samples, 34.7% AES cases and 36 % viral fever showed positive results for the presence of anti-JEV IgM antibodies, which was statistically insignificant (p=0.017). Of 267 cases, 38.9% of males were positive for anti-JEV IgM antibodies, and 29.2% of females showed positive results for anti-JEV, which was statistically insignificant (p=0.098). The seropositivity was found to be highest in TUTH (36.6%) and lowest in BZH (32.2%), which was statistically insignificant (p=0.78). Similarly, the highest seropositivity was observed in the age group 15-50 years (40.2%) and the lowest in the age group above 50 years (25%), which was statistically insignificant (p=0.07). The overall seropositivity rate was 34.8% (Table 2).

Table 1

Socio-epidemiological distribution of cases

Characteristics	No. of samples
AES cases	242(90.6%)
Viral fever cases	25(9.4%)
Male	154(57.7%)
Female	113(42.3%)
Bheri Zonal Hospital	90(33.7%)
Lumbini Zonal Hospital	35(13.1%)
Tribhuvan University Teaching Hospital	142(53.2%)
Total number of specimens	267(100%)

Table 2

Characteristics of the laboratory-confirmed JE cases

Characteristics	No. of samples	Statistics
Age * (years)		χ^2=5.32
<15	13 (29.5%)	p=0.07
15-50	64 (40.2%)	
>50	16 (25%)	

Sex		
Male	60 (38.9%)	χ^2=2.73
Female	33 (29.2%)	p=0.098
Clinical diagnosis		
AES	84 (34.7%)	χ^2=0.017
Viral fever	9 (36%)	p=0.89
Hospitals		
BZH	29 (32.2%)	χ^2=0.47
LZH	12 (34.2%)	p=0.78
TRUTH	52 (36.6%)	
Total	93(34.8%)	

Discussion

The study provides an analysis of seroepidemiology of Japanese encephalitis and epidemiological trend of Japanese encephalitis in some selected hospitals in endemic and non-endemic area of Nepal. In comparison to the national surveillance system only a small number of studies have been undertaken in some hospitals. In Nepal, most JE cases occur in the lowland plains of the Terai region that borders India during and after the annual monsoon season from May to October (Partridge et al., 2007). JE is one of the most critical forms of viral encephalitis worldwide, causing a severe public health problem in Nepal and Southeast Asia (EDCD, 2001). Nepal reported the first confirmed JE case in 1978, and the number of cases has increased. Presently, about 1000-3000 cases are reported annually in Nepal, with an increasing trend (Joshi, 1983). JE has spread to the inner Terai and high hills, including Kathmandu Valley (altitude 1300 m), where the first outbreak of JE was reported in 1995 (Zimmerman et al., 1997).

The present study coincides with a similar study done by Bajracharya (2001), which demonstrated that 57% of the patients were male and 43% female. The present study also shows similarities with the study done by Dumre (2006), which demonstrated 55.8% male and 44.2% female. The present study also agrees with the study of Khanal (2008), which demonstrated 51.3% male and 48.7% female. Males have a higher chance of being bitten by mosquitoes due to their extensive outdoor work, whereas females are commonly restricted to household work (indoor work).

The seropositivity of this study was consistent with previous findings from Nepal. Studies by Dumre (2006), Bista and Shrestha (2005), Pandey et al. (2003), and Sherchand et al. (2001) also reported a similar positivity rate. This study is contrary to some of the previous findings from Nepal. A survey in Bheri Zonal Hospital by Bajracharya et al. (2001) demonstrated a positivity rate of 47.2%, which was quite higher than the present study. Similarly, a study conducted in Bheri Zonal Hospital by Khanal (2008) showed a positive rate of 41.4%, which is higher than the present study. The difference observed

could be because these studies were done in the hyper-endemic region of Nepal, where the positive detection rate could be higher than the national figure.

The overall seropositivity rate of this study was quite high than the national figure (21.6%) (EDCD, 2005/2006). The positive difference between the previous reports and the present finding could be due to the small sample size and localized nature of the study, contrary to the wide coverage of surveillance samples. The negative cases could be due to overdiagnosis or other causes of acute encephalitis syndrome. The finding of this study was consistent with previous research in Nepal (Bundo & Igarashi, 1985; Bista & Shrestha, 2005; and Pandey et al., 2003). However, the present finding did not correlate with the 13.2 % positivity reported by Kubo et al. (1993), 15.4 % by Kubo et al. (1996), and 13.3 % using HI and 22 % using the neutralization test by Ogawa et al. (1992). The present study shows a higher positivity rate than the above reports, possibly due to variations in geographical distribution and the techniques used.

In this study JE was more common in males (38.9%) than females (29.2%) which was in accordance with a study conducted previously in Nepal (Akiba et al., 2001), which could be due to differences in exposure to the vector. Females spent more time indoors during peak feeding hours, while males were outdoors (Partridge et al., 2007). In this study, adults showed a higher seropositivity (40.2%) than children. This could be due the mass vaccination of children <15 years with the SA14-14-2 JE vaccine has shifted JE incidence from children to age groups >15 years (Joshi et al., 2005). The British Military Hospital in Dharan administered three doses of BIKEN-killed lyophilized vaccine to 1,152 individuals as a part of the JE vaccination program that started in 1983, (Henderson, 1984). The SA 14-14-2 live-attenuated JE vaccine is highly effective and safe in Nepal (Bista et al., 2001). In a 1999 Western Terai study, 224,000 vaccine doses resulted in 99% protection for children shortly before the seasonal increase in JE cases. Another study showed 98.5% protection 12-15 months post-immunization, and a third study 5 years later found 96.2% protection (Tandan et al., 2007). In 2006, a JE vaccination program was conducted in six endemic districts, vaccinating 87.74% of the targeted population. Studies in Nepal reported high protection rates after vaccination, providing strong reassurance about the safety of the JE vaccination in the country (EDCD, 2006/2007; Wierzba et al., 2008).

The seropositivity was highest in TUTH (36.6%), followed by LZH (34.28%), and BZH (32.2%). The number of positive cases and seropositivity was higher in TUTH because of the clinically confirmed cases of JE. TUTH is a referral hospital in the country, so many cases were referred to this hospital for treatment. The seropositivity of anti-JEV IgM in TUTH is contrary to previous studies done in TUTH. The present finding does not correlate with the 13.2 % positivity reported by Kubo et al. (1993) and 15.4 % by Kubo et al. (1996). The present study shows a higher positivity rate than the above reports,

possibly due to variations in the techniques used. The seropositivity for anti-JEV IgM in BZH is contrary to the previous findings by Bajracharya et al. (2001) 43.1%; Sherchand et al. (2001) 28.7% and Khanal (2008) 40.8% which stated more seropositivity than the present study. However, the study conducted by Pandey et al. (2003) stated that 37.3% were positive for anti-JEV IgM, which is nearly similar to this study. Statistically, there is a significant difference between different hospitals and the occurrence of the disease ($P > 0.05$; $\chi 2 = 0.47$).

The age group 15-50 years (40.2%) were found more affected than the age group <15 years (29.5%). Although, the incidence of JE is high in children, but more sero-positivity was observed in adults this might be due to the effective vaccination in children. The vaccine is not yet administered to adults >15 years in Nepal and the adults are more prone to JEV infection. Statistically, the association between age and the occurrence of disease was found insignificant (**$p=0.07$, $\chi^2=5.32$**). Due to vaccination among children the incidence of disease in children is decreased and the incidence of the disease has been shifted to the population over-15 year's age group (Joshi et al., 2005). This study was in accordance with the previous findings that show a higher prevalence rate among adults than children. However, our study contradicts earlier findings in Nepal. Bista and Banerjee (2000) reported over 40% of cases in the 5-15 age group, while Dumre (2006) found the highest number of JE-positive cases in the same age group. This difference could be attributed to our smaller sample size and the localized nature of the research.

Conclusion

In 2007/2008, 267 serum specimens were collected from the three hospitals suspected AES and viral fever patients and tested for anti-JEV IgM antibody by IgM capture ELISA. The overall seropositivity was 34.8%, higher than the national figure (21.6%). Males and adults were more affected than females and children, respectively. Most of the JE cases were confirmed at Tribhuvan University Teaching Hospital.

Recommendations

Japanese Encephalitis (JE) testing is limited to a few hospitals. For accurate diagnosis, diagnostic facilities should be expanded in every hospital in JE-endemic regions. Continuous surveillance and research for JE should be strengthened, and mass vaccination campaigns should be implemented in endemic and non-endemic regions. Other preventive measures, including bed nets, should also be considered.

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