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A study on efficacy and safety of SA-14-14-2 vaccine against Japanese Encephalitis in Virudhunagar district

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Abstract:

Objective: To find out the prevalence, efficacy and safety of SA-14-14-2 vaccine against Japanese Encephalitis (JE). **Materials and Methods:** A total of 60 children in the age group of 6 to 10 yrs, belonging to 5 schools in 5 blocks (12 from each) of Virudhunagar District of Tamilnadu were selected for vaccination. Three samples from each child, one sample prior to vaccination, second sample four weeks after vaccination and third sample twelve weeks after vaccination were taken. Pre and post vaccination samples were screened for antibodies to the JE virus by MAC ELISA and antibody titre was determined by Haemagglutination Inhibition (HAI) test. **Results:** Among the 60 pre vaccinated samples, only 2 (3.33%) showed IgM antibodies. Samples collected after 4 weeks and 12 weeks of vaccination, 24 (40.68%) and 58 (98.30%) showed sero conversion to JE respectively. The antibody titre of all the samples were determined by HAI technique. In the prevaccinated IgM positive samples, one male child from Pandalkudi Primary Health Centre (PHC), one female from Mallanginar PHC showed HAI titre of 1 in 160 confirming the presence of subclinical case prevalence in these two Blocks. The efficacy of the vaccine was proved by the seroconversion in the samples collected in all five blocks (PHC) at Virudhunagar by HAI Evaluation of safety of vaccine was analysed by noting the local irritation and systemic toxicity that occured after the vaccination and it was shown that swelling was present in 1 child (8.33%) in Narikudi, headache was present in 1 child (8.33%) in Pandalgudi. No cases of encephalitis / meningitis, seizure were detected in any block.

Key words: Haemagglutination Inhibition (HAI) titre; Japanese Encephalitis (JE); MAC ELISA; SA-14-14-2 Vaccine; Vaccination

Introduction

Japanese encephalitis (JE), a mosquito-borne flaviviral zoonotic infection, remains a major public health problem in Asia, reportedly causing 16, 000 to 50, 000 acute encephalitic episodes and 5,000 to 10, 000 deaths annually (1). It primarily affects children under the age of fifteen living in rural areas, especially where rice is grown and pigs are reared (2).Seventy percent of those who develop illness either die or survive with a long term neurological disability (3).

Since there is no effective or specific treatment for JE, most authorities agree that the control of JE requires universal childhood immunization (4) and other methods such as mosquito control and pig control which have been used with little success. A widely available inactivated vaccine produced from infected mouse brain tissue is expensive and requires two or three doses to achieve protective efficacy and in practice further booster doses to maintain immunity (5,6). A live-attenuated JE SA 14-14-2 vaccine developed in China was licensed in 1988 (7). Since then, an estimated 120 million children have been immunised with this vaccine with no adverse events reported in several large studies (7–10).

JE has been widespread in India. Outbreaks have been reported from States like Uttar Pradesh, West Bengal, Assam, Andhra Pradesh, Karnataka, Bihar, Tamil Nadu, Haryana and other states throughout the years. As per The Directorate of National Vector Borne Disease Control Programme (NVBDCP) the Case Fatality Rate (CFR) due to JE in India has been around 24 % with wide variations in states.

Following the massive outbreak of JE in 2005 in the districts of Eastern Uttar Pradesh and adjoining districts of Bihar, Vaccination campaigns were carried out in 11 highest risk districts of the country in 2006. Children between the age group of 1 to 15 years were vaccinated with a single dose of SA 14-14-2 vaccine.

The second year of the government's five year strategy targets 23 districts of 9 states. JE vaccination Campaigns kicked off in March 2007 and by July2007, 20 million more children and adolescents were targeted to be immunized against JE.

Table 1: Cumulative district wise JE vaccination coverage was as follows

State	#immunized (millions)	%target Population
Uttar Pradesh	**6.836**	**99.97%**
West Bengal	**1.222**	**56.12%**
Assam	**0.647**	**82.26%**
Karnataka	**0.595**	**82.67%**
Total	**9.3**	**88%**

Since 1997, the mosquito pool from Virudhunagar District was confirmed to be positive for JE virus. Past history had shown that very high number of cases and deaths were reported due to JE in this district. So, Virudhunagar District was selected for Japanese Encephalitis vaccination programme during the year 2007. The vaccination was completed in Virudhunagar District during August 2007.

Aims & objectives

1) To know the prevalence of subclinical cases in the study population

2) To study the Efficacy of the vaccine

2a) By proving the seroconversion in the vaccinees by standard techniques like Haemagglutination Inhibition Test and ELISA

2b) By Case Study

3) to study the safety of the vaccine by toxicity and local Irritation Tests.

Materials and Methods

A total of 60 children in the age group of 6 to 10 yrs, belonging to 5 schools in 5 blocks (12 from each) of Virudhunagar District of Tamilnadu were selected for vaccinations after a physical examination for fitness, and after ruling out contraindications. A total of 178 samples were collected from them, 3 samples from each child, 1 sample prior to vaccination, 2nd sample 4 weeks after vaccination and 3rd sample 12 weeks after vaccination.

This is an interventional type of study to determine safety and efficacy of JE live attenuated SA-14-14-2 vaccine.

This study was conducted at WHO AMES LAB, Institute of Microbiology, Madurai Medical College, Madurai, Tamilnadu. The study period was 3 months from October 2007 – December. 2007. The study population included 60 JE vaccinated children of 6-10 years age group. The total sample size was 178 serum samples.

Written informed consent was obtained from the Parents/Guardian of vaccinated children. Ethical Clearance was obtained from the Institutional Ethical Committee.

Inclusion Criteria:

- 6-10 years of age group in both sexes.

Subjects without

- fever / malnutrition / acute infectious disease.
- active untreated tuberculosis
- any cardiac, liver & kidney diseases.
- any allergy & Convulsions.
- any H/o hypersensitivity to Kanamycin or Gentamycin.
- a history of immunization with any other live attenuated vaccine within the past one month.
- Person not on any immunosuppressive therapy.

Exclusion Criteria:

Subjects with

- acute infectious disease, tympanitis & Cardiac, liver, Kidney diseases.
- a proven or suspected hypersensitivity to Kanamycin or Gentamycin.
- Subjects on immuno suppressive therapy
- Pregnancy
- Known or suspected impairement of immunologic function.
- History of serious chronic disease.
- Acute medical illness with or without fever within last 72 hours or an axillary temperature > 37.5^{o}C at the time of inclusion.
- History of documented and suspected encephalitis, encephalopathy, meningitis.
- Immunization with JE vaccine prior to enrollment.
- If received any vaccine, other than the study vaccine within 2 weeks prior to or scheduled to receive a non study vaccination during the conduct of this trial.
- History of seizures including history of febrile seizures, or any other neurologic disorder.

Vaccine:

Live attenuated SA 14-14-2 JE Vaccine made in Chengdu Institute of Biological Products, Chengdu, CHINA, was used for vaccination. This vaccine was supplied in 5 dose vials as a lyophilized powder and was reconstituted with 2.5ml of sterile diluents (PBS containing 2% serum). After reconstitution, it turns into a transparent Orange, red or light pink liquid. The reconstituted vaccine was not used after 2 hours of reconstitution. Vaccine was stored and transported between 2^{o}C to 8^{o}C and protected from light.

Schedule:

One dose (0.5ml) containing not less than 5.4 log PFU of live JE virus. The vaccine was injected subcutaneously in the upper arm of children in 1-15 years of age. sterile water was used for cleaning the skin before injection. The vaccine was administered in the 1st week of August 2007. The children were followed up for 5 days for side effects or reactions.

Blood Specimen:

Blood samples of 60 children were collected approximately 1 week prior to the vaccine to determine their antibody status to JE virus. These samples were treated as prevaccination samples. The samples were again collected approximately 4 weeks & 12 weeks after the vaccination.

Collection & Storage of Blood Specimen:

All the blood specimens (5-10 ml each) were collected by venepuncture. Serum was separated within 48 hours and the sera were stored over ice till they were transported to Madurai Medical College (MMC), Institute of Microbiology, WHO AMES LAB, Madurai which is situated at a distance of 50 km from Virudhunagar. At MMC, the sera were stored at minus 20^{o}C.

Processing

I. Pre and post vaccination samples were screened for antibodies to the JE virus by MAC ELISA manufactured by NIV PUNE.

II. The antibody titre in the pre and post vaccination samples were determined by haemagglutination inhibition test using suckling mice brain derived JEV as antigens which was prepared in house.

Results

During the year 2007, 94% of 1-15 year population of Virudhunagar District was immunized with live attenuated JE SA 14-14-2 vaccine amounting to 4,83,537 beneficiaries.

In our study a total of 178 serum samples were collected from 60 children, selected from 5 Block PHCs, 12 from each Block PHC. Three samples were collected from each child, 1 sample prior to vaccination, 2nd & 3rd sample after 4 weeks & 12 weeks of vaccination. As one female child from Mallanginar Block PHC refused to take post vaccinated blood samples, only 178 samples were collected.

All the 178 blood samples, 60 pre-vaccinated and 118 post vaccinated were screened for IgM antibodies to JE virus with NIV PUNE MAC ELISA KIT. Among the 60 pre vaccinated samples, only 2 (3.33%) showed IgM antibodies to JE virus. Among the samples collected after 4 weeks and 12 weeks of vaccination, 24 (40.68%) and 58 (98.30%) showed sero conversion to JE respectively (Table-2).

The antibody titre of all the 178 sampleswere determined by HAI technique. In the prevaccinated IgM positive samples, one male child from Pandalkudi PHC, one female from Mallanginar PHC showed HI titre of 1 in 160 confirming the presence of subclinical case prevalence in these two Blocks.

Place wise analysis of HAI titre of the samples collected 4 weeks and 12 weeks after vaccination are presented in Table-3, Table-4 respectively.

Thus the efficacy of the vaccine was proved by the seroconversion in the samples collected in all 5 Block PHCs at Virudhunagar by HAI .

The efficacy of the vaccine was further confirmed by the case study also. The number of cases and deaths reported in the year 2006-07 before vaccination were compared with the number of cases and deaths in the year 2007-08 . It was found that there were 3 cases and 1 death from September 2006 to August 2007 in the age group of 1-15 years at Virudhunagar District. Whereas there was no case and no deaths reported from September 2007 to August 2008 in Virudhunagar District after vaccination showing that this vaccine has protected the population of 1-15 years in this district with marked rise in antibody titre in the vaccinated individuals.

Further evaluation of safety of this JE SA 14-14-2 vaccine was analysed by noting the local irritation and systemic toxicity occured after the vaccination and it was shown that swelling was present in 1 child (8.33%) in Narikudi, headache was present in 1 child (8.33%) in Pandalgudi. No cases of encephalitis / meningitis, seizure were detected in any Block.

Discussion

A Study on Efficacy and Safety of SA 14-14-2 Vaccine against Japanese Encephalitis in Virudhunagar District was conducted in 5 Block areas from October 2007 to December 2007.

As the population selected in the study was only the school going children, the age group involved in the study was from 6-10 years. Similar study by Rao, C. V. R. Mohan et al 1993 also involved school going children of this age group at South Arcot District . As the ratio of school going males exceeded females in this District, the sample collection was also more in the males in this study.

In this study, it was shown that 3.33% subclinical cases were present in the population with IgM antibody in prevaccinated samples. Similar findings were also found in the study by Rao, C. V. R. Mohan et al which showed 42.48% subclinical cases in South Arcot District (11). In Tamil Nadu, Virudhunagar District, Villupuram, Thiruvarur, Thanjavur and Perambalur Districts have already proved to have JE cases and deaths reported from 1991. Similar study by A.Gajanana et al 1998 showed that in the non transmission season there may be considerable decline in seropositivity of general population (12). As the samples in this study were collected before the transmission season perhaps the decline in the number of subclinical cases.

In this study, it was found that 40.68% vaccinees showed sero convertion after 4 weeks and 98.3% after 12 weeks of vaccination . Similar study by Young Mo Sohn et al 1999 and Xin YY et al 1998 also showed 85 -100% seroconversion in their vaccinees after a single dose of SA-14-14-2 vaccine which is in accordance with this study(13,8). Indian Paediatrics 2001 showed that IgM antibodies after JE infection arises by 3rd day of onset and maximum at 7 days and lasts upto 3 months, but the studies on immunization by Theodar F Tsai et al showed that 94 – 100% seroconversion can be expected only after 2

primary doses of SA 14-14-2 vaccine at intervals of 1 month and 2.5 month (14). In this study also even though 1 dose was given, the seroconversion was 98.3% after 12 weeks. Thus there is variation in seroconversion after viral infection and immunisation. The viral vaccines are prepared by serial cell culture passages and neuro attenuation, hence the nucleotide sequence of the vaccine gets varied. So the immune response in the disease may not be similar after vaccination.

In this study, it was found that Narikudi Block PHC was the only one where there was no seroconversion after 4 weeks. The article by Theodar F Tsai showed that lower seroconversion rates were mainly due to variation in vaccine dilutions (14). In this vaccine, the expected infectious titre for the minimal standard of vaccine infectivity should not be less than 106.7 TCID50 per ml. As Narikudi is the only block which showed no seroconversion after 4 weeks, it is obvious that there might be some technical deficiencies in vaccine dilutions.

After 12 weeks, it was noted that 7 children showed marked increase in titre, whereas 14 showed decrease in titre and 3 showed no change in titre. It was already shown in studies in Asia that the persistence of vaccine-derived immunity are complicated by natural infections with dengue, West Nile virus, or other flaviviruses and reexposure to JE virus itself, all of which act to reinforce and broaden vaccine-derived immunity to JE virus. Perhaps this might have occured in this population also because this area is also endemic for dengue and westnile and other flavi viruses.

In this study it was shown that one child had no seroconversion at all. It was already shown by Mohammed Zeeshan et al that failure of immune response in their vaccination studies was due to nutritional status, site of administration of vaccine and genetic factors (16). These factors might be the reason for no immune response in one child in Kanniseriputhur.

In this study seroconversion was diagnosed by identifying HAI antibodies and IgM antibodies (ELISA). Thus these 2 tests were used to study the efficacy of the vaccine. This demonstration of HAI antibodies for seroconversion was already done by Rao, C. V. R. Mohan et al and also proved by Gajanana et al. The HAI test has several advantages making it particularly appropriate for subclinical infections as well as post infection phase. It is a sensitive accurate indicator needing only a small quantity of serum or plasma. HAI antibodies develop faster than the neutralizing antibodies and previously infected person may show measurable antibody responses. In this study also HAI technique detected subclinical cases and post vaccinated cases in different periods that is after 4 weeks and 12 weeks.

Similar study by Bista M.B et al also showed that JE vaccine was highly efficient (99.3%) in preventing JE when administered before exposure to virus (15). His study with 20 children confirms the present study. Similar study by Konishi E et al 2002 also evaluated the protective capacity of the approved inactivated JE vaccine by comparing the ratio with those reported for unvaccinated population (17).

The safety of the vaccination was evaluated by the toxicity study it was shown that except for one case with swelling, no other toxic symptoms were elicited, proving the safety of the vaccine. Similar studies revealed that only 5 -10% local reaction , no acute encephalitis and no hypersensitivity reactions were seen in the vaccinees and the vaccine was proved to be effective and safe (7-10,18).

Conclusion

The live attenuated vaccine has excellent immunogenicity and protective efficacy. Its shorter dosage schedule and low production cost make it an attractive option for endemic countries in southeast Asia. The vaccine could first be implemented in those countries with endemic JE who could otherwise not afford an extensive JE vaccination program. Long-term studies on efficacy, immunogenicity, and severe adverse effects in non-endemic populations would make it a more convincing option for travelers. Further studies on long-term immunogenic protection would help determine ideal booster schedules. Although careful surveillance can be difficult, it must be maintained to determine the continued safety of each of these vaccines.

The SA-14-14-2 live JE vaccine has been prequalified by WHO. It is the first JE vaccine with a pediatric indication to reach prequalification and now enables procurement of the vaccine by United Nations agencies. There are also promising new vaccines undergoing clinical trials that show potential for wide scale use. Vaccine schedules, surveillance of adverse events and setting up effective vaccination programmes are important issues that need addressing to maximize the impact of the available vaccines.

Table 2: Seroconversion in pre & post vaccinated samples by Elisa

Block PHC	**Samples collected prior to vaccination (No. of positives/ Total No. of samples (%)**	**Samples collected after 4 weeks of vaccination**	**Samples collected after 12 weeks of vaccination**
1. Narikudi	0/12	0/12	12/12(100%)
2. Reddiapatti	0/12	10/12(83.33%)	12/12(100%)
3. Pandalkudi	1/12 (8.33%)	2/12(16.7%)	12/12(100%)
4. Kanniseriputhur	0/12	4/12(33.3%)	11/12(91.7%)
5. Mallanginar	1/12(8.33%)	8/11(72.72%)	11/11(100%)
Total	2/60 (3.33%)	24/59 (40.68%)	58/59 (98.30%)

Table 3: Place wise analysis of HAI titre of the samples collected after 4 weeks of vaccination

Area	No:	HAI Titre No:(%)									
		NIL	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	Total
Narikudi	12	-	-	-	-	-	-	-	-	-	0
Reddiapatti	12	-	-	-	-	4(33.3%)	6(50)	-	-	-	10(83.3)
Pandalgudi	12	-	-	-	--	--	-	2(17)			2(17)
K.S.puthur	12	-	--	-	-	2(17)	2(17)	-	-	-	4(33.3)
Mallanginar	11	-	-	-	2(18.2)	-	5(45.5)	-	-	1(9)	8(72.7)

Table 4: Place wise analysis of HAI titre of the samples collected after 12 weeks of vaccination

Area	No:	HAI Titre No:(%)									
		NIL	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	Total
Narikudi	12	-	2(17)	4(33.3)	2(17)	2(17)	2(17)	-	-	-	12(100)
Reddiapatti	12	-	-	2(17)	-	-	4(33.3)	6(50)	-	-	12(100)
Pandalgudi	12	-	-	2(17)	6(50)	2(17)		2(17)			12(100)
K.S.puthur	12	1(8.3)	3(25)	4(33.3)	2(17)	2(17)	-	-	-	-	12(100)
Mallanginar	11	-	-	4(36.4)	2(18.2)	4(36.4)	1(9)	-	-	-	11(100)

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