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Immunogenicity and safety of updated Thai Red Cross Regimen - Intradermal Purified Vero cell Rabies Vaccine in animal exposure cases in a tertiary setup in India

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ABSTRACT

Introduction: IDRV as PEP have been successfully introduced in many developing countries in Asia and Africa subsequent to WHO recommendation in 1992. India has adopted IDRV in Updated Thai Red Cross regimen since 2006.

Aims & Objectives: To examine the safety and immunogenicity of PVRV in U-TRC regimen.

Materials & Methods: One hundred adult patients were studied between January 2011 to December 2011. Venous blood was collected on day 0,7,14,28,90 and 365 and Viral Neutralising Antibody(VNA) titre was assessed at Human Biologicals Institute, Hyderabad.

Results: The results indicated that a significant number of patients receiving ID regimen had developed protective VNA titre of ≥ 0.5 IU/ml by day 7 and all have seroconverted by day 14 that persisted till year end. The immunogenicity was not affected by co-administration of ERIG. Adverse reactions reported in 56% of vaccinees were mostly minor.

Conclusion: It was concluded that PVRV is safe and highly immunogenic for post exposure rabies vaccination in the intradermal route.

Key words: Post-Exposure Prophylaxis, Rabies Prevention, Rabies Vaccine, Updated Thai Red Cross Regimen, Viral Neutralising Antibody,

Introduction

Rabies continues to be a public health problem. More than 99% of all human rabies deaths occur in the developing world [1]. Although effective and economical control measures are available, the disease has not been brought under control throughout most of the affected countries. Estimated annual human mortality in India due to rabies is 25,000 to 50,000; the actual number of deaths may be 10 times more than reported [2]. Fortunately this is preventable and the basis of prevention as post exposure treatment encompasses three modalities i.e. local wound care, administration of rabies immunoglobulin (RIG) and active immunisation by any of the modern cell culture vaccines. Globally each year, 10 million people require post exposure treatment.

In 1992, the World Health Organisation recommended the two site intra dermal method (Thai Red Cross Regimen) for post exposure treatment. By this method (2-2-2-0-1-1), two doses of vaccine are administered at two sites intradermally on days 0, 3 and 7 and one dose of vaccine is administered at a single site on days 30 and 90. In 1997, WHO expert committee on rabies recommended that each intradermal dose should be 0.1ml of vaccine containing 0.5 to 2.5 IU of Viral antigen per dose [1,3].

The use of reactogenic and less potent nerve tissue vaccine(NTV) has been withdrawn and since 2004 was replaced by the state government of Odisha with cell culture vaccine which were administered by intramuscular Essen regimen.The high cost combined with the gap between

demand and supply acted as a deterrent for its continuous and regular supply. The Drug Controller General of India in 2006, approved IDRV (Intradermal Rabies vaccine) by Updated Thai Red Cross (U-TRC) regimen (2-2-2-0-2).

The Technical Committee set up by the Department of Health and Family Welfare came out with a guideline for IDRV [2,4].The State Government of Odisha in 2007 approved the use of IDRV for post exposure prophylaxis. Since 2008, the Anti Rabies Clinic at Department of Community Medicine, VSS Medical College has been using IDRV by the Updated-Thai Red Cross regimen.

Rabies vaccines are potent, but many factors affect immunity, including genetic characteristics, nutrition, concomitant diseases, and concomitant use of other drugs besides other confounding factors hitherto known or unknown[5].The present study was undertaken to access the immunogenicity and safety of PVRV administered through intra-dermal route as per Updated Thai Red Cross Regimen (2-2-2-0-2) as post-exposure prophylaxis in Category II and III patients.

Materials and Methods

The study was carried out at Anti Rabies Vaccination Clinic of VSS Medical College Hospital between December-2010 to January-2012. All the eligible 218 cases reporting to the clinic during initial two months of the study, were enrolled. A detailed medical history was recorded for each patient that included age, weight, height, sex, ethnic group and concomitant illness and treatment including past vaccinations against rabies. The circumstances of the exposure, the site and type of the inflicted wound, the surgical interventions done if any, the species and current status of the animal involved were also recorded.

All patients enrolled were of WHO category II or III cases presenting within 24hours of exposure to known or potentially rabid animals. The subjects were selected between 18 to 70 yrs of age, who were otherwise likely to be available for follow up for the whole length of the trial and were willing to provide consent as per the ICH- GCP guideline. Informed written consent was obtained from all subjects. The study protocol was duly approved by the Institute Ethics Committee of the hospital.

Excluded from the study were patients with a history rabies immunization, immunosuppressant, malignancy, allergic reaction to antibiotics or suffering from chronic debilitating diseases. Subject with history of seizure activity, pregnant and nursing mothers, chloroquine or anti malarial treatments, history of drug or alcohol abuse and recent history of blood or plasma transfusion were excluded from the study. Subjects with history of exposure to any animal bite, currently included in some other trial or who have participated in any other clinical trial within the past 3 months were also excluded from this study.

There were 118 drop outs ; 81 patients denied consent , 15 were aged below 18years ,2 were pregnant ladies, 3 were having chronic debilitating diseases, 3 had past ARV administration, 8 demonstrated detectable VNA on day-zero samples, 6 were lost for complete follow up.

One hundred subjects fulfilled the inclusion and exclusion criteria and were studied for the 365 day long study period with a grace of 15 days.

Post exposure prophylaxis regimen: Prior to IDRV inoculation each patient was subjected to assessment of vital parameters and a brief general examination to rule out concomitant diseases. All the category III patients were co-administered ERIG (Equine rabies immunoglobulin) [6]. Each patient received two 0.1 ml injection of PVRV intradermally (using 1ml disposable syringe with 26 gauge needle via Mantoux technique) over the deltoid area on days 0, 3, 7, 28 according to the U-TRC Regimen [7,8].

Adverse reactions were asked and looked for at each visit.

Vaccine and Immunoglobulin: The test vaccine used was the commercial lot of PVRV (Abhayrab) manufactured by the Indian Immunologicals Limited. It was a freeze dried vaccine licensed for IM use. One immunizing dose (0.5ml) contained the protective activity of equal to or greater than 2.5 IU. The vaccine contains rabies virus (L. Pasteur 2061/ vero) propagated on vero cell line, inactivated with beta-propiolactone with thiomersal (0.015%) added as preservative [9].

Immunoglobulin used for the study was a commercial lot of Equirab, equine rabies immunoglobulin manufactured by

Bharat Serum and Vaccine limited in 5ml vials with concentration of 300 IU/ml.

Assay of rabies virus neutralizing antibody (VNA): Venous Blood sample (5ml) was collected from each patient on days 0, 7, 14, 28, 90 and 365 by a 10 ml disposable syringe, serum transferred into a sterile test tube with a label (marking consisted of patient ID, date and day of sampling since day zero e.g. 008/D3/17.03.2011), stored at sub zero temperature at the ARV clinic and dispatched to the laboratory of Human Biological institute, Hyderabad with adequate cold chain. The serum was assayed for the presence of VNA which was expressed in IU/ml.

Outcome Measures: The main outcome measure was assessment of immunogenicity by estimation of rabies viral anti-rabies antibody (VNA) titres at 0, 7, 28, 90 and 365 days by Rapid Fluorescent Focus Inhibition Test (RFFIT)[10]. Participants were also all asked if they experienced any untoward effects of the vaccine does. *Statistical Analysis:* The VNA titres were tabulated and analysed. Titre below the detectable limit of less than 0.05 IU/ml were denoted a value of zero. Seroconversion rate was analyzed on days 0, 7, 28, 90, and 365. Seroprotection was analyzed on days 28,90 and 365. Geometric mean titres of antibodies was calculated at day 0,7,14,90 and 365. Significance of post vaccination GMT in comparison to pre vaccination titre was assessed. The subjects were considered to be protected if the antibody levels are ≥0.5 IU/ml. Reported solicited and unsolicited adverse events were summarized, using frequencies and percentages, by event severity and duration.

Results

Patient demographics: The demographics of the patients studied are listed in Table: 1. Seventy seven patients had wounds of WHO category III, 23 patients of WHO category II, and none of category I.

Exposure and wound treatment: More than 91% of all wounds had been inflicted by dogs, besides other animals like cats(6%),monkeys(2%) or mongoose(1%).

More than half (54%) of the wounds involved lower extremities. However, 32% of patients were bitten in the face, head, neck or fingers. Sixty seven patients had incurred bleeding wounds at various body sites and surgical procedure was not required for any of them. Wound washing was proper only in 17% cases while the rest of the cases were offered the same on arrival at the clinic.

In all cases of Category-III exposures, equine rabies immunoglobulin (ERIG) (40 IU/kg of body weight) was infiltrated in and around the site of the wounds. Medications administered to patients during the study included: tetanus toxoid, tetanus anti serum or tetanus immunoglobulin, antibiotics, analgesics, antihistaminics and antipyretic. None of the subjects had detectable VNA titre on day zero. The cohort of immunized subjects having protective VNA titre rose from 56% on D-7 to reach 100% by D-14.All the subjects maintained the protective VNA titre of above or equal to 0.5IU/ml till D-365.The peak GMT was attained around D-28 which continued to decline marginally till the end of one year.

Table: 1. Patients demographics.

Total Subjects	Male/female Ratio	Mean age in years(range)	Mean body mass index(range)
100	84:16	28(18-69)	21.3(16-31.4)

Immunogenicity

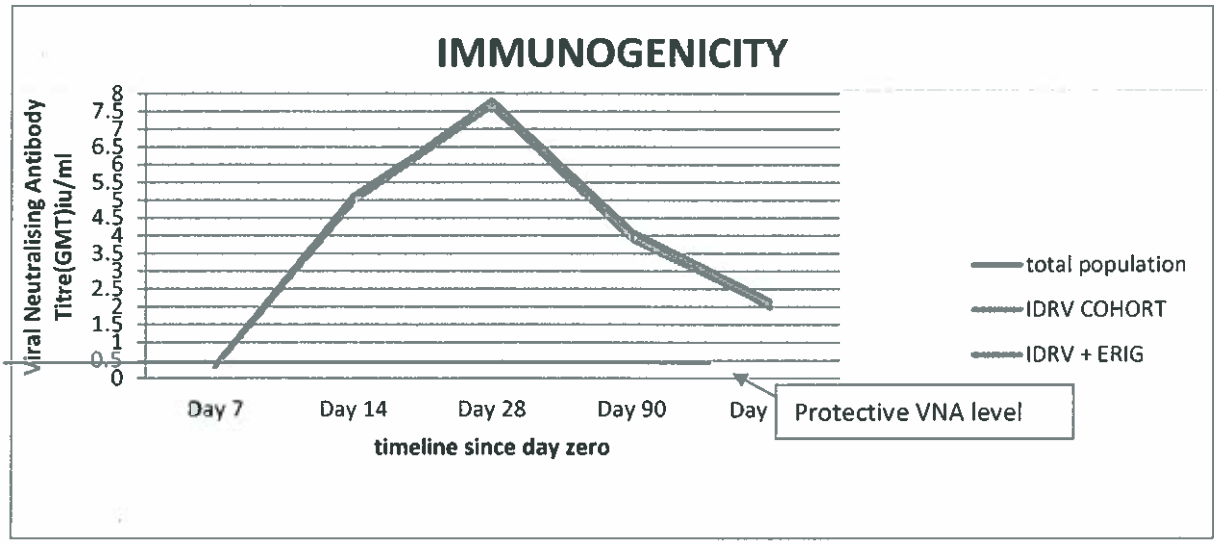

Table: 2. Rabies Viral Neutralising Antibody Titre.

Study cohorts	Antibody Titre	Day 0	Day 7	Day 14	Day 28	Day 90	Day 365
Total study Population	N	97	89	88	92	96	97
	Seroconversion %	0	62.9	100	100	100	100
	GMT IU/ml	--	0.35	5.01	7.81	4.12	2.15
	95% C.I.		0.10-2.20	2.80-6.57	3.09-9.60	4.03-10.46	2.00-3.57
IDRV cohort	N	22	19	19	21	20	22
	Seroconversion %	0	59.3	100	100	100	100
	GMT IU/ml	--	0.32	4.96	7.65	3.89	2.01
	95% C.I.		0.12-2.16	2.68-5.89	2.86-9.47	3.99-9.67	1.97-3.24
IDRV+ ERIG Cohort	N	77	70	69	71	76	75
	Seroconversion %	0	63.8	100	100	100	100
	GMT IU/ml	--	0.35	5.11	7.69	4.11	1.98
	95% C.I.		0.14-1.76	2.11-5.99	3.21-9.12	4.13-9.54	2.14-3.11
Chi-square test between cohorts	for sero-conversion	--	NS	NS	NS	NS	NS

Abbreviations: N-number of patients; IDRV-intradermal rabies vaccine; ERIG-equine rabies immunoglobulin

The GMT (geometric men titre) of VNA was 0.35 IU/ml on D-7 which was below the protective level. By D-14 the GMT of VNA increases to 5.01IU/ml i.e. above the protective VNA.

Seventy seven patients, all with category-III exposure had received sero-vaccine therapy. The remaining 23 patients had category-II exposure and received IDRV only. The GMT of VNA of both the cohorts of patients demonstrated

comparable trends with no statistically significant differences($p > 0.5$) at each point of the timeline of GMT assay throughout the 365 day study period. Day-zero serology. As many as 41 samples of the 600 collected and dispatched were rejected due to contamination.

Safety: As many as 64% subjects were detected to be having adverse reactions to IDRV. All were minor and transient mostly in the form of local effects. No serious adverse reactions were identified and reported during the one year follow up period. (Table: 3)

Table: 3. Adverse events to IDRV (N=100)

Local Effects	Subjects	Systemic Effects	Subjects	Overall Effects	Subjects
Mild Pain	58%	Headache	12%	No adverse effects at all	36%
Swelling	23%	Fever	8%	Any Adverse effects	64%
Erythema	11%	Dizziness	4%		
Itch	12%	Malaise	7%		
Echymosis	2%	Nausea	2%		
Any local effect	33%	Regional adenopathy	1%		
		Any systemic effect	14%		

Abbreviations: IDRV-intradermal rabies vaccine; N-number of patients.

Discussion

Safe and highly efficacious rabies vaccines produced in various cell cultures and embryonated eggs have been commercially available for last many years. But, in developing countries where rabies is enzootic such vaccines are too costly and in short supply as compared to the demand. However, intradermal rabies vaccination (IDRV) using selected cell culture vaccines have been established as an efficacious and economic alternative to the standard intramuscular (IM) regimens. IDRV has been successfully introduced for post-exposure prophylaxis (PEP) in developing countries such as India, Philippines, Srilanka and Thailand. It is estimated that in the absence of post-exposure prophylaxis, about 3,27 000 persons would die from rabies in Africa and Asia each year [2].

The World Health Organization (WHO) standard assay for determining antibody level is the rapid fluorescent focus inhibition test (RFFIT) and is used to determine the degree of immunity after vaccination against rabies. WHO has specified minimum titre of 0.5 IU/ml of serum as the protective level defining vaccine efficacy [11].

As many as 56% of vaccinees produced VNA beyond the protective level by D-7 even though the titre on D-0 was negligible in all of them. This emphasizes that RIG is not indicated later than D-7 to Category-III cases.

WHO states, to declare a regimen or vaccine to be adequately immunogenic, most vaccinees must achieve protective VNA level by day 14 of a post-exposure regimen, with or without simultaneous administration of rabies immunoglobulin and irrespective of age. The present series demonstrated 100% seroconversion rate by D14 both with or without RIG [9,10,12].

Rabies immunoglobulin(RIG) for passive immunization is administered at the beginning of rabies PEP, to previously unvaccinated persons to provide immediate antibodies until the patient responds to rabies vaccination by actively producing antibodies. If RIG is not given with the first dose of vaccine, it can be given up to Day 7 of the vaccine series. After Day 7, RIG should be avoided due to possible interference with the developing vaccine immune response. ERIG is administered at a dose of 40 IU/kg body weight for all age groups. No more than the recommended dose of

RIG should be used due its potential to partially suppress active immunization. As much as possible of the calculated dose of RIG should be infiltrated into the subcutaneous tissue and/or muscle around the wound site(s). Any remaining amount of RIG should be administered intramuscularly at an anatomic site distant from vaccine administration. RIG should never be administered in the same syringe or at the same anatomical site as vaccine. In the absence of a bite or other known site of virus introduction, the full dose of RIG should be administered at a site distant from vaccine administration. Regardless of the interval between exposure and initiation of PEP, both RIG and vaccine should be administered if indicated in persons not previously rabies immunized [2,5,9,13]. There is a theoretical probability of interference of VNA titre by the ERIG con-currently administered with IDRV to category –III subjects. A note of caution has been issued against excessive doses of RIG by Warrell et al [6]. Briggs et al had reported a lower VNA GMT in subjects receiving RIG[16]. But,the present series reflected no significant impact of RIG administration on the immunogenicity of IDRV. Seroconversion rate or GMT in category-II and III cohorts treated with IDRV alone or IDRV plus ERIG were comparable and similar with no statistically valid difference identified.

The immunogenicity against rabies persisted as late as one year since IDRV in all subjects in the present series as reflected by a GMT on day-365.Some other studies have reflected persistence of the protective GMT as late as up to 5years[15]. Thus the requirement of 1 or 2 boosting doses of PEP for all re-exposure cases need a re-look. As such WHO advocates, Post-Exposure Prophylaxis for previously vaccinated persons already received a full course of either Pr-EP or PEP regimen with ID/IM schedule of a potent CCV need to be administered two booster doses, intramuscularly (0.5ml/1ml)/intra-dermally (0.1 ml at 1 site) on days 0 and 3. Proper wound toilet should be done though treatment with RIG is not necessary [9].

TRC regimen involves 5 dosings (0,3,7,28,90 :2-2-2-1-1) of IDRV. It produced protective VNA as early as D-14 in almost all vaccinees under PEP. U-TRC regimen includes only 4 dosings (0,3,7,28:2-2-2-2).VNA response is still adequate and satisfies WHO mandate of seroconversion by D-14 in all the subjects.

In our study no deaths were reported amongst the study population till the one year study period indicative of the fact that IDRV dosing with 0.1ml of PVRV produced an antibody level sufficient for providing protection against this fatal disease.

Adverse reaction occurring at the site of injection, were mild and typical of those reported by other intradermal vaccine studies, and were resolved with treatment.

Conclusion

It can be concluded that PVRV is a highly immunogenic vaccine when administered in 0.1ml intra dermal doses in post exposure treatment of the WHO cat-II and cat- III patients, with immunogenicity reaching its acceptable levels by day 14. The WHO intra dermal regimen for post exposure treatment with PVRV requires only 15% of the amount of vaccine needed to treat a patient with IM regimen [1].

Recommendations: The intradermal regimen is an attractive option for physicians in developing countries with limited resources. By significantly reducing the cost of post exposure treatment in developing countries, more patients will be able to afford purified tissue culture vaccine, and will not have to suffer the vaccination reactions and failures associated with the older but less expensive nerve cell vaccines [7].The only bottleneck in utility ,acceptability and feasibility of the regimen happens to be the proficiency of the paramedical staff in the administration of intradermal vaccines besides an established cold chain.

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Conflict of Interest: The authors declare that the trail has been conducted as a phase – IV trail of PVRV Abhayrab manufactured by Indian Immunologicals Limited, India.

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