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Research Article

Safety and immunogenicity of intradermal rabies vaccination for post exposure prophylaxis

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ABSTRACT

Background: The affordability to anti-rabies vaccine (ARV) for intramuscular administration in post exposure prophylaxis (PEP) is a major constraint. Therefore, in countries, where there are financial constraints, WHO recommends intradermal rabies vaccination (IDRV) that reduces the quantity and cost of vaccination. The aim of the study was to evaluate the safety and immunogenicity of IDRV implemented under National pilot project.
Methods: A longitudinal study was conducted at anti rabies clinic (Government referral hospital), India where IDRV is implemented. The study included 515 animal bite cases who received PEP as recommended by WHO. ARV was administered intradermally using updated Thai red-cross regimen.
Results: The incidence of adverse drug events was 9.7% and all resolved without any complication. The geometric mean concentration of rabies virus neutralizing antibodies among the vaccinees was 11.89IU/mL on day 14, which was above the WHO recommended titers of ≥ 0.5IU/mL.
Conclusions: IDRV was found to be safe and immunogenic in PEP.

Keywords: Animal bites, Intradermal rabies vaccination, Safety, Immunogenicity

INTRODUCTION

Rabies is a fatal encephalitis that occurs in >100 countries throughout the world. It is transmitted to humans and other animals through close contact with saliva from infected animals i.e. bite, scratches, licks on broken skin and mucous membranes. Although a number of animals serve as vectors for transmission, dogs are the main source of human infections and poses a potential threat to >3.3 billion people worldwide.[1] Timely and correct post exposure prophylaxis (PEP) for these animal bite victims is necessary to prevent rabies. Proper wound management and simultaneous administration of rabies immunoglobulins (RIG) in all category III exposures combined with prompt administration of potent cell culture vaccines (CCV) is effective in preventing rabies, even after high-risk exposure.[2]

Since their development, more than four decades ago, CCVs have proved to be safe and effective in preventing

rabies. These vaccines are intended for both pre- and post-exposure prophylaxis and have been administered to millions of people worldwide.[3] But, the affordability to CCVs for intramuscular administration during PEP is a major constraint in developing countries of Asia and Africa. Therefore, World Health Organization (WHO) recommends intradermal route of vaccination with CCVs for these countries to reduce the quantity of vaccine and the cost of vaccination.[2]

In India, animal bites in humans are a major public health problem and an estimated 17.4 million animal bites occur annually which accounts to an incidence of 1.7%.[4] Considering the large number of animal bite cases in the country and huge demand for CCVs, there is a need to introduce intradermal rabies vaccination (IDRV). Therefore, following the recommendations of WHO and ICMR, the drug controller general of India (DCGI) approved intra dermal administration of rabies vaccines using updated TRC regimen in 2006.

Department of health and family welfare, Government of India, under 11th five year plan, implemented pilot project on prevention and control of rabies in humans in 5 cities through national centre for disease control (NCDC) as the nodal agency. The main strategy is to make IDRV operational and to facilitate wider coverage of PEP with the available quantity of vaccines and make PEP cost effective.[5] Bangalore is one of the project areas where it is implemented & Banashankari corporation referral hospital is one of the six centers in Bangalore, where pilot project is implemented. The present study was done to evaluate the safety and immunogenicity of intradermal rabies vaccination among animal bite victims in this hospital.

METHODS

This longitudinal study was conducted for a period of one and half years at anti rabies clinic, Banashankari corporation referral hospital, Bangalore, India where IDRV is implemented. 515 animal bite cases, who reported to the study centre for post exposure prophylaxis during the study period, were included in the study based on the inclusion and exclusion criteria. A thorough and detailed enquiry was done among all the study subjects to rule out taking any rabies vaccine either as pre exposure prophylaxis (PrEP) or PEP and history of any animal bite in the past. Similarly, any concomitant medical conditions / treatments were ruled out.

A standard case record form was maintained for each bite victim that included details of socio-demographic profile, type of exposure and post exposure prophylaxis provided at the study centre and the telephone number for further follow up.

The study was initiated, following clearance from the institutional ethics committee and was conducted in accordance with ICH - GCP guidelines. All the bite victims were given post exposure prophylaxis (PEP) including wound wash, anti-rabies vaccine and equine rabies immunoglobulin (ERIG) in all category III bites. Anti rabies vaccine was administered intradermally using updated Thai red cross regimen i.e., 2 doses of 0.1 ml vaccine given over both the deltoid muscle on days 0, 3, 7 and 28. The vaccines used during the period of study were Rabipur (Purified chick embryo vaccine, manufactured by Novartis vaccines) and Abhayrab (Purified vero cell rabies vaccine, manufactured by Human biological institute).

Assessment of safety

Following vaccination, all the subjects were observed for half an hour for possible immediate local/ systemic adverse drug events (ADEs). At the end of half an hour, reactogenicity was recorded, only if the subject spontaneously complained of a problem to a question on general wellbeing i.e., unaided recall. The subjects were given a follow up card to indicate if they had any late adverse events and was recorded in the subsequent visits i.e., on Day 3, 7 and 28.

Assessment of immunogenicity

Blood samples were drawn from a sub sample of 95 subjects who consented on Day 14 for estimation of rabies virus neutralizing antibody (RVNA). 5ml of venous blood was drawn from each patient under aseptic precautions and the sera were separated and tested for RVNA by rapid fluorescent focus inhibition test (RFFIT) at the Department of neurovirology, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India which is a WHO collaborating centre for reference and research on rabies.

Estimation of rabies virus neutralizing antibody (RVNA)

Modified RFFIT was done as per WHO recommended procedure. The cell line used was BHK 21 (ATCC CCL 10) and 96 well tissue culture plates (Sigma) and BHK21 adapted CVS 11 strain of rabies virus. The reference serum used was an in house serum calibrated against 2nd international reference standard having a titer of 30 IU/mL (obtained from National Institute of Biological standards, UK). Briefly, doubling dilutions of serum samples and reference serum (after heat inactivation at 56 C for 30 min in a water bath) in duplicate were made in 96 well plates using IMDM (Sigma Cat No.17633) To each 100 µl of serum dilution 100 µl of CVS (100 FFD_{50}) was added and the plate to was incubated at 37 C for one hour. A confluent monolayer of BHK 21 cells were trypsinized and re- suspended in 10 ml of IMDM with 10% FCS (Sigma, cat No. F2442). Cell control and virus controls were also included. To each well of the 96 well plates 100 µl of cell suspension was added and the plate was incubated at 37 C in a CO2 incubator (Sanyo, Japan). After 24 hours the cells were fixed in cold acetone for 30 minutes and stained by direct FAT using commercially

available rabies N conjugate (light diagnostics USA, Cat No. F199). The plates were then observed under an inverted fluorescence microscope (Nikon Eclipse). The highest dilution of serum showing 50% inhibition of fluorescence foci was taken as end point dilution. The titer was converted to IU/mL in comparison with reference serum.

All the study subjects were followed up for 1 year to know their survival status.

All the biting dogs could not be traced or caught for laboratory examination due to logistical difficulties.

Statistical analysis

The data was analysed statistically by computing percentages, geometric mean concentration (GMC), range, geometric standard deviation (GSD), Standard error (SE), 95% confidence interval (CI) for GMC.

RESULTS

All the belonged to lower or lower 515 animal bite victims were included in the study, majority of them were in the age group of 16-45 years (46.21%) followed by 7-15 years (27.77%) with the median age being 17.48 years and inter quartile range of 11- 37 years. 351 (68.16%) were males and 164 (31.84%) were females. Most of the study subjects i.e., 215 (41.75%) were illiterates and belonging to lower socio-economic status (62.91%) (Table 1).

Table 1: Socio demographic characteristics of animal bite victims.

Characteristics		Number	Percentage
Age	0 – 6	54	10.48
	7-15	143	27.77
	16 - 45	238	46.21
	46 – 60	52	10.10
	> 60	28	5.44
Sex	Male	351	68.16
	Female	164	31.84
Education	Illiterate	215	41.75
	Primary school	83	16.12
	Middle school	44	8.54
	High school	83	16.12
	Intermediate	48	9.32
	Graduate/Postgraduate Professional degree	37	7.18
		5	0.97
Socio-economic status*	Upper	12	2.33
	Upper middle	28	5.44
	Lower middle	142	27.57
	Upper lower	324	62.91
	Lower	9	1.75

*Modified Kuppuswamy Socioeconomic Status classification

In the present study, the biting animals were dog (97.28%), cat (1.55%) and monkey (1.17%). The site of bite was on lower limb (68.93%), upper limb (21.17%), trunk (6.60%), head and neck (2.72%) and multiple sites (0.58%). Majority (75.53%) of the animal bite victims had category III exposure. 67% of the study subjects had done wound wash with soap and water. None of the biting animal was followed due to logistical reasons (Table 2).

Table 2: Distribution of study subjects according to the details of exposure.

Characteristics		Number (n=515)	Percentage
Biting animal	Dog	501	97.28
	Cat	8	1.55
	Monkey	6	1.77
Type of dog	Pet dog	177	34.37
	Stray dog	324	62.91
Category of exposure	III	389	75.53
	II	126	24.47
Site of bite	Head & neck	14	2.72
	Trunk	34	6.60
	Upper limb	109	21.17
	Lower limb	355	68.93
	Multiple site	3	0.58
Type of wounds	Abrasion	251	48.74
	Laceration	56	10.87
	Puncture wounds	176	34.17
	Mixed wounds	32	6.21
Wound wash following bite	Yes	345	67
	No	170	33
Application of local irritant	Yes	79	15.34
	No	436	84.66

The incidence of adverse drug events (ADEs) was found to be 9.7%. The ADEs were pain at the site of injection, induration, erythema, itching & fever. All the ADEs were mild and resolved without any complications (Table 3).

The RVNA response among all the subjects on day 14 was adequate as per WHO recommendation of $\geq$0.5 IU/ml with the geometric mean concentration (GMC) of 11.89 IU/mL. All the study subjects were healthy and alive after one year of completing PEP (Table 4).

Table 3: Adverse drug events among the study subjects.

Adverse drug events	Number (%)
Erythema	11 (2.1%)
Itching	16 (3.1%)
Pain	11 (2.1%)
Induration	4 (0.8%)
Fever	8 (1.6%)
Total	50/515 (9.7%)

Table 4: Immunogenicity among the study subjects.

No. of subjects	GMC (IU/mL) (day 14)	GSD (IU/mL)	SE (GMC)	95% CI	
				Lower bound	Upper bound
95	11.89	1.32	0.20	11.50	12.28

GMC- Geometric Mean Concentration; GSD- Geometric Standard Deviation; SE-Standard Error, CI- Confidence Interval

DISCUSSION

India is highly endemic for rabies and has the largest number of animal bites in the world. Cell culture vaccines given intramuscularly are the main stay of PEP for animal bite victims. Intradermal administration of cell culture rabies vaccines offers an equally safe and immunogenic alternative to intramuscular rabies vaccination and requires less volume of vaccine and is recommended by WHO in resource constraint countries. Thus, intradermal rabies vaccination reduces the direct cost of vaccine by 60-80% compared with standard intramuscular vaccination.[1]

The present study showed that, intradermal rabies vaccination is safe for post exposure prophylaxis. Similar results were shown in a study conducted by Madhusudana et al with ADEs of 9.5% with the use of purified cell culture rabies vaccine (PCECV), and study by Sudarshan, et al with PCECV using KIMS regimen also showed a total ADEs of 3.1.[6,7] Similarly, Sampath et al also showed that, the total ADEs of 5.50% for purified vero cell rabies vaccine.[8] Therefore, the safety of study vaccines was comparable to other studies and it confirms that IDRV is safe for PEP among animal bite victims.

World Health Organization recommends a minimum RVNA titer of $\geq$0.5 IU/mL of serum for protection against rabies from day 14 onwards.[1] The objective of vaccination in post-exposure prophylaxis (PEP) is to stimulate the immune system to produce antibody titers of at least 0.5 IU/mL by day 14 and persist for a long time in animal bite cases. In the present study, all animal bite victims had protective RVNA titers of ≥0.5 IU/mL on day 14 with the Geometric Mean Concentration of 11.89. Similarly, a study conducted by Khawplod et al showed GMC titers of 9.14IU/mL on day 14.[9] Another study conducted by P Suntharasamai et al, showed the GMC of 9.07IU/mL on day 14, showing protective levels.[10] Similarly, a study conducted by Madhusudana et al using TRC regimen showed GMC of 4.3IU/mL on day 14.[6] In a study by Sudarshan, et al using KIMS regimen (2-2-2-2-2) also showed GMC of 4.17IU/mL on day 14.[7] The immunogenicity of the intradermal rabies vaccines in the present study were similar to other studies and it confirms the immunogenicity of IDRV among animal bite victims.

CONCLUSION

In conclusion, the present study showed that, the intradermal rabies vaccination using Updated TRC regimen (2-2-2-0-2) was safe and immunogenic as PEP in animal bite victims. Therefore, IDRV will have an important role in effective post exposure prophylaxis and thereby eliminating rabies in India and other Asian countries.

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