The study site was the Rabies Clinic of the Institute of Preventive Medicine, Hyderabad. It is a State government operated facility independent from the manufacturer of *Abhayrab.* The regular medical and nursing staff of this facility were the designers and investigators for this project. The Rabies clinic of this institute treats over 4000 patients for potential rabies exposures monthly. Approximately, 20-25 human rabies cases are also diagnosed annually. Estimates by local public health authorities are that approximately 5% of dog bite victims reporting for treatment have been bitten by rabid animals. The vast majority of patients receive Semple vaccine at no charge. Only a small proportion (1-2%) of Class III exposures are also given rabies immunoglobulin. This is due to the lack of funding and the fact that patients have to pay for this product which the majority cannot afford and also due to the fact that RIGs are not available throughout the year.

2. Materials and methods

The clinical study was approved by the Institutional Ethics Committee (IEC) of the Institute of Preventive Medicine, Hyderabad. The IEC took into consideration the fact that the vaccine was evaluated in non-exposed healthy individuals prior to being used in the present study.

Informed written consent was obtained from all the patients before enrolment in the study.

Study subjects were divided into four groups as follows:

Group I consisted of 60 healthy volunteers aged 20-56 years (mean 41) and with a male/female ratio of 52 : 8. They were given a course of pre-exposure rabies vaccine using *Abhayrab* 0.5 ml (one ampoule) intramuscularly into deltoid muscle on days 0, 7 and 21 (Table 1).

Group II patients consisted of 75 subjects with WHO Category II injuries [2] (mostly bites). They received *Abhayrab* 0.5 ml intramuscularly into deltoid muscle on days 0, 3, 7, 14, 30 and 90 without immunoglobulin.

Group III patients consisited of 67 subjects with WHO Category III injuries [2] (mostly bites) who received the same regimen as Group II and also without rabies immunoglobulin.

Group IV patients consisted of 88 subjects with WHO Category III injuries (multiple, facial and hand bites) [2] who also received equine rabies immune globulin (ERIG) 40 IU/Kg which was injected into and around bite wounds as recommended by WHO.

It is not known how many of the patients had actually been the victims of rabid animals since these were not available for testing.

The *Abhayrab* vaccine used was batch AYB 26/99, potency of 10.82 IU per 0.5 ml dose. The ERIG used was manufactured by Pasteur Merieux (Imorab) with a concentration of 200 IU/ml (Batch No. T5060-4).

Blood was collected for neutralizing antibody determination from all patients on days 0, 14, 30, 90 and 365. The sera of the volunteers, who received pre-exposure vaccination (Group I), were tested on days 0, 14, 35 and 365. Neutralizing antibodies were determined using coded duplicate samples and the Rapid Fluorescent Focus Inhibition Test (RFFIT) [5] at the Pasteur Institute of India, Coonoor. Results were decoded in the presence of Dr. A.M. Ghanekar by Dr. Suhasini V. Reddy.

Compliance among subjects in Group I (the volunteers) for the full course and blood collection was 35%. That for Groups II-IV was 65%, 52% and 64% respectively. None of the patients in Groups II-IV died of rabies by the end of the second year of follow up. Adverse side effects were mild to minimal and consistent with previous studies of other WHO recognized tissue culture rabies vaccines [6-14].

Antibody titers were above the WHO recommended protective level (0.5 IU/ml) [2] on days 14, 30, 90 and 365 (Table 1) except for one patient, a 33 year old healthy man who had a borderline low titer (0.46 IU/ml) on day 14. He had received ERIG and was found to have a titer of 2.00, 4.82 and 1.55 by days 30, 90 and 365, respectively.

3. Results and discussion

All Group I volunteers demonstrated a lasting immune response that was well above the 0.5 IU/ml WHO mandated minimum level on days 14, 35 and 365 (Table 1). Potentially rabies exposed patients in Groups II, III (without ERIG) and IV (with ERIG) also demostrated neutralizing antibody titers above the 0.5 IU/ml with the exception of one patient in Group IV. He had a lower titer on day 14 which rose to an acceptable levels by days 30, 90 and 365. It is noteworthy that geometric mean antibody titers (GMT) were 7.04, 9.47 and 8.42 IU/ml in Groups II-IV, respectively, by day 90 prior to the administration of the booster vaccine injection. The day 90 booster was administered as a safety measure since antibody titers were not yet known to the investigators at that time. It appears that the 90 day booster dose will not be required for *Abhayrab.* It had been used with other new tissue culture rabies vaccines in Europe and the Americas when they were first introduced but was later abondoned [15].

Adverse side effects were few and comparable or fewer to those seen with other tissue culture rabies vaccines [6-14].

Table 1
Serum neutralizing antibodies (RFFIT) in volunteers and patients bitten by dogs vaccinated with Abhayrab

Day	Group I	Group II	Group III	Group IV
0				
GMT	<0.05	<0.05	<0.05	<0.05
n	60	75	67	88
Range	–	–	–	–
n<0.5	60	75	67	88
7				
GMT	–	0.83	0.78	0.61
n	–	75	67	88
Range	–	<0.05-8.00	<0.05-4.20	<0.05-4.20
n<0.5	–	61	53	81
14				
GMT	12.69	13.53	15.78	10.34
n	56	72	65	82
Range	0.53-64.00	1.00-66.51	1.00-66.51	0.46-66.51
n<0.5	0	0	0	1
35 (Group I), 30 (Group II-IV)				
GMT	18.19	15.03	8.04	11.66
n	52	66	62	81
Range	2-32	2.10-64.00	1.50-32.00	2.00-64.00
n<0.5	0	0	0	0
90				
GMT	–	7.04	9.47	8.42
n	–	48	39	59
Range	–	1.60-33.33	1.05-56.51	1.05-66.51
n<0.5	–	0	0	0
365				
GMT	12.26	4.15	7.82	5.80
n	21	49	35	56
Range	0.56-32.00	1.05-31.11	1.05-66.51	0.92-66.51
n<0.5	0	0	0	0

The cut-off point for the RFFIT test was 0.05 IU/mL. N <0.5 are the number of subjects with antibody titers under 0.5IU/mL. Vaccination schedule : Group I: days 0, 7 and 21; Group II-IV: 0, 3, 7, 14, 30 and 90.

They consisted of minor irritations (0.2%) or mild pain at injection sites (4.06%). Only very few patients complained of systemic symptoms such as fatigue (0.33%), dizziness (0.06%), headache (0.13%) or mild fever (0.67%). All recovered with symptomatic treatment only (analgesics and antihistamines) and without having to abandon or alter the PET regimen. Among the group of 88 patients who also received ERIG on day 0, only one had an early local injection-site reactions (erythema and pruritis starting within 1 or 2 days). One subject had a mild serum sickness-like illness starting on day 8. Both resolved with symptomatic treatment; demonstrating again the safety of highly purified equine rabies immune globulin [16]. We conclude that Abhayrab, a new rabies vaccine developed in India, is a highly immunogenic and safe product.

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