Protective Antibodies after Vaccination with Human Diploid Cell Rabies Vaccine*

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SUMMARY **The level of protective antibodies after pre-exposure prophylaxis through human diploid cell rabies vaccination was determined using the standard mouse neutralization test. In subjects who were not previously vaccinated against rabies, the antibody level after immunization with 1 millilitre of vaccine intramuscularly on days 0, 7 and 28 was higher than in those who received 0.1 millitre intracutaneously at four sites on day 0, followed by single-site injections on days 3 and 7. To achieve a booster effect, one injection, either one millilitre intramuscularly or 0.1 millilitre intracutaneously, gave guite similar results. However, the antibody levels of both groups were higher than in those who had received a full course of Semple or suckling mouse brain rabies vaccines for post-exposure prophylaxis.**

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Rabies is still an unresolved public health problem in Thailand. Approximately 300 cases of human deaths from rabies have been recorded annually since 1957.[1] About 30,000 people are treated each year at Thai Health Authorities Services for post-exposure prophylaxis against rabies,[2] in addition to an unknown number of patients who visit private clinics for the same purpose. Three types of rabies vaccine for humans are now available in this country, viz., the locally prepared 5% sheep brain or Semple vaccine, 2% suckling mouse brain (SMB) or Fuenzalida vaccine, and the imported human diploid cell strain vaccine (HDCV) of Institut Merieux, France. Among these, HDCV has gained wide acceptance for providing greater immunogenicity and fewer adverse reactions.[3-5] HDCV has been recommended for use in two or three doses subcutaneously (s.c.) or intramuscularly (i.m.) for pre-exposure prophylaxis and five to six doses for post-exposure prophylaxis.[6] The smaller doses given intracutaneously (i.c.) also induced high titres of neutralizing antibody.[7-9]

In this study, the kinetics of protective antibodies induced by HDCV in full doses and in small doses for pre-exposure prophylaxis were studied for the purpose of safety surveillance, and therapeutic implications.

MATERIAL AND METHODS

Subjects

All subjects vaccinated came from a group of healthy hospital personnel whose work exposes them to risk of rabies contact either in the laboratory or in patients' wards.

Group 1. *Primary immunization using conventional methods.* Four male and six female subjects, 18 to 40 years of age (mean 28 ± 6) who did not previously receive any rabies vaccination were injected with HDCV 1 millilitre intramuscularly in the deltoid area on days 0, 7 and 28. Blood samples were taken on days 0, 7, 14, 28 and 56.

Group 2. *Primary immunization with small intracutaneous doses.* Ten female student nurses, 18 to 20 years of age (mean 19 ± 0.5) with no history of rabies vaccination; they were injected with HDCV 0.1 millilitre intracutaneously at four sites on the volar surface of both arms and the anterior aspects of both thighs on day 0, followed by 0.1 millilitre intracutaneously at one volar site on days 3 and 7. Blood specimens were taken on days 0, 7, 14, 28, 98, 210 and 364.

Group 3. *Booster vaccination with one conventional dose.* Five male and 4 female subjects, 24 to 32 years of age (mean 33 ± 7) who had a history of immunization with HDCV (pre-exposure prophylaxis of two doses on days 0 and 28 a year or more previously) were given one booster injection of HDCV 1 millilitre intramuscularly in the

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Table 1 Neutralizing antibodies following HDCV vaccination

Group	No. of subjects	Neutralizing antibodies (IU/ml) on specific days							
		0	7	14	28	56	98	210	364
1	10	< 0.01	0.01 – 0.04 (0.03 ± 0.01)	2.97 – 130.86 (49.46 ± 37.12)[a]	23.76 – 150.86 (82.54 ± 37.91)[b]	59.87 – 190.07 (127.29 ± 32.00)	–	–	–
2	10	< 0.01	0.01 – 0.02 (0.02 ± 0.00)	1.18 – 14.97 (7.15 ± 4.54)	8.40 – 37.71 (21.12 ± 9.82)	–	8.18 – 17.25 (12.06 ± 3.52)	2.04 – 9.43 (4.49 ± 2.75)	1.48 – 4.31 (2.64 ± 1.17)
3	9	0.51 – 3.74 (1.97 ± 1.06)	11.88 – 38.71 (20.01 ± 8.85)	32.72 – 69.01 (49.36 ± 11.43)	59.87 – 130.86 (77.25 ± 24.03)	–	–	–	–
4	10	1.48 – 4.31 (2.64 ± 1.17)	11.88 – 23.76 (16.71 ± 4.17)	32.72 – 69.01 (45.42 ± 13.15)	29.93 – 95.03 (62.22 ± 16.96)	32.72 – 69.01 (45.57 ± 11.42)	–	–	–

a and b showed a statistically significant difference compared with groups 1 and 2 ($p < 0.01$)

deltoid area. Blood specimens were taken on days 0, 7, 14 and 28.

Group 4. *Booster vaccination with small intracutaneous dose.* The subjects of group 2 were re-vaccinated with 0.1 millilitre of HDCV intracutaneously as a yearly booster. Blood specimens were collected on days 7, 14, 28 and 56 after injection.

For comparison, sera from people who received Semple or suckling mouse brain rabies vaccines as post-exposure prophylaxis were also studied in parallel as groups 5 and 6.

Group 5. *Primary vaccination with the full course of Semple vaccine.* This group consisted of one female and 14 male subjects, 36 to 59 years of age (mean 44 ± 9). They received daily 2 millilitres of Semple vaccine subcutaneously for 14 days and a booster on days 24, 34 and 104.

Group 6. *Primary vaccination with the full course of suckling mouse brain vaccine.* Subjects comprised two females and 16 males, 22 to 55 years of age (mean 47 ± 9). The schedule of immunization was similar to that of group 5, but 1 millilitre of suckling mouse brain vaccine was used instead.

The sera from subjects in groups 5 and 6 were examined on days 0, 31 and 104.

Antibody determination

All sera were kept at -20°C until rabies antibody titres were performed using the standard mouse neutralization test[10] with the 100 LD_{50} CVS strain as the challenged virus. The neutralizing antibody titres were calculated to international units per millilitre (IU/ml) by reference to standard antiserum (State Serum Institute, Copenhagen, Denmark) included in all tests.

Vaccines

HDCV from the Institut Merieux (lots W 0527 and V 0505) was used for vaccination. Semple and suckling mouse brain vaccines were from the Pharmaceutical Organization of Thailand.

The study was conducted during the period 1981-1983.

RESULTS

The geometric mean titres, standard deviation and range of NT Ab calculated to IU/ml in all groups are as shown in Tables 1 and 2.

In the groups 1 and 2 that received primary immunization with HDCV, none of the subjects had any rabies antibodies on day 0. An acceptable level of NT Ab was detected on day 14 in both groups. Group 1 (HDCV 1 ml, i.m. on days 0, 7 and 28) showed significantly higher antibody levels than group 2 (0.1 ml, i.c. x 4 on day 0 and 0.1 ml i.c. on days 3 and 7). On day 28, after two injections, NT Ab in group 1 was 82.54 ± 37.91 IU/ml and after the third injection, NT Ab on day 56 was 127.29 ± 32.

In comparison with group 2, after the last injection on day 7, NT Ab reached a peak of 21.12 ± 9.82 IU/ml on day 28 and then declined (as shown) in follow-up tests made 3, 7 and 12 months later. However, after one year, all subjects still had an antibody level higher than the protective level (Figs. 1 and 2).

In the groups that received booster immunization, the subjects in group 3 had the antibody level on day 0 ranged from 0.51 to 3.74 IU/ml. Subjects in group 4 (previously group 2) who, before receiving the

Table 2 Neutralizing antibodies after Semple and suckling mouse brain post-exposure prophylaxis.

Group	No. of subjects	Neutralizing antibody (IU/ml) on specific days		
		0	31	104
5	15	< 0.01	0.19 – 9.43 (3.07 ± 2.40)	0.09 – 1.08 (0.34 ± 0.30)
6	18	< 0.01	0.23 – 11.84 (2.79 ± 3.33)	0.19 – 1.08 (0.42 ± 0.33)

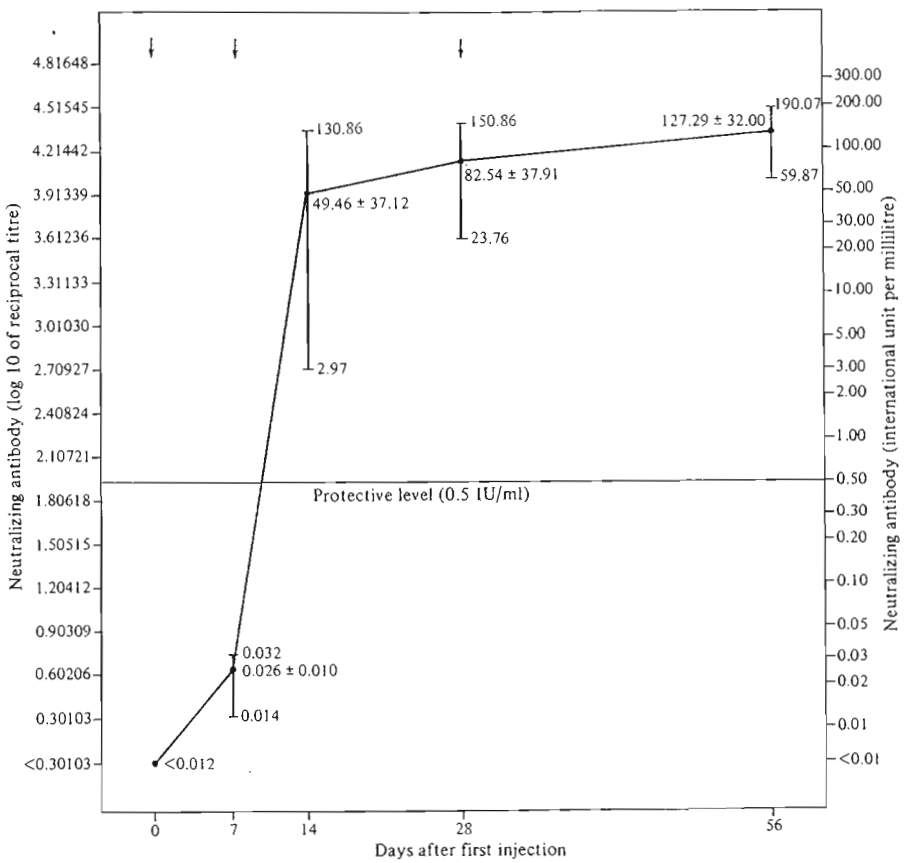

Fig. 1 Neutralizing antibody response of 10 subjects (group 1) after primary immunization with three intramuscular doses of 1.0 ml of human diploid cell rabies vaccines (Institut Merieux, France) on days 0, 7 and 28. (Arrow indicates day of injection)

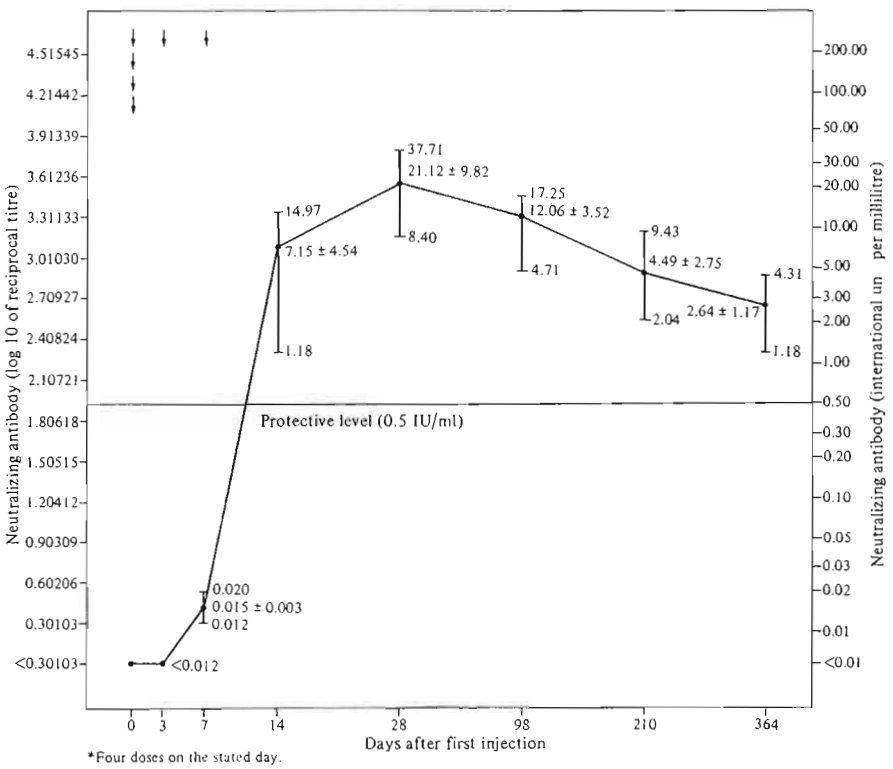

Fig. 2 Neutralizing antibody response of 10 subjects (group 2) after primary immunization with six intracutaneous doses of 0.1 ml of human diploid cell rabies vaccine (Institut Merieux, France) on days 0*, 3 and 7. (Arrow indicates day of injection)

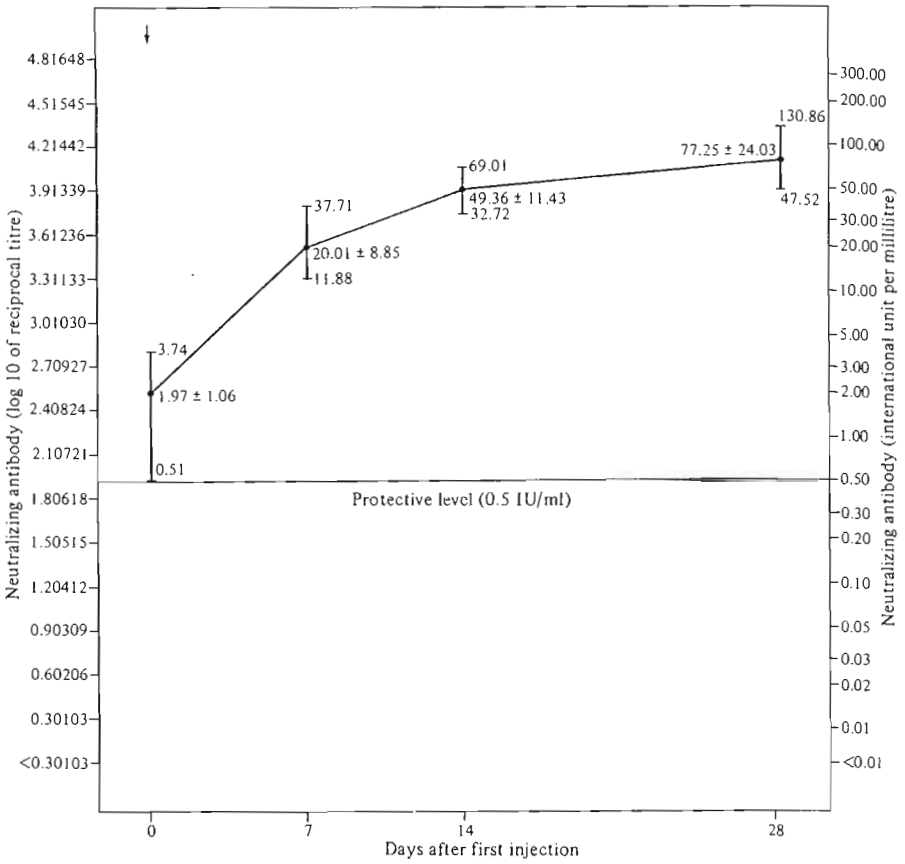

Fig. 3 Neutralizing antibody response of nine subjects (group 3) after booster immunization with one intramuscular dose of 1.0 ml of human diploid cell rabies vaccine (Institut Merieux, France) on day 0. (Arrow indicates day of injection)

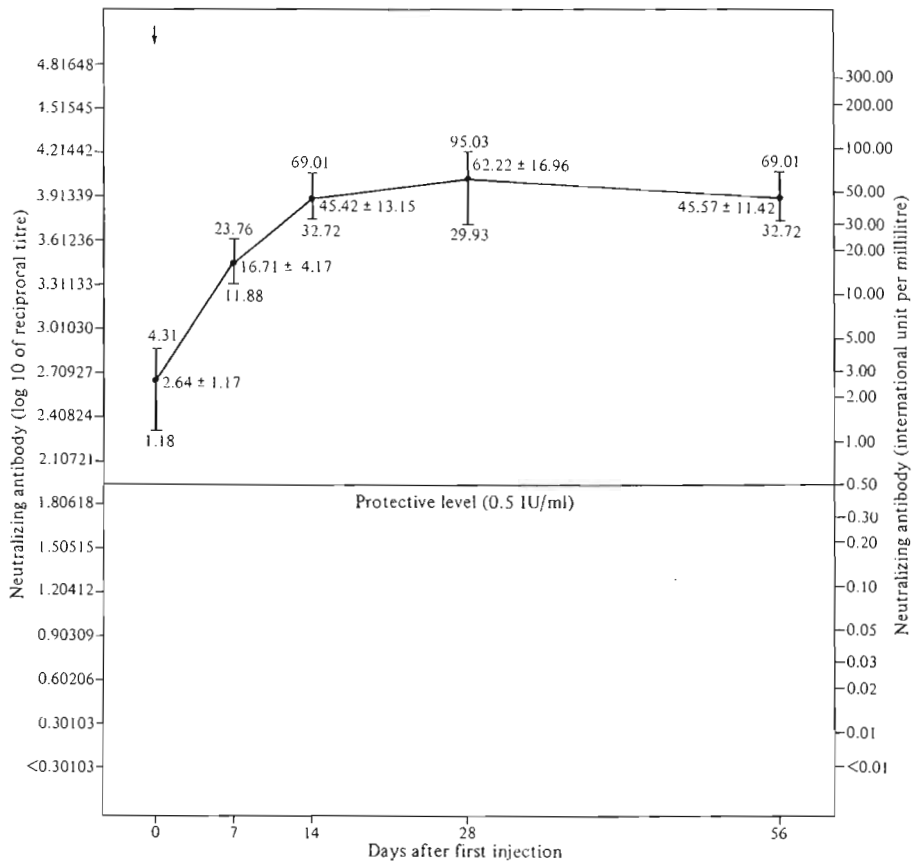

Fig. 4 Neutraliing antibody response of 10 subjects (group 4) after booster immunization with one intracutaneous dose of 0.1 ml of human diploid cell rabies vaccine (Institut Merieux, France) on day 0. (Arrow indicates day of injection)

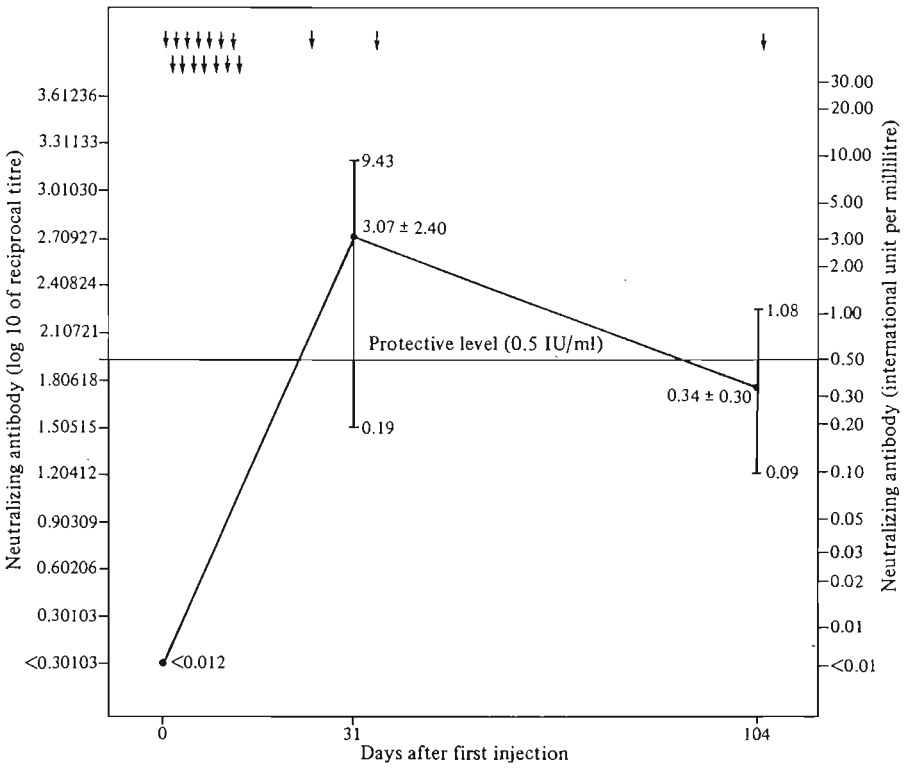

Fig. 5 Neutralizing antibody response of 15 subjects (group 5) after primary immunization with 14 daily doses of 1.0 millilitre Semple rabies vaccine (local products) and booster immunization given in three doses on days 24, 34 and 104. (Arrow indicates day of injection)

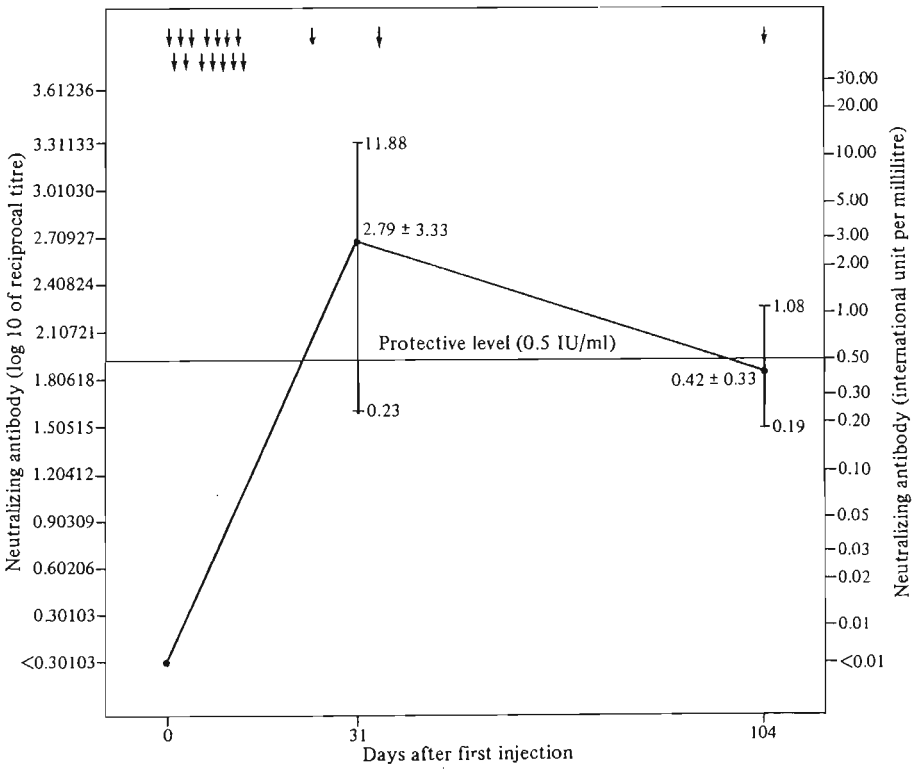

Fig. 6 Neutralizing antibody response of 18 subjects (group 6) after primary immunization with 14 daily doses of 1.0 millilitre of suckling mouse brain rabies vaccine (local products) and booster immunization given in three doses on days 24, 34 and 104. (Arrow indicates day of injection)

booster, had antibodies ranging from 1.48 to 4.31 IU/ml. After one booster dose, a satisfactory level of anamnestic response was shown on day 7, and reached a peak in four weeks (Figs. 3 and 4).

In comparison with the antibody levels in groups 5 and 6 following their post-exposure course with Semple or suckling mouse brain rabies vaccine, those immunized with HDCV showed superior results. In the Semple vaccine group, the antibody levels of one subject on day 31 and eight subjects on day 104 were lower than the protective level; antibody levels were 3.07 ± 2.40 and 0.34 ± 0.30 IU/ml respectively. In the suckling mouse vaccine group, antibody levels were 2.79 ± 3.33 and 0.42 ± 0.33 IU/ml on days 31 and 104 respectively. An unacceptable level was found in four subjects on day 31 and in 12 subjects on day 104. The trends of antibody response are shown in Figures 5 and 6.

DISCUSSION

The measurement of neutralizing or protective antibodies using mouse neutralization or rapid fluorescent focus inhibition (RFFIT) tests[11] is currently the only available indicator for evaluating the protective immune response. The results of both these tests are comparable. The methods of assessing the role of other immune mechanisms, i.e. cell mediated immunity and interferon in providing protection from rabies has not yet been established.

Our results are in agreement with findings in the United States which showed the effectiveness of immunization with small intracutaneous or subcutaneous doses.[12] The antibody levels of our subjects were much higher than those observed in previous reports; this could be due to the use of a different method, mouse neutralization versus RFFIT, or to ethnic differences. However, our results confirmed the previous studies, strongly suggesting that HDCV can be used intracutaneously or subcutaneously for primary pre-exposure immunization and intracutaneously for obtaining booster response.[12,13]

In developing countries where rabies is endemic, wide-scale use of pre-exposure prophylaxis in this economical way should be considered for persons at high risk of exposure to rabies.

In post-exposure treatment, the aim is to generate the rapid production of a high antibody level that is maintained throughout the possible incubation period. Regarding how high and how long that level should be, the World Health Organization recommends that post-exposure rabies vaccination should be aimed at achieving a neutralizing titre of 0.5 IU/ml;[14] in about 10 to 20 per cent of rabies patients, the incubation period is longer than three months.[15,16]

Our results show that the use of HDCV meets these criteria even in the group receiving small doses while those immunized with Semple and suckling mouse brain vaccine did not. Successful post-exposure treatment using multiple-site intracutaneous HDCV has been reported recently [17] While waiting for the development of less expensive tissue culture vaccine,[18] economical intracutaneous HDCV regimens might be introduced carefully in more post-exposure trials.

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