Original Article

ANTIBODY TITERS IN ANIMAL BITE VICTIMS AFTER POST EXPOSURE VACCINATION WITH INTRADERMALLY ADMINISTERED PURIFIED VERO CELL RABIES VACCINE USING MODIFIED THAI RED CROSS REGIMEN

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Abstract

Objective: To determine the seroconversion following rabies vaccination by intradermal route in cases of animal bite attending Anti rabies center, Lahore for post exposure prophylaxis.

Study Design: Cross sectional descriptive study.

Place and Duration: Antirabies center, Birdwood road Lahore, Microbiology laboratory, office of Bacteriologist, Government of Punjab, Lahore.

Patients and Methods: Victims of all ages and both sexes having exposure with suspected rabid animal within 24 – 72 hours were included, fulfilling inclusion and exclusion criteria, over 3 months period from February to April 2011. Patients of Category II and III wounds were included. Purified vero cell vaccine (PVRV) with antigenic content > 2.5 ml was used for intradermal vaccination according to modified Thai Red Cross regimen (2-2-2-0-2). Each victim received 0.1 ml intradermal dose on each deltoid on day 0, 3, 7 and 28th day of bite. Blood samples from victims were taken on day 0, 14 and 35. Antibody titers were estimated by ELISA kit.

Results: Fifty cases were studied including 20 children. Male female ratio was 4:1. Optimum seroconversion (> 0.5 IU/ml) was achieved in all cases by day 14. Antibody levels increased further (> 4 IU/ml) in 92% cases on day 35. Geometric mean titers were 3.2 IU/ml and 6.2 IU/ml on day 14 and 35 respectively.

Conclusion: Intradermal route for cell culture rabies vaccine for postexposure prophylaxis in animal bite victims was efficacious and safe. The smaller dosage of vaccine was economically affordable by patients in referral centers.

Key words: Rabies, intradermal vaccine, Thai Red Cross regime, Purified vero cell vaccine.

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Introduction

Rabies is a fatal zoonotic disease transmitted to humans and other animals by the bite of an infected animal. The causative agent is neurotropic virus of the Rhabdoviridae family present in the saliva of the rabid animal.[1-3] More than 55000 people die of this disease worldwide annually.[2,3] Majority of these cases occur in

developing countries.[3] In Pakistan rabies is an under reported disease. Unfortunately no community based data on incidence of rabies is available, yet an estimated figure reports 7 to 9.8 cases per million population per year from Karachi.[4] Few known studies on epidemiology of rabies in Pakistan.[5-7] Reveal that the incidence of animal bite cases is also rising alarmingly. According to a WHO report (2010), recorded cases of dog bite from the basic health units of Pakistan were more than 97000.[8]

For more than 20 years WHO has recommended the replacement of nervous tissue vaccine(NTV) with more efficacious and safer cell/tissue culture vaccines (TCV) for post exposure prophylaxis (PEP).[9] Nervous tissue vaccines have been found to produce excessive local reactions at the site and are less immunogenic.[10] However these vaccines are still widely used in the country.[7] Tissue culture vaccines are also available in Pakistan yet these vaccines are quite expensive and are not affordable by majority of the animal bite victims who belong to the poor socio economic class.

WHO currently recommends intrademal regimens with cell culture vaccines used for IM route in countries where both availability and / or affordability of rabies vaccines is limited.[10,11] This method is specially recommended for post exposure prophylaxis in specialized centres where a large number of post exposure treatments are given each day.[11] Two intradermal (ID) vaccination regimens for PEP are currently recommended by WHO. These include 8-0-4-0-1-1 schedule also known as "Oxford regimen" for the use with Human diploid cell vaccine(HDCV) and purified chick embryo cell vaccine (PCECV) and the 2-2-2-0-1-1 regimen, also known as Thai Red Cross regimen to be used with purified vero cell vaccine (PVRV) and purified chick embryo cell vaccine (PCECV) (11,12). Thai Red Cross regimen consists of intradermal injection of 0.1 ml of PVRV or PCECV at two locations (one in each upper deltoid region) on day 0, 3, 7 and one at day 28 and one at day 90. Only the WHO recommended vaccines that full fill the criteria of immunogenicity, safety and efficacy are recommended for PEP by intra dermal route.[12,13] Regional studies conducted earlier have proved the immunogenicity and safety of intradermal regimens of modern cell culture vaccines.[16-20] On the basis of these studies, many Asian countries have adopted the use of intradermal route for PEP and have successfully incorporated it into their national rabies prevention programs.[10] Although cell culture vaccines are being administered at few public and private sector hospitals by intradermal route,[7] yet there is no data available regarding the efficacy and safety of these vaccines in Pakistani population.

Anti rabies centre, Institute of Public Health, Lahore is the largest centre of Punjab, dealing with nearly 11000 new cases of animal bite per year. Cell culture vaccines were introduced in affording patients by intramuscular route (Essen regimen)[13,14] few years ago. However due to financial constraints, only few affording patients could get cell culture vaccine. Majority of patients were immunized with NTV. Intradermal route with TCV was introduced recently. The present study was aimed to determine the antibody titer in the recipients of cell culture vaccine by ID route.

Objective

To determine the seroconversion following rabies vaccination by intradermal route in cases of animal bite attending Anti rabies center, Lahore for post exposure prophylaxis.

Study Design

Cross sectional descriptive study.

Materials and Methods

Patients included in study were chosen amongst the victims of animal bites attending Anti-rabies Centre, office of Bacteriologist, Govt. of the Punjab, Institute of Public Health, Lahore for post exposure treatment (PET). Victims of all ages and both sexes having exposure with suspected rabid animal within 24 – 72 hours were included in study. Patients were examined by the doctor in-charge of the centre and wounds were categorized according to WHO Criteria into Category I, II or III.[13,14] Patients of Category II and III wounds were included in the study. Decision to administer vaccine alone or vaccine and Rabies immune globulin (RIG) was made according to the category of the wound. Pregnant women and patients having history of previous rabies immunization, acute infectious disease, immunosuppressive therapy or receiving immunoglobulins previously were excluded from the study.

Informed written consent was obtained from all victims or their parents in case of young children. Information regarding age, sex, body weight, time since exposure to the animal, specie of animal, its status whether killed or alive were recorded on a performa.

Post Exposure Treatment (PET)

Wounds were thoroughly washed with soap and water. Purified vero cell vaccine (PVRV) (Sanofi Aventis) purchased from the market was used. Antigenic content was > 2.5 unit / 0.5 ml as per label. The vaccine was reconstituted with 0.5 ml of diluent provided by the manufacturer and was used within six hours of reconstitution. Reconstituted vaccine was kept in a refrigerator at 4°C. Any left – over reconstituted vaccine was discarded at the end of the clinic time. A staff nurse and a vaccinator were trained for intra dermal technique. Vaccine was administered according to modified Thai-Red Cross (TRC) regimen (2,2,2,0,2).

Each victim received 0.1 ml intradermal dose on each deltoid on day 0, 3, 7 and 28th day of bite.[18] Modified Thai Red Cross regimen was used eliminating day 90 dose / booster and replacing it with 2 doses each 0.1 ml on both deltoids on day 28. This schedule had already been used in earlier study to avoid poor compliance on day 90.[15]

Single intradermal dose of the vaccine 0.1 ml (PVRV) is 1/5th of reconstituted vaccine (0.5 ml). In this way 2 vials of PVRV sufficed for 5 intradermal doses (for 5 persons) on any one day. In total, 2 vials of vaccine were required for each victim to complete the Thai Red Cross regimen in comparison with 5 vials used in IM Essen regimen. In young children injections were given in anterolateral thigh**.** All patients with Category III wound were advised RIG as well.

Sample Collection and Detection of Anti Rabies Antibodies

Blood samples (5 ml venous blood) from victims were taken on day 0, 14 and 35. All the victims were instructed to report on day 14 and day 35 so that the blood samples could be taken on these days. Sera were separated, labeled, coded and stored at –20°C till further analysis.

Three patients had titres up to the safe level on day 0 without H/O exposure or immunization, and thus excluded. Five patients did not report on day 14 and were also excluded. Fifty cases successfully completed the study.

Evaluation of Rabies Antibodies

Rabies virus antiglycoprotein antibodies were detected by indirect immunoenzymatic technique using commercially available ELISA kit (Platellia Rabies II kit, Bio-Rad Germany). The assay was performed in Microbiology laboratory, O/O Bacteriologist, Govt. of Punjab, Lahore. Negative and positive controls were run and the optical density (OD) readings were validated for quality of detection. For quantitative analysis of the patient samples, a reference curve was constructed using the corrected ODs of quantification standards and used to calculate the titers of unknown sera.

Results

Study cases (n = 50) were categorized into age group of children 3 – 15 years of age,[20] adults 16 – 50 years of age[25] and age > 50 years.[5] There were 40 male and 10 female cases. Twenty nine (58%) victims had category II bites, whereas 21 (42%) had category III wounds (Table 1). More than 80% of all wounds were inflicted by dogs. However other animals implicated were cats, horse, donkey, cow and monkeys. All the subjects developed titers above 0.5 IU/ml by day 14.

Table 1:
Patient demographics and wound Category (n = 50).

Age Group	No. of Victims	Male / Female Ratio	Wound Category	
			Category II	Category III
Children (3 – 15 yrs)	20	17: 3	11	9
Adults (16 – 50 yrs)	25	18: 7	14	11
Older Age Group > 50 years	5	5:0	4	1
Total	50	40:10	29	21

Table 2: Anti rabies antibody titres on different days in study cases (n = 50).

Antibody Titre IU/ml	Day 0 No. of Cases (%)	Day 14 No. of Cases (%)	Day 35 No. of Cases (%)
< 0.5	50 (100%)	0	0
0.5 – 0.9	0	6 (12%)	0
1 – 3.9	0	36 (72%)	4 (8%)
4 and above	0	8 (16%)	46 (92%)

Table 3: Geometric mean titres of rabies antibodies (n = 50).

Day	Geometric mean titre IU/ml	Seroconversion (%)
0	0.19	0
14	3.2	100
35	6.2	100

Detection of antibodies on day 35 showed further improvement in the titers (Table 2). Geometric mean titer on day 14th after first intradermal dose was found to be 3.2 IU/ml and 6.7 IU/ml on day 35 (Table 3).

Minimal local adverse reactions were observed in the form of erythema (5 cases) and induration (4 cases) settling within three days.

Rabies immunoglobulin (RIG) was advised to all cases in category III wounds; however it was administered only in nine patients. Twelve patients did not receive RIG due to non affordability.

Patients were followed up for 3 months; all exposed cases remained healthy.

Discussion

WHO recognizes a level of ≥ 0.5 IU/ml being an adequate level of virus neutralizing antibodies for the protection against rabies which should be achieved by day 14 of vaccination.[13] In the present study all the victims of animal bite vaccinated intradermally with 0.1 ml of PVRV achieved antibody titres above 0.5 IU/ml by day 14. The antibody titres further increased on day 35. These findings were in agreement with findings reported earlier. Thai Red Cross intradermal PET schedule was assessed by Chutivongse et al[16] in severely bitten and proven rabid animals using PVRV. Antibody titre determination in a randomly selected sub group showed seroconversion in all 10 patients by day 14. All patients were followed for one year post exposure. There were no deaths and the efficacy of the regimen was found to be hundred percent. Briggs et al[17] reported rabies anti body titre > 0.5 IU on day 14 in subjects of study group who received PVRV intradermally by Thai Red Cross regimen (TRC). Khwaplod et al[18] also observed adequate neutralizing antibody titres by day 14 employing 2 site and 8 site regimens. They also reported that by either method, safe titres were acquired by day 14, yet significantly higher titres were not observed on day 5 and 7 by either regimen. They suggested that administration of RIG around wound remains an important component of PET on first day of severe exposures. Madhusudana et al[19] also reported immunogenicity of TRC post exposure vaccine regimen in Indian population using both PVRV and PCECV reporting safe seroconversion levels on day 14 and further onwards with minimal side effects. Warrell et al[21] also reported antibody levels > 0.5 IU/ml in the study group receiving PVRV as per TRC schedule. Ambrrozaits et al[22] evaluated a new four site intradermal schedule (4-0-2-0-1-1) in healthy subjects using PVRV and PCECV also reported that all subjects developed rabies virus neutralizing antibody (RVNA) titres above 0.5 IU/ml by day 14.

Tentawichein et al[23] however reported failure of the multiple intrademal site regimen in HIV patients with low CD_4 count and recommended IM regimen or further boosters of ID doses following evaluation of VNA titres.

In the present study, only 9/21 patients of category III wounds received RIG infiltrated around the wound. Antibody titres in these patients were low as compared to other sera on day 14 but remained within protective level. These findings are similar with those reported by Briggs et al.[17]

In the present study, sera were evaluated for the presence of rabies virus antibodies using commercially available ELISA test. The titres in the present study were comparable to results of the study by Mala et al[20] who also reported immunogenicity of TRC post exposure vaccine regimen by ELISA method using PCE-

CV. Welch et al[25] reported a low degree of variability between ELISA assay and rapid fluorescent focus inhibition test (RFFIT) except in samples with high RFFIT value. Simani (26) however reported lower mean titres of rabies antibodies when the test was performed by ELISA as compared to RIFFIT. Although RIFFIT and fluorescent antibody virus neutralization (FAVN) tests are considered gold standard for detection of rabies antibodies, performance of these tests however requires specialized reference laboratories with expertise in these assays which was not available in our centre.

The economical advantage of using intradermal regimen was quite evident, as only two vials of the vaccine (PVRV) were required for each patient as opposed to five vials per patient if the PET was given by intramuscular Essen schedule. Similar advantage has been observed with the usage of other WHO recommended vaccines given intradermally.[17,19,20,26] ID route reduces the cost of PEP to 60%, thereby making it affordable to the dog bite victims who mostly belong to poor socio economic strata in developing countries like Pakistan. These ID regimens require sharing of vaccine ampoules between patients, therefore these regimens can be successfully used in referral centers.

It is concluded that intradermal regimen for administering anti rabies vaccine was efficacious, giving adequate protective antibody titers in recipients as postexposure prophylaxis. The economical advantage of small dosages of vaccine used by ID route makes it affordable and feasible in a referral center in a developing country like Pakistan. Based on our findings, it is recommended that nervous tissue vaccine may be replaced by cell culture vaccine by intradermal route in referral centers at national level. Furthermore, effective dog control strategies should be implemented to decrease the rabies reservoir.

References

1. What is rabies?
2. WHO rabies fact sheet updated July 2013.
3. Yousaf MZ, Qasim M, Zia S, et al. Virology Journal, 2012, 9: 50 page 1-5.
4. Wasay M, Salahuddin N, Khatri IA. Tetanus and rabies eradication in Pakistan; a mission not impossible. JPMA, 2008 April; Vol. 58 (4): 158-159.
5. Zaidi SMA, Labrique AB, Khowaja S, et al. Geographic Variation in Access to Dog-Bite Care in Pakistan and Risk of Dog-Bite Exposure in Karachi: Prospective surveillance using a low – cost Mobile Phone System. Negl Trop Dis. Dec. 2013; 7 (12): e2574.
6. Iqbal MS, Rana MN, Saeed M, et al. Epidemiology of dog bite cases visiting dog bite centre Lahore. PPJ. 2003; Vol. 27 (1): 5-8.
7. Salahuddin N, Jamali S, Ibraheem K, et al. Awareness about Rabies Post Exposure Prophylaxis in Pakistan among Patients and Health Care Workers: Results from an Asian Rabies Expert Bureau Study, JCPSP. 2011, Vol. 21 (8): 491-494.
8. WHO Pakistan Rabies control programme WHO Eastern Mediterranean.
9. Eighth report of the WHO expert Committee on Rabies, Geneva World Health Organization, 1992; 24 – 25 (WHO Technical Report Series, No. 824).
10. WHO expert committee on rabies second report (WHO Technical Report Series, No.982) 2013.
11. WHO Expert Consultation on rabies; First report Geneva WHO 2005 (Technical Report Series No. 931).
12. WHO report of informal discussions on intradermal application of Modern rabies vaccines for human post exposure treatment, Geneva 22 Jan 1993 (TRS 824).
13. WHO Guide for post exposure prophylaxis, 2014.
14. WHO Guide for post exposure prophylaxis, 2010.
15. Khawplod P, Wilde H, Sirikwin S, et al. Revision of Thai Red Cross intra-dermal rabies post exposure regimen by eliminating 90 day booster injection. Vaccine, 2006 Apr 12; 24 (16): 3084-6.
16. Chutivongse et al. Post exposure prophylaxis for rabies with antiserum and intradermal vaccination. Lancet. 1990 April 14; 335 (8694): 896-8
17. Briggs DJ, Banzhoff A, Nicolay U, et al. Antibody response of patients after post exposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. Bulletin of WHO, 2000; 78 (5): 693-8.
18. Khwaplod P, Wilde H, Tepsumethanon S, et al. Prospective immunogenicity study of multiple intradermal injections of rabies vaccine in an effort to obtain an ealry immune response without the use of immunoglobulin. Clin Infect Dis. 2002 Dec. 15; 35 (12): 1562-5.
19. Madhusudana SN, Sanjay TV, Mahendra BJ, et al. Comparison of safety and immunogenicity of purified chick embryo cell rabies vaccine (PCECV) and purified vero cell rabies vaccine (PVRV) using the Thai Red Cross intradermal regimen at a dose of 0.1 ML. Hum Vaccine, 2006 Sep-Oct;2(5):200-4.
20. Mala C, Ichhpujani RL, Bhardwaj M, et al. Safety and immunogenicity of the intradermal Thai red cross (2-2-2-1-1) post exposure vaccination regimen in the Indian population using purified chick embryo cell rabies vaccine. IJMM. 2005 Jan – March; 23 (1): 24-28.
21. Warrell MJ, Riddell A, Mee Yu L, et al. A simplified 4-site economical intradermal Post exposure rabies vaccine regimen: A randomized controlled comparison with standard methods. Neg Trop Dis. 2008 April; 2 (4).

22. Ambrozaitis A, Laiskonis A, Balciuniene L, et al. Rabies post exposure prophylaxis vaccination with purified chick embryo cell vaccine (PCECV) and purified vero cellrabies vaccine (PVRV) in a four – site intradermal schedule (4-0-2-1-1), An immunogenic, cost effective and practical regimen. Vaccine, 2006 May 8; 24 (19): 4116-21.
23. Tentawichein T, Jaijaroensab W, Khawplod P, et al. Failure of multiple site intradermal post exposure rabies vaccination in patient with human immunodeficiency virus with low CD4 T lymphocyte count. Clin Infect Dis. 2001 Nov. 15; 33 (10): E122-4.
24. Welch RJ, Andreson BL, Litwin CM. An evaluation of two commercially available ELISAs and one in – house reference laboratory ELISA for the determination of human anti rabies virus antibodies. J Med Microbiol. 2009; Vol. 58: 806-810.
25. Simani S. Comparison of Three Serological Tests for Titration of Rabies in Immunized Individuals, Arch Iranian Med. 1999; 3: 125-27.
26. Warrell MJ, Nicolson KG, Warell DA, et al. Economical multiple site Intradermal vaccination with human diploid cell strain vaccine is effective for post exposure rabies prophylaxis. Lancet. 1985 May 11; 1 (8437): 1059-62.