J. of Obst. & Gyn. of India Vol. 52 No. 2 : March/April 2002 Pg 48-52

Administration of Purified Verocell Rabies Vaccine During Pregnancy: Results of a Controlled Clinical Trial

Sudarshan MK , Mahendra BJ., Madhusudana SN., Ashwanthnarayan DH

Department of Community Medicine and Rabies Epidemiology Unit, Kempegowda Institute of Medical Sciences, Bangalore 560 004.

Summary

In this prospective study 29 pregnant women and 17 non-pregnant women (paired and matched controls) received post-exposure rabies prophylaxis with Purified Verocell rabies Vaccine, PVRV (verorab) by Essen regimen as approved by World Health Organization. The pregnant women group was regularly monitored throughout the pregnancy by Obstetricians, Ultrasonologists and Physicians and seroconversion was monitored throughout the pregnancy.

In all, 106 doses of PVRV administered to pregnant women and 59 to the control group did not produce any adverse effects. The health of the pregnant women was normal throughout and they delivered babies which were healthy, free from any congenital defects and had normal growth and development during infancy as assessed by paediatrician. The vaccine was immunogenically efficacious in both the groups as all women had protective rabies neutralizing antibody titres ($\geq$ 0.5 IU/ml) from day 14 till day 365 of follow up. In conclusion, Purified Verocell rabies Vaccine (PVRV, Verorab) by Essen regimen is safe and efficacious in pregnant women.

Introduction

Rabies is endemic in India as about 30,000 persons die annually which accounts to 60% of 50,000 deaths globally (WHO, 1997). The principal vector is the dog and it is estimated that there are about 22. 25 million dogs in India (for a population of about 1000 million) thus constantly exposing the people to the risk of rabies and often pregnant women are also exposed to the infection following dog bites. The Semple vaccine, prepared out of sheep brain and inactivated by Beta-Propiolactone (BPL) continues to be the mainstay of anti-rabies treatment even today. It involves a series of upto 14 paintul daily injections (primary course) in the anterior abdominal wall and in pregnant women it is injected in the thigh or interscapular region. However, modern anti rabies vaccines are also available in the market viz. Human Diploid Cell vaccine, HDCV (MIRV), Purified Chick Embryo Cell (PCEC, Rabipur) vaccine and Purified Verocell rabies Vaccine (PVRV, Verorab and Verovax-R) which are administered in the arm intramuscularly. These vaccines are approved by World Health Organization (whereas the Semple's vaccine is recommended for discontinuation) and pregnancy is no contraindication to their administration. But many obstetricians are reluctant to administer anti-rabies vaccines to pregnant women fearing endangering the foetus and affecting the health of the pregnant women and possible adverse influence on the outcome of the pregnancy. Infact, all anti-rabic vaccines, including even the Semple vaccine are inactivated vaccines and considered safe in pregnancy. But very few documented studies are available demonstrating their safety and efficacy in pregnancy. Till date these include case reports from Germany (Cates W. Jr., 1974 and Tescharek R et al, 1990), Varner et al, 1982, detailed report from Thailand (Chutivonge & Wild 1989 and 1995) and very recently from India by us (Sudarshan et al, 1999).

Hence this non-randomized controlled prospective clinical trial was aimed to demonstrate the effect viz. safety and efficacy of Purified Verocell Rabies

Vaccine (Verorab) in pregnant women with reference to their health, intrauterine growth of foetus, outcome of pregnancy, growth and development of the babies born and immune response.

Materials and Methods

Selection of pregnant women and controls: twenty nine pregnant women with history of animal bites and seeking anti-rabies treatment were included after obtaining informed consent and as per the guidelines of the ethics committee. Confirmation of early pregnancy in one case was done by ELISA test of urine sample. All of them were subjected to an ultrasound examination (ALOKA – 630 machine, with three probes of 3.5 megahertz curved probe, 3.5 megahertz linear probe and 2.5 megahertz probe) by sonologist, first at the time of recruitment and the second at 20th week of pregnancy or later as per the advice of the obstetrician in the KIMS Hospital.

Seventeen non-pregnant women viz. women in reproductive age group, unmarried or widowed or married and using any family planning method with history of animal bites and seeking anti-rabies treatment were also recruited after matching them with the cases (viz. pregnant women) for age, socio-economic status (as per modified Kuppuswamy Scale) and number of doses of PVRV received (either 3 or 5 doses).

Anti-rabies Treatment: All of them received the treatment as per the WHO guidelines and received five doses of PVRV (Verorab) intramuscularly in deltoid as per Essen regimen on days 0, 3, 7, 14 and 30 (WHO, 1992). Wherever needed all the wounds were washed under running tap water with detergent soap and applied with weak tincture iodine or spirit depending on the site. The vaccine was stopped after 3 doses if the biting animal was found normal and alive after 10 days observation period. However, none received rabies immunoglobulin (RIG) as Equine RIG was not available and Human RIG was too expensive and women could not afford it.

Follow up: All the subjects viz. pregnant women and the control group were continuously monitored clinically by the obstetricians, physicians and ultrasonologists in the hospital and by house visits by resident internees particularly for any possible adverse events. In case of pregnant women, as far as possible institutional delivery was encouraged and following delivery all the babies born were regularly followed up particularly for growth and development by pediatricians in the KIMS Hospital. Estimation of Rabies Neutralizing Antibody Titres: The patient's blood was collected on day 0 (before vaccination), 14, 90, 180 and 365 after vaccination. Rabies neutralizing antibody titres were determined by Mouse neutralization Test (MNT) as advocated by WHO at National Institute of Mental Health and Neurosciences, Bangalore. Similarly, blood samples were collected from some babies born after obtaining permission from their mothers (who received PVRV during pregnancy) on days 0 (day of birth), 14, 30 and 90.

Results

Profile of Subjects: Majority of the pregnant women (n=29) were young adults viz. mean age 23.4 years (range 18-35 years). They belonged to the upper and middle socio-economic class. Eighteen (62.1%) were multiparous and 13 (44.8%) were in second trimester of pregnancy at the time of recruitment. Only 5 (17.2%) women gave history of abortions or complications in their previous pregnancies. However, 4 (13.8%) cases were lost to follow up due to migration from the town. The controls viz. non-pregnant women (n=17), were all young adults viz. mean age 23.5 years (range 18-35 years). Majority belonged to upper and middle class (76.4%).

Anti-rabies treatment: In case of pregnant women except for one case of cat bite all were exposed to dog bites. The classification of bites according to WHO guidelines revealed that 23 (79.3%) subjects belonged to category II exposure and 6 (20.7%) to category III exposure. In 20 (68.9%) subjects the biting animal was alive at the end of 10 days observation period necessitating only 3 doses of vaccine on days 0, 3 and 7. In case of control group all were exposed to dog bites, 14 (82.4%) belonging to class II and 3 (17.6%) to class III. In 13 (76.4%) cases only 3 doses of PVRV were given as the dog was healthy and alive after 10 days of observation.

Acceptability and Tolerance of the Vaccine (Table I): All subjects viz. pregnant women and controls accepted the vaccine when the risk of rabies and the known safety and tolerance of the vaccine in the general population and the protocol of treatment were explained to them. During the course of treatment none of the pregnant women complained of any side effects to the vaccine which was well tolerated. However, in the control group 2 women (11.7%) experience transient, minor, systemic side effects viz. fever and headache, which were mild and self limited and subsided without any treatment.

Safety of the vaccine in relation to pregnancy (tables II, III and IV): In addition to the incidence of adverse event to the vaccine in pregnant women, possible effects of the vaccine on the intrauterine growth of the foetus, outcome of pregnancy and on the growth and development of the

babies born were also studied.

Table – I
Adverse Reactions to PVRV (Verorab) in Pregnant Women and their matched controls

Particulars	Pregnant Women	Controls
No. of Subjects	29	17
No. of doses of PVRV (Verorab) received	106	59
Local reactions	Nil	Nil
Systemic effects	Nil	2

Two women complained of transient fever and headache which were self limiting and subsided without any treatment.

In 29 pregnant women (Table – II) a total of 72 (67.7%) doses of PVRV were injected upto 20 weeks of gestation of which precisely 30 (28.2%) doses were injected during the first trimester viz. the crucial period of organogenesis. There were no congenital anomalies either prior to or during the course of pregnancy or later in the babies born to these pregnant women. Besides, another 48 (45.2%) doses were injected during 2nd trimester, the period of fetal growth and development and 28 (26.6%) doses were injected during 3rd trimester, the period of fetal maturation. The intrauterine growth, development and maturation was normal in all subjects as monitored by periodic ultrasound examinations.

Table II
Doses of PVRV (Verorab) Administered During Different Gestational Age of Pregnant Women (N=29)

Gestational Age in weeks	Doses of PVRV injected No.	%
4 (n=3)	5	4.7
8 (n=3)	7	6.6
12 (n=8)	18	16.9
16 (n=7)	18	16.9
20 (n=10)	24	22.6
24 (n=4)	06	5.7
28 (n=3)	10	9.5
32 (n=7)	10	9.5
36 (n=5)	08	7.6
Total	**106**	**100.0**

n=number of pregnant women

Note: As administration of Verorab was a course of injections given on day 0 (1st injection), 3, 7, 14, and 30 the figures in parenthesis indicate the actual number of pregnant women who received a specific number of Verorab doses (injections during a particular gestational age.

The outcome of pregnancy (Table III) in these vaccinees was normal and uneventful and correspond well to the prevailing obstetric outcome situation in the local population. All the pregnant women were otherwise healthy and are alive even today.

Table-III
PVRV (Verorab) in Pregnancy: Outcome of Pregnancy in Vaccines

No. of Subjects	29
Lost to followup	1
No. of deliveries	25
• Safe vaginal	22
• Caesarean	3
No. of abortions	Nil
No. of miscarriages	Nil
No. of still births / fetal deaths	Nil

Table-IV
Effect of PVRV (Verorab) on Babies Born to Pregnant Vaccinees

No. of pregnant vaccines	25
No. of babies born	26
No. of twins born	1 pair
Sex of babies born	13 boys and 13 girls
Birth Weight (babies)	
• Mean	2.7 kg
• Range	1.6 – 4.5 kg
Apgar Score (babies)	
> 6/10 at 1st min	26
> 8/10 at 10th min	26
Head circumference	
• Mean	35.1 cms
• Range	32-39 cms
Chest Circumference	
• Mean	33.6 cms
• Range	32-39 cms
Length of baby	
• Mean	48 cms
• Range	45-50 cms
Congenital anamolies	Nil
Growth and Development Milestones	Normal
No. of deaths during Infancy (0-1 year)	Nil

The babies born to these pregnant women were normal as revealed (Table – IV) by their birth weight, Apgar scors, head and chest circumference and length

at birth. These babies were regularly followed up in the KIMS Hospital by pediatricians and had normal growth and development and are healthy and alive even today.

Immune response to the vaccine: All the subjects viz. both pregnant women and the control group had protective level of rabies neutralizing antibody titre (≥ 0.5 IU / ml) from day 14 till day 365 irrespective of having 3 or 5 doses of vaccine. Paradoxically, the titres were slightly higher in pregnant women (except on day 180) as compared to the control women though the difference was not statistically significant ($p>0.2$). A profound boosting effect was also observed in 2 pregnant women who had taken some rabies vaccine in the past.

In some babies with whom it was possible to test their serum, protective levels of antibodies were present at birth and persisted till 3 months.

Discussion

Human rabies is practically a 100% fatal disease. Consequently, there are no contraindications to post-exposure rabies vaccination including pregnancy. In clinical practice, live viral vaccines are contraindicated in pregnancy for their possible teratogenic effect. But all anti rabic vaccines including the Semple's (Sheep brain) vaccine are inactivated (by BPL) vaccines and are generally considered safe in pregnancy. Besides, the potential benefit of anti-rabies vaccination in pregnancy as a life saving treatment is clearly justified despite certain potential risks perceived by lay people and shared to some extent even by some obstetricians. There are also rare and occasional instances of medical termination of pregnancies performed following post-exposure rabies vaccination in pregnant women. This is mostly because of lack of adequate and concrete evidence of safety of anti-rabic vaccines in pregnancy. Hence, many medical professionals including obstetricians are often hesitant or reluctant to administer anti-rabic vaccines to pregnant women.

Post-exposure treatment of pregnant women with semple vaccine is highly cumbersome as inoculations to anterior abdominal wall is contraindicated and multiple doses of large quantities (upto five ml per dose) of vaccine have to be administered over lateral aspect of thighs or interscapular regions. Besides, the injections are painful and cause adverse local reactions like pain, erythema, itching, induration and abscess formation. They are also known to give rise to severe neuro-paralytic reactions in 1:5000 to 1: 10,000 cases. Hence, in pregnant women Cell Culture Vaccines (CCVs) like Human Diploid Cell Vaccine (HDCV), Purified Chick Embryo Cell (PCEC, Rabipur) vaccine, Purified Verocell Rabies Vaccine (PVRV, Verorab or Verovax - R) are preferred as these are given intramuscularly in the deltoid region in small quantities (0.5 ml or 1 ml depending on the type of vaccine). They have excellent safety records in the general population and are approved by WHO for use (WHO, 1992). But very few studies have reported their safety and efficacy in pregnancy addressing their possible effects on the health of the woman, foetus, outcome of pregnancy and growth and development of the babies born. This is even more pertinent in case of PVRV, which is derived from a continuous cell line like verocells, which is potentially tumerogenic though as per the WHO requirement the levels of substrate DNA in the final vaccine is less than 100 picograms per dose, which is the accepted safe limit (WHO, 1987).

Finally, studies of antibody response to vaccine during pregnancy were limited to two case reports only, till the present authors published the partial results of this trial (Sudarshan et al, 1999).

Earlier studies by Chutivongse et al (1989 and 1995) with PVRV and Escharek et al (1990) with HDCV and Varner et al (1982) have reported that post-exposure vaccination with modern CCVs was safe and no death occurred due to rabies in their subjects. However, minor side effects like pain, itching and induration were observed in few cases.

In this study, PVRV was found to be free of any side effects in pregnant women and had total compliance thus demonstrating excellent tolerance and acceptability. The reported incidence of mild transient systemic effects in 2 (11%) non-pregnant women is well within the normal limits of tolerance and acceptability.

Most importantly the vaccine did not produce any adverse effect on the intrauterine growth, development and maturation of the foetus or on the outcome of pregnancy and the babies born were normal, free from any congenital defects and had normal growth and development during their infancy and one year follow-up period. This confirms the safety of the vaccine during pregnancy.

Lastly, regarding immunogenecity of the vaccine, it was hypothesized that in pregnancy, wherein there is an altered immune status this would probably result in reduced immune response to PVRV resulting in lower rabies neutralizing antibody titres. On the contrary slightly higher antibody titres were obtained in pregnant women (except for day 180) though this was not significant statistically ($p<0.2$). The detailed reports are published elsewhere (Sudarshan et al 1999). Overall

the post-exposure prophylaxis with PVRV (Verorab) administered by Essen regimen was as immunogenic in pregnancy as in general population.

To conclude rabies vaccination with PVRV (Verorab) is safe in pregnancy and immunogenically efficacious by Essen regimen with good antibody response. The results of this study with similar reports from other parts of the world should alleviate apprehensions amongst medical professionals particularly obstetricians in advocating PVRV (Verorab) during pregnancy for post-exposure rabies prophylaxis.

Acknowledgement

The authors thank Aventis Pasteur India Limited (formerly Pasteur Merieux Serums and Vaccins), India for complimentary doses of vaccine (Verorab) and research grant. We also thank the obstetricans of KIMS Hospital and Jayanagar General Hospital, Ultrasonologists, Pediatricians and Assistant Professor of Statistics, Dr. Gangaboriah of Kempegowda Institute of Medical Sciences, Bangalore for their valuable professional support.

References

1. Cates W. Jr. Obstet. Gynec., 44: 893, 1974.
2. Chutivongse S and Wilde H: Vaccine 1989, 7: 546 1989.
3. Chutivongse S, Wilde H, BenjaVengkulchai Manecrat. Clin Int. Dis., 20: 818, 1995.
4. Fescharek R., Quast U., Dechert G. Vaccine 8, 409 1990.
5. Sudarshan MK, Madhusudana SN and Mahendra BJ: J. Commun. Dis. 31: 229, 1999.
6. Sudarshan MK, Madhusudana S or Mahendra BJ., Ashwath Narayana DH,· Jayakumary I and gangabariah. Post-exposure rabies prophylaxis with Purified Verocell Rabies Vaccine: A study of immuno response in pregnant women and their matched controls. Ind. J. public health, XXXXIII: 2: 76-78, 1999.
7. Varner MW, MC Guiness GA, Galask RP: Am. J Obstet Gynecol; 143: 717, 1982.
8. World Health Organization: Requirements for rabies vaccine (inactivated) for human use produced in continuous cell lines. Tech. Rep. Series 760 Annex 9, Geneva, Switzerland, 1987.
9. World Health Organization: Expert committee on Rabies, 8th Report, Tech. Rep. Series, 824, 53-55, Geneva, Switzerland, 1992.
10. World Health Organization: World Survey of Rabies,, No. 33, Geneva, Switzerland, 1997.