

COMPARATIVE IMMUNE RESPONSES OF CHILDREN AFTER INTRADERMAL AND INTRAMUSCULAR RABIES VACCINATION

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Background: Rabies is a cause of death to people within 100% of Case Fatality Rate. Approximately 55.000 people died because of rabies each year, the vast majority of these deaths happen in Asia and Africa. This study aims to find out comparative immune responses of intradermal (ID) and intramuscular (IM) vaccination in children. **Method:** This was an experimental study to determine antibody response of ID and IM rabies vaccines with randomized pre and posttest control group design. ID and IM vaccination were carried out in 4 replication for each group. A number of 16 children were recruited for each group. Enzyme Linked Immunosorbent Assay (ELISA) was applied to determine titers antibody on day 0, 7, 21, and 28 after vaccination. **Results:** This study found that titer antibody induced by ID vaccination was lower than IM vaccination. However, the different is not statistically significant in both groups ($p > 0.05$). Titers antibody on day 7 after vaccination were 3.08 ± 2.09 IU/ml intradermally and 4.22 ± 3.02 IU/ml intramuscularly. On day 21 and 28 after intradermal vaccination, titers antibody were 6.78 ± 3.52 IU/ml and 12.53 ± 5.92 IU/ml, respectively. Intramuscularly, antibody titers were 9.76 ± 4.86 IU/ml on day 21 and 14.98 ± 7.76 IU/ml on day 28. **Conclusion:** ID vaccination is safe and can be used as an alternative vaccination for rabies in human. In addition, 0, 7, 21 ID vaccination methods can be recommended for use to control rabies cases in Indonesia because that methods induce protective immune response.

Keywords: Antibody; titer; Antirabies; vaccine; immune response.

INTRUDUCTION

Rabies virus infection is still a public health problem throughout the world, including Indonesia. Rabies is zoonotic disease caused by virus, transmitted from animals to humans and attack central nervous system. Rabies causes of death in people with CFR (Case Fatality Rate) 100%. Approximately 55.000 people died because of rabies each year, the vast majority of these deaths happen in Asia and Africa.^{1,2} At this time the rabies continues to spread and difficult to control, not only in developing country but also in developed countries. For decades, Bali is one of the few provinces in Indonesia declared free of rabies. On 28 November 2008 for the first time case of rabies found in Bali, namely in the area of Bukit Ungasan, Kuta district, Badung regency and on December 1, 2008 an rabies outbreak was declared.³

Until now, the methods used to control rabies in animals and in human are vaccination, in human mostly by intramuscular routes. Intramuscular vaccination face problems when applied to poor

and big population country because vaccine price is expensive. These problems cause difficulties in achieving vaccination coverage of greater than 70%.⁴ Recently, intradermal vaccination has been recommended by the World Health Organization (WHO) and has been successfully used to eradicate rabies in many countries, especially for controlling of rabies in India, Pilippines, Thailand, and Srilangka.¹ Intradermal vaccine that have been guaranteed for its safety is the Rabies Virus Glycoprotein (VRG).

Indonesia Ministry of Health in year 2000 has recommended the use of the type vero rabies vaccine intradermally. The rationale to perform intradermal vaccination is cost efficiency and higher vaccination coverage, but until now the intradermal vaccination has not been implemented. Several studies conducted overseas indicate that the ID Anti Rabies Vaccine (VAR) induces antibody titers less than half of the ID VAR. Sukathida in his study comparing ID and IM vaccination found there was no significant difference in antibody formation after of these two types of vaccinations intradermal.⁵

The study aims to evaluate the application of ID vaccination. It is expected to improve vaccination coverage by more than 70% in Indonesia. Therefore, It is necessary to conduct a

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study to determine the antibody titer of intradermal vaccines compare to intramuscular vaccines.

PATIENTS AND METHOD

This was an experimental study with a randomized pre and posttest groups design. The treatment is divided into two groups, intradermal and intramuscular vaccination. Each group consisted of four replications. The vaccines were gained from Verorab vaccine, a commercial vaccine (lot no. G1543-2) from Sanosi Pasteur-France. A total of 16 healthy children injected intradermally, each 0.1 ml on days 0, 7, 21 (Thai Red Cross modified), and 16 healthy children injected two sites intramuscularly, each 0.5 ml on days 0, 7, 21 (Zagreb methods). Subjects were children between 11 to 13 years old.

Antibody titers were measured using Enzyme Linked Immunosorbent Assay (ELISA) B. V. Europ Veterinary Laboratories (lot no H1006-AB03) in accordance with manufacture procedures described. The procedure; 100 ml of serum samples (diluted 1:100 in dilution buffer) was added to each well of the ELISA plates that coated with glycoprotein-rabies virus and incubated at 37⁰ C for 60 minutes. Afterwards, the wells were washed 3 times with ELISA wash buffer, 100 ml of anti-human IgG-horse radish peroxydase was then added into each well and incubated again at the same temperature for 1 hour. After that, wells were washed 2 times, and 100 ml of buffer A and buffer B were added to the respective wells, incubate at room temperature (21⁰ C) for 10-15 minutes. The absorbance was recorded by ELISA reader at 450 nm.

Data were analyzed by Analysis of Variance (ANOVA), followed by Least Significant difference (LSD) if there were significant differences amongst groups.

RESULTS

ID vaccination induced antibody titer lower than IM vaccination. ID antibody titer on day 7 was 3.08 ± 2.09 IU/ml; on day 21 was 6.78 ± 3.52 IU/ml; and on day 28 was 12.53 ± 5.92 IU/ml. On the other hand, IM vaccination induced antibody titer of 0.31 ± 0.49 IU/ml on day 7; 9.76 ± 4.86 IU/ml on day 21; and 14.98 ± 7.76 IU/ml on day 28. The data were presented in Table 1, 2 and Figure1.

Tabel 1

Mean Rabies Humoral Antibody Titres on day 0 and on days 7, 21, and 28 after ID and IM

Days	Humoral Antibody Titer (IU/ml)	
	ID	IM
0	0.29±0.06	0.31±0.49
7	3.08 ± 2.09	4.22 ± 3.02
21	6.78 ± 3.52	9.76 ± 4.86
28	12.53 ± 5.92	14.98 ± 7.76

Table 2
Mean of Antibody titer (IgG) in ID compare to IM Vaccination

Days	Antibody Titer (IU/ml)		p*
	ID	IM	
0	0.29±0.06	0.31±0.49	0.987
7	3.08 ± 2.09	4.22 ± 3.02	0.445
21	6.78 ± 3.52	9.76 ± 4.86	0.056
28	12.53 ± 5.92	14.98 ± 7.76	0.103

*significance at $p < 0.05$

DISCUSSION

Anti-rabies antibodies examined in this study were the antibody produced before and after vaccination in which produced a humoral immune response. Humoral response form can be used as an indicator of vaccine protection. According to the World Health Organization, rabies vaccine can protect when titer value > 0.5 IU / cc.⁶

In this study, the humoral immune response after intradermal vaccination showed an increase at day 7; 15 people were protective (94%) protection and 1 was not protective (6%). On days 21 and 28, antibody titer increased significantly and showed protective ability 100%. When compared between intradermal and intramuscular vaccination on day 7, there wasn't significant differences of antibody titer both them ($p = 0.445$). On day 21 the antibody titer comparison between ID and IM vaccination was not significantly different ($p = 0.056$). On day 28 there was no significant difference in antibody titer values after ID and IM vaccination ($p = 0.103$). This study also showed that antibody titers in intramuscular vaccination on days 7, 21, and 28 are higher than ID vaccination, in contrast to Widyaningsih research, i.e. ID vaccination produced antibody titers higher than intramuscular vaccination. It is possible because the dose used in this study (0.1 ml) was less than dose in Widyaningsih research (Thai Red Cross method).⁷ Achieving protection on ID vaccination at 7 days despite a small dose (0.2ml) compared to IM (1.0 ml) because skin cells are APC more Lenggarhans cells than muscle cells. There are differences in pattern of antibody responses induced by ID and IM vaccine. In ID and IM vaccination, antibodies titer reached maximum peak on day 28. These showed the same result with research done by Lang J, Briggs, Khawplod, and Windyaningsih. Research by Lang, et al in Indonesia (1997) showed that from 93 cases of HPR bites vaccinated intramuscularly with VAR (PVRV Zagreb method), the number of cases dropped to 61 on day 28 after vaccination. Means of antibody titer on day 0 (prior to VAR) was 0.03 IU/ ml, and increased to 19.13 IU/ ml on day 28.⁸

Briggs, et al. (2000) investigated 75 people that received VAR intradermally in 72 hours after HPR bites. The means of antibody titer on day 0

was not measured, but on day 7 was 0.32 IU/ml (59 subjects), has not reached the minimum protective level. On day 14 achieved the minimum protective level (28.9 IU/ ml), and on day 30 began to decline i.e. 10.9 IU/ ml.⁹ Khawplod, et al studied cases of bites received Intradermally VAR 2.2.2 Method (day-0, 3, and 7 each given VAR 0.1 in the right and left deltoid). The means of antibody titer on day 0 was 0.06 IU/ ml, on day 7 was 0.08 IU/ ml with 71% sero-conversion. On day 14, sero-conversion reached 100%, with mean of Antibody titer was 5 UI/ml. HPR bite cases who received Intramuscular vaccination, antibody titer was 0 IU/ml on day 0; 0.08 IU/ ml on day 7 with 29% sero-conversion; 3.81 IU / ml on day 14 with sero-conversion reached 100%.¹⁰

Widyaningsih (2007) found that of the 33 subjects, antibody titer was 0.08 IU/ml before intramuscularly vaccinated; 0.18 IU/ml on day 7 after vaccination; 1.95 IU/ml on day 21; and reached 2.80 IU/ml on day 28. Of the 51 bite cases, intradermally vaccination produced antibody titer 0.44 IU/ml on day 0; 0.59 IU/ml on day 7; 2.40 IU/ml on day 21; and 2.86 IU/ml on day 28 after vaccination. Statistically, there wasn't significant difference between ID and IM before day 7, but there was a significant difference on day 21 and 28 ($p > 0.05$).⁷

All of the studies reported that the protective Antibody levels have been reached on day 14 with titer range from 3.5 to 28, 9 UL/ ml. On day 28 in the case of HPR bite that intradermally vaccinated, antibody titer decreased.⁹ On the other hand, Windyaningsih found that antibody titer increased on day 28 with range between 3.58 to 10.9 IU/ml.⁷

CONCLUSION

On day 7 the mean antibody titers reached protective titers in both intradermal vaccination and intramuscular vaccination. IgG titers equal between intradermal and intramuscular vaccination. Similarly, at day 21 and 28 IgG had same titer between intradermal and intramuscular vaccination. Intradermal vaccine is safe and can be used as an alternative vaccine to combat rabies in human and intradermal vaccine 0, 7, 21 methods can be recommended for use to control rabies cases in Indonesia because that method induce protective immune response.

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