ARTICLE @ VIROLOGY

Analysis of Neutralizing Antibody Titers within One Year After Treatment for 282 Category III Rabies Exposed Patients

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ABSTRACT

The Rabies Virus (RABV), a member of the Lyssavirus genus within the Rhabdoviridae family, is a significant zoonotic pathogen that causes fatal encephalitis in mammals. Although rabies is incurable, the disease can be prevented through effective post-exposure prophylaxis (PEP). This study investigates the effects of rabies immunoglobulin (RIG) or/and vaccine administration on the production of RABV neutralizing antibodies in patients following exposure to RABV. We analyzed 282 patients who experienced grade three exposures to RABV due to incidents involving dogs, cats, or other animals between January 2019 and December 2023. Patients received either RIG or vaccination, and blood samples were collected within one-year post-treatment to assess the presence of RABV neutralizing antibodies. Our findings indicated a 100% seroconversion rate among patients. The geometric mean concentration (GMC) of neutralizing antibodies varied significantly across different age groups, with children and adolescents demonstrating higher GMC compared to adults. Notably, patients who received both RIG and multiple vaccine doses exhibited elevated GMC levels compared to those receiving a single vaccine dose. Timely PEP with RIG or/and vaccination following RABV exposure is effective in inducing seroconversion. This study highlights the influence of age on the immune response and underscores the importance of optimized PEP, particularly for high-risk populations. Further research is warranted to determine the long-term protective effects of PEP and its role in rabies prevention strategies.

Article History: Submitted: 28/05/2025; Revised: 30/05/2025; Accepted: 13/06/2025

DOI:10.21092/jav.v14i2.118

Key Words: Rabies Virus; Post-Exposure Prophylaxis; Rabies Immunoglobulin; Neutralizing Antibodies; Zoonotic Disease

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Introduction

The rabies virus (RABV) is a member of the *Lyssavirus* genus within the *Rhabdoviridae* family, capable of infecting the central nervous system of both humans and animals, ultimately leading to fatal encephalitis in mammals [1]. Rabies is a zoonotic, acute, naturally occurring infectious disease caused by the RABV that impacts the central nervous system. It is primarily transmitted to humans through bites or scratches from infected mammals, as well as through the licking of mucous membranes or fresh wounds. This disease remains a significant public health concern in many regions worldwide [2-4]. Annually, rabies is responsible for approximately 59,000 deaths globally, with over 90% of these fatalities occurring in Africa and Asia; notably, around 40% of the victims are children under the age of 15 [5].

To this day, rabies remains a disease that demands serious attention, particularly in communities across Africa and Asia [6]. According to the World Health Organization, Category III exposure to rabies includes one or more of the following conditions: single or multiple transdermal bites or scratches; contamination of mucous membranes or broken skin with animal saliva (e.g., licking); contact of the rabies virus with open wounds or mucous membranes; and exposure caused by bats.

Although rabies is essentially incurable once clinical symptoms appear, the incubation period from infection to disease onset typically ranges from 15 to 90 days [7]. Consequently, rabies can be prevented even after exposure through timely and effective post-exposure prophylaxis (PEP), which primarily includes wound washing with soap and water, flushing with virucidal agents, induction of active immunity via vaccination, and provision of passive immunity with rabies immunoglobulin (RIG) [8–10].

This study aims to evaluate the effects of immunoglobulin and vaccine administration in patients exposed to rabies. A total of 282 patients who experienced grade three exposures to rabies through incidents involving dogs, cats, or other animals and sought medical attention between January 2019 and December 2023 were treated with rabies immunoglobulin or rabies vaccine. The vaccine was manufactured by Liaoning Cheng Da Biotechnology using a Vero cell line and the Pasteur vaccine strain PV2061 of rabies virus (RABV). It is inactivated with β-propiolactone (BPL), lyophilized, and reconstituted in 0.5 ml of physiological saline. Blood samples were collected within one year after treatment to detect RABV--neutralizing antibodies and to investigate factors influencing antibody production.

Materials and Methods

1. Samples

Blood samples were collected from 282 patients exposed to rabies who had been vaccinated or/and treated with RIG, volunteered for the study, and the samples were obtained within one year after vaccination.

The neutralizing antibody detection was performed at the Rabies Detection Center of Wuhan Institute of Biological Products Co., Ltd.

2. Cells and viruses

BSR cells from the WIBP cell bank. The cells were propagated in Modified Eagle's Medium (Gibco, Waltham, MA, USA), which supplemented with 10% fetal bovine serum (FBS), 100 mg/ml streptomycin and 100 IU of penicillin (Gibco, Waltham, MA, USA), and cultured at 37°C in a 5% CO_2 incubator.

The rabies virus strain CVS-11 was kindly provided by the Shanghai Institute of Pasteur.

3. Rapid fluorescent focus inhibition test

The detection of rabies antibodies was performed using the rapid fluorescent focus inhibition test (RFFIT).

The specific procedure is as follows: Serum samples were heat-inactivated at 56°C for 30 minutes and then serially diluted threefold with Minimum Essential Medium (MEM). These diluted sera were mixed with an equal volume of CVS-11 virus in a 96-well plate. For comparison, reference standard (National Standard for Rabies Human Immunoglobulin, National Institutes for Food and Drug Control, China), negative control (Human Rabies Virus Negative Serum, SINOPHARM Wuhan Blood Products Co., Ltd., China), and positive control (Rabies Immunoglobulin, SINO--PHARM Wuhan Blood Products Co., Ltd., China) were included. The mixture was incubated for 1 hour at 37°C with 5% CO_2. BSR cells (5×10^4 cells per well) were added and cultured for 24 hours. The supernatant was subsequently removed, and the cells were fixed with 50μL of acetone for 30 minutes at 4°C. A diluted fluorescent antibody (Rabies DFA II Reagent, Minipore, USA) was then added, and after incubation in the dark for 1 hour, the supernatant was removed, and the cells were washed three times with 150μL of PBS. The results were analyzed by photographing the plate using a fluorescence chemiluminescence analyzer (ImmunoSpot® S6 Fluoro-Spot Line, CTL, USA). Finally, the titer of the test sample (IU/mL) was calculated using the following formula:

$$\text{Titer}=10^{(J-K)} \times L$$

where:

J = The log ED_{50} of the standard

K = The log ED_{50} of the test sample

L = The titer of the standard, in IU/mL

For more details, please refer to the second method in General Rule 3512 of the 2020 edition of the Chinese Pharmacopoeia, Part IV. According to the WHO, rabies virus neutralizing antibody (RVNA) titers of ≥0.5 IU/mL are considered adequate for rabies protection.

4. Statistical analysis

Geometric Mean Concentration (GMC) of neutralizing antibody titers were calculated using Prism 9.0 software (GraphPad Software, San Diego, CA, USA). Mann-Whitney test was used to determine the significance of differences. Significance was indicated when the value of $P < 0.05$.

Results

1. Analysis of neutralizing antibodies in rabies Patients of different age groups

A total of 282 category III exposed patients were tested for RABV neutralizing antibodies. The results (see table 1 and figure 1) indicated a seroconversion rate of 100% among all 282 rabies patients.

Specifically, the geometric mean concentration (GMC) of rabies neutralizing antibodies was 51.2 IU/ml for the 6 patients aged 0-5 years, 30.1 IU/ml for the 18 patients aged 6-17 years, 20.1 IU/ml for the 250 patients aged 18-60 years, and 32.6 IU/ml for the 8 patients aged 61-80 years.

Table 1. Analysis of PEP Treatment in Rabies Patients of Different Age Groups

Age (years old)	Number	Number of positive	Percent of positive (%)	GMC (IU/ml)	95% CI	Range
0-5	6	6	100	51.2	19.8-132.2	10.7-121.4
6-17	18	18	100	30.1	19.8-45.8	6.2-171.8
18-60	250	250	100	20.1	17.7-22.8	1.2-285.3
61-80	8	8	100	32.6	10.7-99.3	3.6-171.8

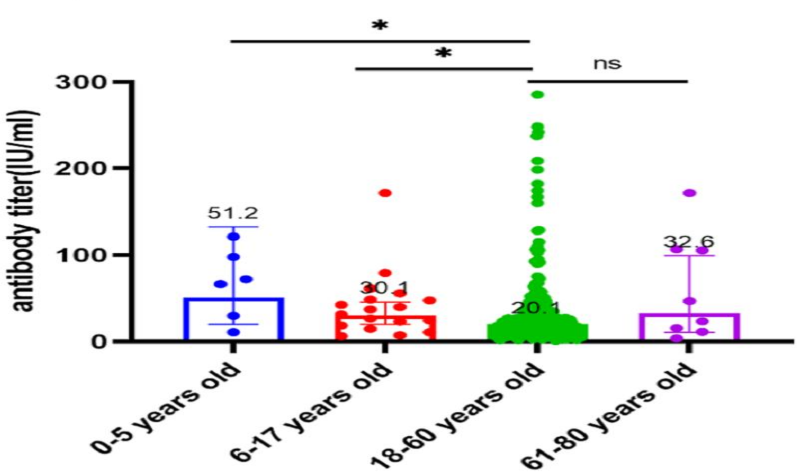

Figure 1. Analysis of PEP Treatment in Rabies Patients of Different Age Groups

The GMC exhibited variation across different age groups, with the highest to lowest concentrations observed in the following order: 0-5 years, 61-80 years, 6-17 years, and 18-60 years, respectively.

Notably, the GMC for patients aged 0-5 years and 6-17 years was significantly higher than that for those aged 18-60 years (P= 0.0144, P=0.0270). These findings suggest that age influences the production of rabies neutralizing antibodies in patients.

2. Analysis of neutralizing antibodies in rabies patients aged 18-60

A further classification of the immuniza--tion status of 250 patients aged 18-60 years was conducted to explore the impact of rabies immunoglobulin administration and the number of vaccine doses on the production of rabies neutralizing antibodies.

The results (see table 2 and figure 2) indicated that 196 patients who did not receive rabies immunoglobulin but were vaccinated exhibited a seroconversion rate of 100%. Among these, 74 patients received only one dose of the vaccine, while 122 patients received multiple doses, with GMC of rabies neutralizing antibodies measuring 19.4 IU/ml and 20.0 IU/ml, respectively, revealing no statistically significant difference.

Additionally, 54 patients received both rabies immunoglobulin and vaccination, all achieving a seroconversion rate of 100%. Within this group, 34 patients received one dose of the vaccine, resulting in a GMC of 16.1 IU/ml, whereas 20 patients received multiple doses, yielding a GMC of 34.9 IU/ml, which was significantly higher than that observed in patients who received only one dose of the vaccine (P=0.0071).

These findings suggest that both vaccination and the administration of rabies immunoglobulin after rabies exposure can induce rabies-neutralizing antibodies, achieving a seroconversion rate of 100%. Furthermore, multiple doses of vaccination and repeated administration of rabies immunoglobulin can further enhance the production of these neutralizing antibodies.

Discussion

Post-exposure prophylaxis with timely vaccination is the only effective method to prevent rabies after exposure. This study investigated the seroconversion and rabies neutralizing antibody levels in 282 patients who received purified Vero-cell vaccine or RIG after rabies exposure. The results demonstrated that patients across various age groups achieved a 100% seroconversion rate following timely PEP; however, the geometric mean concentrations of antibodies varied. Notably, children exhibited higher antibody concentrations than all the other groups, corroborating findings from previous studies[11,12]. The immune systems of children are in the developmental stage and typically exhibit stronger initial immune responses compared to those of adults. For example, one year after vaccination, antibody concentrations in children aged 1-4 years and 12-17 years were found to be 3.10 and 1.74 times higher, respectively, than those in adults. This enhanced response may be attributed to the greater proliferative and differentiation capacities of B cells and T cells in children[13].

In contrast, immunosenescence, which is characterized by a decline in immune cell function, significantly impairs the ability of older adults to mount robust immune responses. Studies have shown that individuals aged ≥60 years exhibit significantly lower antibody levels compared to those <20 years, with protective efficacy

Table 1. Analysis of PEP Treatment in Rabies Patients of Different Age Groups

Age (years old)	RIG injection	Vaccination dose	Number	Number of positive	Percent of positive (%)	GMC (IU/ml)	95% CI	Range
18-60	No	1	74	74	100	19.4	15.8-23.7	2.5-208.6
		>1	122	122	100	20.0	16.6-24.1	1.2-285.3
	Yes	1	34	34	100	16.1	10.8-24.0	1.8-237.6
		>1	20	20	100	34.9	22.3-54.5	10.2-248.3

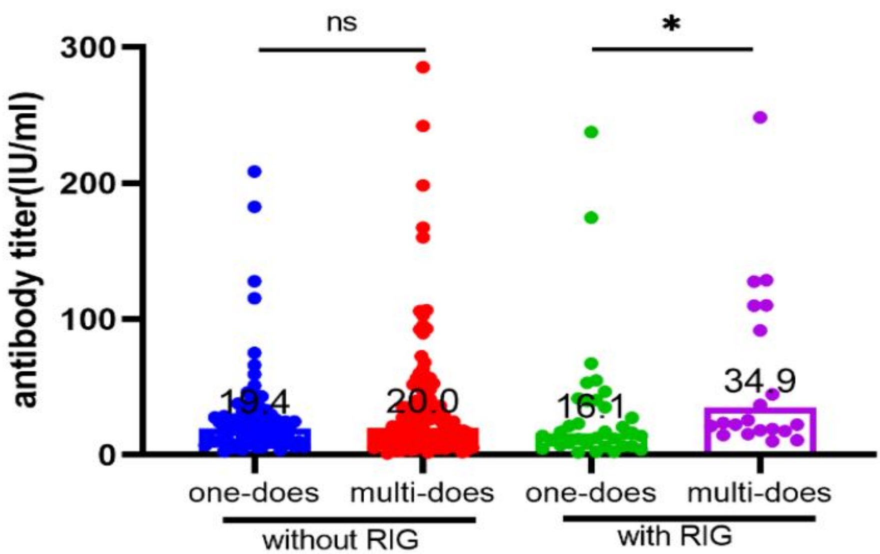

Figure 2. Analysis of PEP Treatment in 18-60 Years Old Rabies Patients

rates of 78.34% versus 96.56%, respectively. This decline is primarily due to the weakened capacity of B cells to produce high-affinity antibodies and the reduced helper function of T cells, both of which negatively impact the persistence of antibodies [14-15].

In this research, the substantial differences in sample sizes among age groups may have influenced the statistical analysis of the data. Consequently, a detailed analysis of immunization status was performed on a selected RIG sample of 250 individuals aged 18-60 years. The findings revealed that within the 18-60 age group, 74 patients attained a 100% seroconversion rate after receiving only one dose of the vaccine. Furthermore, multiple doses of vaccination and repeated administration of RIG can enhance the GMC to some extent. Currently, RIG is available only in a few major cities in Asia, resulting in very few of the millions of exposed patients receiving this life-saving treatment. In China, the majority of human rabies cases occur in rural areas where public health services are relatively underdeveloped, and the economic conditions of patients' families are often poor, potentially leading to incomplete PEP treatment and subsequent treatment failure [16-17]. To alleviate the economic burden and reduce the demand for RIG, the World Health Organization (WHO) recommends that individuals at risk of exposure in countries with endemic canine rabies should be considered for pre-exposure rabies vaccination, thereby decreasing the need for RIG following exposure [18].

In developing countries like China, the limited promotion of rabies prevention knowledge, combined with the high costs associated with implementing widespread pre-exposure vaccination programs, leads to a low rate of pre-exposure vaccinations. To enhance the effectiveness of post-exposure prophylaxis, it is essential for the country to actively promote awareness of pre-exposure vaccination, facilitating timely access for those who can afford it. Furthermore, after potential exposure, treatment must be strictly administered in accordance with the World Health Organization recommended protocols.

Admittedly, our study has certain limitations. Firstly, we only analyzed the levels of rabies virus neutralizing antibodies in patients who received treatment within one year PEP. The absence of antibody level data beyond this time frame limits our ability to fully assess the durability of the immune response. Secondly, the relatively small sample sizes in some age groups (0-5 years old and 61-80 years old) may have influenced the robustness of our statistical analysis.

Nevertheless, our study provides valuable evidence supporting the effectiveness of timely treatment following rabies exposure. It objectively demonstrates that age significantly influences antibody titers after exposure and treatment. Our findings indicate that timely vaccination can achieve clinically protective antibody levels, suggesting that, in certain circumstances, additional administration of rabies immunoglobulin may not be necessary. However, it is crucial to emphasize that each case is unique, and individuals should always follow the guidance of healthcare professionals.

Abbreviations

RABV, Rabies Virus;
PEP, Post-exposure Prophylaxis;
RIG, Rabies Immunoglobulin;
RVNA, Rabies Virus Neutralizing Antibody;
GMC, Geometric Mean Concentration.

Statements

About the Patient Consent, All patients provided written informed consent before participating in the study.

Funding

This work was supported by the Ministry of Science and Technology of People's Republic of China (2020YFC0842100, 2020YFC0860600).

Author contributions

Jie Yang: Investigation, Data analysis, Writing; *Anna Yang:* Methodology, Data analysis; *Deqin Pang:* Methodology, Data analysis; *Guangming Zhang:* Methodology; *Shengli Meng:* Supervision, Reviewing; *Jing Guo:* Supervision, Reviewing.

Competing interests

The authors declare no financial and non-financial competing interests.

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