

Efficacy of antibody levels in different post-exposure rabies vaccination programs

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ABSTRACT

Objectives. Rabies is a fatal acute viral zoonotic disease causing encephalomyelitis in humans and many other mammalian animals. Prophylaxis is vital since there is no treatment for rabies. This study was a comparison of antibody levels in patients who were vaccinated following different vaccination protocols. **Methods.** Eighty-five patients who were included in the rabies vaccination program who presented to the vaccination center of our clinic with the complaint of suspicious contact with rabies were included the study. In 61 (71.8%) patients a 2-1-1 vaccine program (Zagreb regimen) was implemented, and in 24 (28.2%) patients, a 5-dose rabies vaccine and rabies immune globulin (RIG) in a dose of 40 IU/kg (Essen regimen) was applied. **Results.** In patients on the 2-1-1 vaccine program, antibody levels on the 21st day were greater than 0.5 IU/ml in 49 (80.3%) patients. Antibody levels on the 28th day in the group that received the 5-dose rabies vaccine and rabies immune globulin administration was greater than 0.5 IU/ml in 17 (70.8%) patients. The difference between the two groups of vaccination programs was not statistically significant ($p=0.344$). Seroconversion rates for approximately one month after the last dose of vaccination in the serum samples were 90% and 75% in the groups with 2-1-1 vaccination, and RIG and 5-dose vaccinations, respectively. The differences were not statistically significant ($p=0.071$). **Conclusions.** Identification of similar seroconversion rates suggests that the 2-1-1 vaccination program may be a good alternative option to the standard vaccination program when RIG is unavailable.

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Keywords: Antibody; Immunization Schedule; Rabies

Introduction

Rabies is a viral zoonosis, preventable with the vaccine. The infection is transmitted from animals to humans with the virus in the saliva through lacerations, scratches, and bites, and proceeds with fatal encephalitis [1]. The incubation period usually ranges from 1 to 3 months after exposure, but can range from days to years. Approximately 10 million people worldwide receive prophylaxis due to animal

bites [2].

Rabies prophylaxis is administered through active and passive immunization. Prompt wound care and administration of rabies immune globulin (RIG) and vaccine are highly effective in prevention from human rabies following exposure. The World Health Organization (WHO) recommends human diploid cell vaccine + rabies immune globulin on initial, 3rd, 7th,

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14th, and 28th days. In 2009, the Center for Disease Control (CDC) provided new vaccine scheme recommendations. As an alternative to the Essen regimen (initial, 3rd, 7th, 14th, and 28th days), similar antibody levels were demonstrated with this regimen without applying the dose on the 28th day [3, 4].

The aim of this study was to determine the developing antibody levels after different vaccination applications and present data to define a preferable method for prophylaxis after contact.

Methods

This study was designed as a single centre. The patients using any drugs, age of under 15 and had chronic diseases were excluded from the study. Patients who presented themselves to the Rabies Vaccine Center of the Department of Infectious Diseases and Clinical Microbiology of the Ankara Education and Research Hospital with the complaint of suspicious contact with rabies, who consented the drawing of blood samples were included in the study. Patients were evaluated and included in the prophylaxis program according to the Rabies Prevention and Control Guidelines of the Republic of Turkey, Ministry of Health, General Directorate of Basic Health Services and WHO recommends [5, 6]. Abhayrab® vaccine (Human Biologicals Institute, India), licensed for active immunization procedures (Wistar rabies PM/WI 38-1503-3M strain), was used at 2.5 IU/dose, applied intramuscularly in 0.5 ml in the deltoid muscle.

For passive immunization, Equirab (Bharat Serums and Vaccines Ltd. India), which is a horse origin rabies antiserum containing 1000 IU/5 ml was applied at 40 IU/kg (Rabies immune globulin, RIG). According to the study design, venous blood samples were obtained twice in each group, such as on the 21st and 28th days of the 2-1-1 scheme and the RIG + 5-dose vaccine plan, respectively, and an average of four weeks after vaccination in both groups. Sixty-one (71.8%) patients received the 2-1-1 vaccine program. The 5-dose rabies vaccine and rabies immune globulin at a dose of 40 IU/kg were applied to 24 (28.2%) patients. Second blood samples were obtained in all patients 31.3 days (range 25-41 days), on average, after the last dose of the vaccination. Blood samples were centrifuged at 3000 rpm for four minutes, and serums were separated. Serums were placed in sterile

Eppendorf tubes and frozen and stored at -20°C. All samples were analysed simultaneously. Human Rabies Virus Antibody (IgG) ELISA Kit (Cusabio Biotech, China) was used for the rabies antibody assays.

Antibody levels were analysed in all patients. The serum antibody level, which has been accepted by WHO was 0.5 IU/ml, was taken as the lower limit of protection [6].

Statistical Analysis

Statistical analysis of data obtained was performed using SSPS for Windows 15.0 package program. The descriptive analysis was performed, was data was expressed as a number, percentage, and mean \pm standard deviation. Using chi-square and Fisher's exact test, values with $p < 0.05$ was set for statistical significance.

Results

Eighty-five patients who were included in the rabies vaccination program and who consented for blood to be drawn were included in the study.

The mean age of the patients was 34.8 ± 13.16 years. Forty-six (54%) were male. The patients were grouped according to the location of the bite on the body or mucosal contact with the animal, and the appropriate prophylaxis was applied. No patient received five doses of vaccine without receiving RIG. Rabies and tetanus prophylaxes were administered together in 57 (67.1%) patients (Table 1).

Among the 85 patients, antibody levels were higher than 0.5 in 27 (69.2%) of female and 39 (84.8%) of male patient respectively. This difference was not statistically significant ($p = 0.086$). Antibody levels in the second blood samples taken after the vaccination were positive in 43 (93.5%) males and 30 (76.9%) females. Among the men three patients (6.5%) and nine (23.1%) patients among women were had lower antibody levels. The difference was statistically significant ($p = 0.029$).

Mean age in the groups, with and without protective antibody levels were 34.5 ± 12.7 years and 35.7 ± 14.9 years, respectively, with no statistically significant difference between the groups ($p = 0.754$). On the 21st day, the antibody levels were higher than 0.5 IU/ml in 49 (80.3%) patients of the 61 patients on the 2-1-1 vaccine program. Corresponding antibody levels were greater than 0.5 IU/ml in 17 (70.8%)

Table 1. Demographics of the patients

General Characteristics	Total n (%)	2-1-1 n=61 n (%)	RIG+ 5 dose n=24 n (%)
Age	34.8 ±13.16	36.6 ±13.5	30.2 ±12.09
Gender			
Female	39 (49.5)	25 (41)	14 (58.3)
Male	46 (54.1)	36 (59)	10 (41.7)
Contact with animal causing risk			
Dog	72 (84.7)	52 (85.2)	0 (83.3)
Cat	13 (15.3)	9 (14.8)	4 (16.7)
Body region of bite or mucosal contact			
Lower extremity	60 (70.6)	47 (77)	13 (54.2)
Upper extremity	19 (22.4)	10 (16.4)	9 (37.5)
Both extremity	5 (5.9)	4 (6.6)	1 (4.2)
Head	1 (1.2)	-	1 (4.2)
Tetanus Prophylaxis	57 (67.1)	43 (70.5)	14 (58.3)
Day of control blood draw	31.3±2.8	31.5±2.9	30.9±2.5

RIG: Rabies immunoglobulin

patients on the RIG and 5-dose rabies vaccine group on the 28th day of the program. This difference between the two groups was not statistically significant ($p=0.344$) (Table 2).

Antibody levels in the second blood samples taken at a mean 31.5 days after the last vaccination (range 25-41 days) were analysed. Protective antibody levels were detected in 55 (90.2%) patients on the 2-1-1 vaccine program. Protective antibody levels were positive in six (9.8%) patients among those who had lower antibody level during the first blood drawn after the last dose of vaccination (on the 21st day), while six (9.8%) continued to be negative. Three (66%) patients of them were male.

Among patients who received rabies immune globulin and 5-dose rabies vaccine, protective antibodies were positive in 18 of 24 (75%) patients from the second blood samples taken at a mean

30.9±2.5 days after the last dose of vaccine. Protective antibody levels were positive in only one (1.6%) patient among those who had lower antibody level during the first blood drawn after the last dose of vaccination (on the 28th day), while six (25%) continued to be negative. Four (66%) patients of them were male. Although protective antibody rate was higher in the 2-1-1 group, this difference was not statistically significant (OR: 3.06, 95% CI: 0.75-12.6, $p=0.071$). Distribution of antibody levels in different vaccination programs was showed in Figure 1.

When considering the effects of tetanus prophylaxis, applied simultaneously with rabies prophylaxis after contact on protection, no statistically significant difference was found between patients with and without tetanus prophylaxis. Among the 85 patients, 44 (77.2%) developed protective antibodies among those with additional tetanus prophylaxis,

Table 2. Antibody levels according to the vaccination programs

	2-1-1 n=61 n (%)	RIG+5 dose vaccination n=24, n (%)	<i>p</i>
After the last dose of vaccination			
Antibody>0.5 IU/mL	49 (80.3)	17 (70.8)	0.344
Antibody<0.5 IU/mL	12 (19.7)	7 (29.2)	
Approximately one month after the last dose of vaccination			
Antibody>0.5 IU/mL	55 (90.2)	18 (75)	0.089
Antibody<0.5 IU/mL	6 (9.8)	6 (25)	

RIG: Rabies immunoglobulin

while 13 (22.8%) had no protection. Among patients with no tetanus prophylaxis, 22 (78.6%) patients similarly developed protective antibodies and six (21.4%) patients had no protection ($p=0.886$). Among the 13 (22.8%) patients with no protection after the last vaccination, who had been administered simultaneous rabies and tetanus prophylaxis, 7 continued to be antibody negative.

Discussion

The rapid fluorescent focus inhibition test (RFFIT) is considered the gold standard assay for detecting rabies antibody. But many other serological techniques are currently used for detecting rabies antibody levels like fluorescent antibody virus neutralization (FAVN), enzyme-linked immunosorbent assays (ELISAs) for humans and animals [7, 8].

Protective antibody levels can be determined with less vaccination. In this way, both the patient visits and the cost of the vaccine can be reduced. A detailed review of the evidence in support of reduced, four-dose schedules for human post-exposure has been published. In the review 12 published rabies vaccination studies during 1976-2008 representing approximately 1000 human subject, all subjects developed rabies virus neutralizing antibodies on day

14 [9].

Literature suggests that age and gender do not statistically significantly affect protective antibodies development [10-12]. There was no statistical association between age and protective antibodies in this study in concordance with the literature. However, the higher rate of protective antibodies in male patients was statistically significant. We attributed this situation to the greater number of male patients in this study. Further research in a larger patient population is required.

Bites on the extremities were the most frequent types of bits in the studies [13-15]. In the current study, the region of the body in which the bites or mucosal contacts occurred was in the lower extremity in 60 (70.6%) patients and the upper extremity in 19 (22.4%) patients. It was in concordance with the literature. It was thought to be because contact between animals and humans occurs at a level close to the ground, corresponding to the head of the animal and the lower extremities of humans. The use of the upper extremity to protect oneself from the animal is thought to be another factor explaining the frequency of extremity bites.

Protective antibody levels in the literature vary between 27% and 100%, with differences according to the day of the assay of antibody titre and the method of vaccination. In this study, protective antibodies

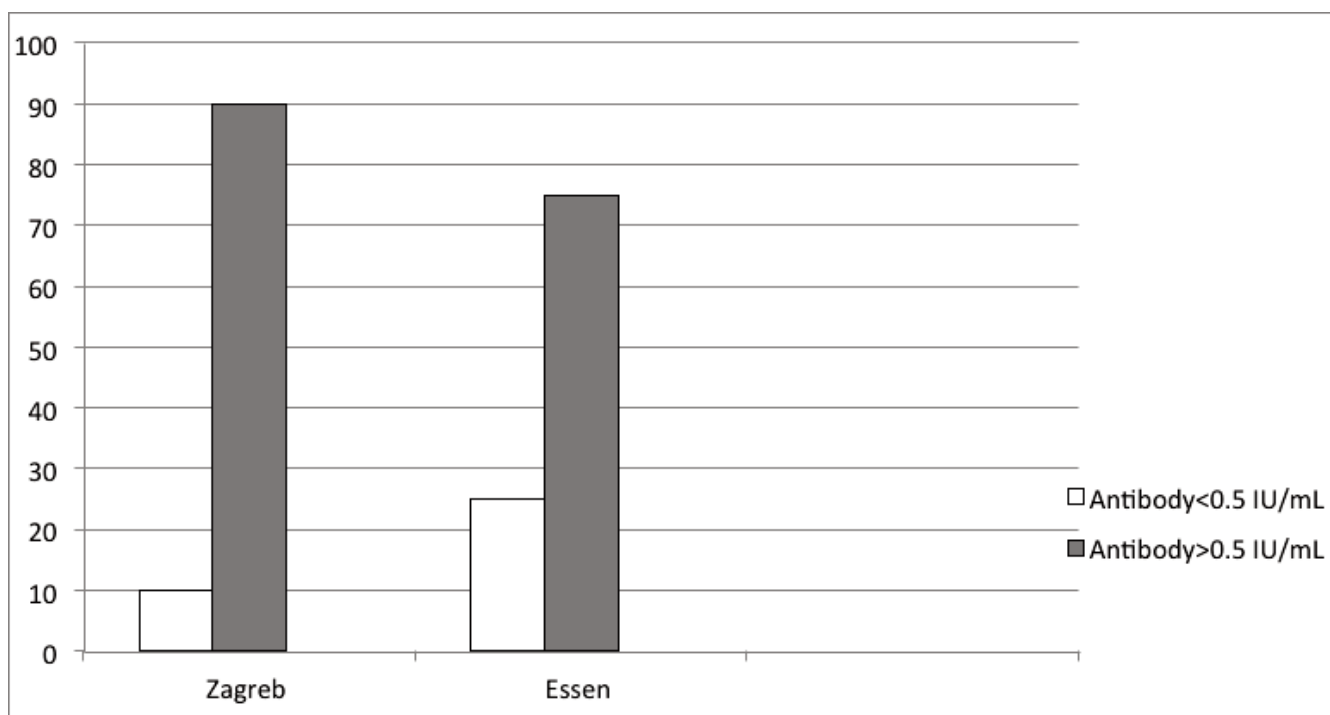


Figure 1. Distribution of antibody levels in different vaccination programs.

rates were determined as 80.3% and 90.2% for each vaccine program. It was compatible with the literature [16-22].

Protective antibodies rate in the late phase, approximately 28 days after the last dose of vaccine was 71% among patients who were administered the rabies immune globulin and 5-dose vaccine. It was in concordance with the literature [17, 18, 22, 23].

The 80% protective antibodies rate that was identified on the 21st day in patients in the 2-1-1 vaccine program was higher than the protective antibodies rate of 70% on the 28th day after RIG and five doses of rabies vaccine application. However, there was no statistically significant difference between the two groups in the protective antibodies rates. The lower rate of protective antibodies in the RIG-administered group was attributed to the differences in the immune responses among the patients, possibility of inappropriate storage conditions or improper application of RIG, or to the differences in the number of patients between the groups.

Serum samples were taken on the 21st and 28th days after the last dose of vaccination has been commonly evaluated in the literature with generally no evaluations after that. The protective antibodies rates developed in the first month, which were 90.2% and 75%, in the 2-1-1, RIG and 5-dose rabies vaccine programs, respectively were compatible with the literature [9, 22, 23]. The difference between the two applications was not statistically significant.

After three doses of vaccine, the protective antibody was detected in both groups. The follow-up period of the patients in the treated group 2-1-1 protective antibody positivity continued to be detected at a higher rate than the other group, but not statistically significant. However different results can be obtained in the other studies with long follow-up period and a large number of patients.

We determined that 12 patients in both groups had no protective antibody levels during the follow-up period. The age and sex have no effect on developing protective antibody, in this study. The patients, who had no protective antibody levels, were not examined for immunodeficiency. Because of being immunosuppression during the vaccination period can be the reason for lower protective antibody levels. Inappropriate storage conditions and applications of vaccine can cause this result also.

Simultaneous tetanus prophylaxis was identified to have been performed in 57 (67.1%) patients. This rate was 32% in a study by Hacibektasoglu *et al.* [24],

Torun [25] detected a 91.3% rate of tetanus prophylaxis. No study evaluating the association between the protective antibody rates and simultaneous tetanus prophylaxis could be found in the literature. In this study, protective antibody rates were not statistically significantly associated with tetanus prophylaxis rates. Further studies are required to be performed on this subject.

Conclusions

Application of prophylaxis after contact does not seem to be sufficient for rabies protection by itself. The percentages of antibody levels that are lower than the 0.5 IU/mL value accepted by the WHO as protective were 10% and 25% in the 2-1-1 and RIG + 5 dose vaccine applications, respectively.

When the lengthened incubation period in rabies is taken into account, the importance of appropriate and rapid wound cleaning is once more revealed. Patients who were bitten by an animal or who had mucosal contact with animals with a risk of rabies should present to health facilities as soon as possible.

The identification of antibody development rates of 75-90% in patients who completed the vaccination programs obligates an analysis of antibody titres again if the patients are exposed to the rabies virus again, and obligates re-vaccination if this value is lower than 0.5 IU/ml. The both regime show similar protective antibody rates and 2-1-1 rabies vaccination regimen is more cost effective. We recommend repeated vaccination in these situations if the period is more than one year after the first vaccination and if antibody titres could not be analysed.

Similar protective antibody rates identified after two different vaccination programs suggest that the 2-1-1 vaccination program can be a real alternative to the classic RIG and 5-dose vaccine applications.

Limitations of this study are ELISA, not gold standard test for detecting antibodies in rabies and the small number of patients.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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