EVALUATION OF THE EFFECTIVENESS OF PREEXPOSURE RABIES VACCINATION IN IRAN

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Background – Rabies is a zoonotic viral disease which infects mammals. It is transmitted to humans and other animals by an infected animal bite. Once the symptoms of the disease develop, rabies is fatal. Rabies is found in all provinces and cities of Iran especially in northern regions, from North-West to North-East of the country. The main objective of this study was to determine the level of antirabies antibody titer in preexposure-vaccinated high-risk individuals working in antirabies treatment centers in Iran and to compare them with WHO standards.

Methods – In this study, the serum antibody titers of 2,490 vaccinated high-risk staff working in antirabies treatment centers were surveyed to determine the level of antirabies antibody titers. The titers were then compared with WHO standards. From June, 1999 through June, 2000, rabies antibody titers were measured by ELISA in WHO Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran. Antibody titers were classified in 3 groups: 1) below 0.5 IU/mL, 2) 0.5 – 10 IU/mL, and 3) above 10 IU/mL.

Results – The antibody titer in the group having received complete vaccination with booster was significantly higher than the group with incomplete vaccination (p < 0.5). The level of antibody titer decreased with time. Our results indicated that this period was about two years for the titers above 10 IU/mL and 5 years for the titers between 0.5 and 10 IU/mL.

Conclusion – A booster dose should be injected within a maximum of 2 years which is very important in raising antirabies antibody titer. We concluded that the interval between the last vaccine injection and the time of sampling inversely correlates to the antibody titer.

Keywords: Effectiveness • Iran • rabies vaccine

Introduction

Rabies is a zoonotic viral disease which infects mammals. It is usually transmitted to animals and humans through close contact with the saliva of infected animals.1,2 Once the symptoms of the disease develop, rabies is fatal in both animals and humans.3

In Iran, rabies has been detected in all provinces especially in North, North-West, and North-East of the country and has a significant role in economic loss and social disruption.4–6 Recommendations of the World Health Organization (WHO) in 1992 concerning preexposure vaccination are as follows:

• Preexposure immunization should be offered to persons at high-risk of exposure such as laboratory staff working with rabies virus, veterinarians, animal handlers, wildlife officers, and other individuals who are living in or traveling to areas where rabies is endemic. Such immunization should preferably consist of three full intramuscular doses of tissue-culture rabies

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vaccine.

- The presence of virus-neutralizing antibodies in vaccinated individuals should be ascertained where feasible using the serum samples collected 1 to 3 weeks after the last dose.
- Periodic booster injections are recommended for persons at continuing risk of exposure to rabies. The following guidelines are recommended for determining when boosters should be administered:
  - All persons who work with live rabies virus in a diagnostic, research, or vaccine production laboratory should have a serum tested for rabies virus-neutralizing antibodies every 6 months and a booster administered when the titer falls below 0.5 IU/mL.
  - All other persons at continuing risk of exposure to rabies should have a serum sample tested for rabies virus-neutralizing antibodies every year; a booster should be administered when the titer falls below 0.5 IU/mL.7–9

Preexposure vaccination of high-risk individuals is very important. Such individuals may become infected unaware of having been exposed and therefore not seek postexposure treatment. A similar case arose in Iran in 1991 leading to death of a 39-year-old veterinary technician. He had no history of animal bites but had inserted his bare hands into the mouth of a rabid cow 40 days prior to development of the symptoms.10

The objective of this study was to determine rabies-neutralizing antibody titers and immunological status in vaccinated high-risk individuals (veterinarians, veterinary technicians and students, and the staff working in health centers) as well as making recommendations about booster dose injection.

**Materials and Methods**

Although human diploid cell culture vaccine (HDCV) was used as the preexposure schedule throughout the country before 1992, it was replaced, since then, by rabies Vero vaccine.11, 12 Both HDCV and rabies Vero vaccine are produced by Aventis Pasteur (Lyon, France).13, 14 From 28 June, 1999 through 24 the June, 2000 the serum samples were obtained from 2,490 preexposure-vaccinated high-risk individuals who worked in 170 anti-rabies treatment centers throughout the country and were sent (by the staff of antirabies treatment centers in cold chains) to WHO Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran. The samples were accompanied by a filled questionnaire including the data regarding the province and city of residence, age, sex, and weight of the donor; dates, route, and type of the vaccine; and dates of booster injection, history of serum injection, and (if applicable) any postexposure treatments.

The criterion used for evaluation of immunological status of vaccinated individuals was rabies-neutralizing antibody titer determined by enzyme-linked immunosorbent assay (ELISA) using Platelia Rabies Kit (code 72,200, Diagnostics Pasteur).9, 15 The test is based on the use of a solid phase, prepared with the glycoprotein extracted from the inactivated and purified virus membrane and an enzymatic conjugate (protein A from Staphylococcus aureus) coupled with peroxidase. Any positive serum reactions (distinguished by yellow color whose intensity depended on the antirabies titers) could be measured by an ELISA spectrophotometer.16, 17 All data from the questionnaires were entered in Epi2000 software and then analyzed by parametric and nonparametric statistical methods.

**Results**

Of the studied 2,490 vaccinated high-risk individuals, 97% were males and the rest (3%) were females.

As shown in Table 1, 1,959 (78.7%) of the subjects were in the 21- to 45-year-old age group. Table 2 shows antibody titers by age group. The antibody titers were classified in 3 groups: 1) below 0.5 IU/mL, 2) 0.5 to 10 IU/mL, and 3) above 10 IU/mL.

The first group (titers below 0.5 IU/mL) showed low antibody titers and immunity against rabies; the second group (titers ranging from 0.5 to 10 IU/mL), moderate antibody titers; and the third group (titers above 10 IU/mL), high antibody titers. Considering the above grouping, approximately half of the samples (50.2%) had moderate levels of

**Table 1. Age and sex distribution of the studied individuals.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 yr</td>
<td>12 (0.48)</td>
<td>54 (2.2)</td>
<td>66 (2.7)</td>
</tr>
<tr>
<td>21 – 45 yr</td>
<td>48 (1.9)</td>
<td>1911 (76.75)</td>
<td>1959 (78.7)</td>
</tr>
<tr>
<td>&gt; 45 yr</td>
<td>5 (0.2)</td>
<td>460 (18.5)</td>
<td>465 (18.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65 (2.5)</strong></td>
<td><strong>2425 (97.5)</strong></td>
<td><strong>2490 (100)</strong></td>
</tr>
</tbody>
</table>
antibody titer whereas 35.5% and 14.3% had weak and optimal levels, respectively.

Based on vaccination status (Table 3), the subjects of the study could be divided into four groups: 1) the subjects who received a complete vaccination regimen plus booster (CB), 2) those with a complete vaccination regimen but without booster (CWB), 3) the subjects with an incomplete vaccination regimen plus booster (IB), and 4) those who received an incomplete vaccination regimen without booster (IWB).

In the present study, 2,416 (97%) of the subjects had received preexposure prophylaxis (PEP) and 48 of them were given postexposure treatment (PET). Most of the individuals had received complete immunization without any booster. The most frequently encountered level of antibody titers were between 0.5 and 10 IU/mL in all groups (Table 3).

The titers above 10 IU/mL were found predominantly in individuals having received complete treatment with a booster compared with those without any booster (p < 0.05).

The time interval between the last dose of the vaccine and the time of sampling was extremely variable ranging from 1 to 21 years in 1,435 (57.6%) (Table 4), several months to 1 year in 821 (33%) (Table 5), several days to 1 month in 75 (3%), and undetermined in the rest (6.4%) of the individuals.

In Table 5, a considerable number of those receiving the last dose of the vaccine less than one year before sampling showed a titer below 0.5 IU/mL. The reason is that the majority of these people had not received the booster and therefore had not finished the course of vaccination.

The level of antibody titer decreased with time. Our results indicated that this period of time was about 2 years for those with titers above 10 IU/mL and 5 years for those having titers between 0.5 and 10 IU/mL.

### Discussion

Considering the high number of exposed persons in all parts of the country,1 large numbers of staff in health centers are involved in management of these cases. Due to contact with wounded exposed individuals, the staff are at high-risk of rabies infection. Regarding the importance of prophylaxis in high-risk individuals, all the staff working in health centers should be vaccinated as preexposure prophylaxis (PEP).18

Antibody titers in the group who had received complete vaccination with a booster were significantly higher than the group with incomplete vaccination (p < 0.5). The important point was the interval between the last dose of the vaccine and the time of sampling which is inversely correlated

<table>
<thead>
<tr>
<th>Antibody titer</th>
<th>PEP</th>
<th>PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>105/22</td>
<td>1/22</td>
</tr>
<tr>
<td>0.5 – 10</td>
<td>242/51</td>
<td>17/90</td>
</tr>
<tr>
<td>&lt; 0.5</td>
<td>126/27</td>
<td>12/63</td>
</tr>
<tr>
<td>Total</td>
<td>473/100</td>
<td>31/165</td>
</tr>
<tr>
<td>&gt;10</td>
<td>1/3.3</td>
<td>1/3.3</td>
</tr>
<tr>
<td>0.5 – 10</td>
<td>1/33.3</td>
<td>1/33.3</td>
</tr>
<tr>
<td>&lt; 0.5</td>
<td>3/100</td>
<td>3/100</td>
</tr>
</tbody>
</table>

**Table 2.** Frequency of vaccinated individuals by different age groups and antibody titers (IU/mL).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Antibody titer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.5</td>
<td>0.5 – 10</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>&lt; 20 yr</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>21 – 45 yr</td>
<td>692</td>
<td>36</td>
</tr>
<tr>
<td>&gt; 45 yr</td>
<td>166</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>884</td>
<td>35.5</td>
</tr>
</tbody>
</table>

**Table 3.** Frequency of different vaccination statuses by antirabies antibody titers.

PEP= preexposure prophylaxis; PET= postexposure treatment; CB = complete + booster; CWB = complete without booster; IB = incomplete + booster; IWB = incomplete without booster.
to the antibody titer. In other words, the level of antibody titer decreases as the time between the last dose and the sampling is increased.

Our data suggest that the antibody titers between 0.5 and 10 IU/mL will decrease within 5 years of the last dose of the vaccine. The maximum decrease has been shown to occur after 10 years. Since maintaining antirabies antibody levels between 0.5 and 10 IU/mL is of vital importance, it is recommended that high-risk individuals receive a booster dose every 5 years. However, in poor economic situations and when the vaccine is unavailable, a booster dose every 10 years is necessary.

According to the results, the failure in adhering to the common WHO protocols on rabies vaccination could be the main reason for undesired levels of antibody in high-risk individuals.

In conclusion, the following recommendations are suggested: 1) all staff of antirabies treatment centers should attend the workshops held in WHO Collaborating Center for Reference and Research on Rabies of Pasteur Institute of Iran; 2) in high-risk individuals, preexposure immunization should be performed according to WHO schedules on days 0, 7, and 28 followed by a booster injection in order to maintain the serum antirabies antibody at immunizing levels; and 3) production of higher

Table 4. Frequency of time intervals between the last dose of the vaccine and blood sampling by antibody titer (IU/mL) in years.

<table>
<thead>
<tr>
<th>Year</th>
<th>&lt; 0.5 No.</th>
<th>0.5 – 10 No.</th>
<th>&gt; 10 No.</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108</td>
<td>149</td>
<td>35</td>
<td>292</td>
</tr>
<tr>
<td>2</td>
<td>129</td>
<td>130</td>
<td>33</td>
<td>292</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>90</td>
<td>11</td>
<td>181</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>73</td>
<td>11</td>
<td>149</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
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<tr>
<td>6</td>
<td>43</td>
<td>48</td>
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<td>90</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>82</td>
<td>9</td>
<td>152</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>27</td>
<td>5</td>
<td>66</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>16</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>20</td>
<td>3</td>
<td>42</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>10</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>13</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>20</td>
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<td>7</td>
<td>0</td>
<td>8</td>
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<tr>
<td>15</td>
<td>4</td>
<td>17</td>
<td>1</td>
<td>6</td>
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<tr>
<td>16</td>
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<td>0</td>
<td>2</td>
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<tr>
<td>17</td>
<td>2</td>
<td>3</td>
<td>0</td>
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<tr>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>610</td>
<td>697</td>
<td>128</td>
<td>1435</td>
</tr>
</tbody>
</table>

Table 5. Frequency of time intervals between the last dose of the vaccine and blood sampling by antibody titer (IU/mL) in months.

<table>
<thead>
<tr>
<th>Month</th>
<th>&lt; 0.5 No.</th>
<th>0.5 – 10 No.</th>
<th>&gt; 10 No.</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>87</td>
<td>41</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>54</td>
<td>25</td>
<td>87</td>
</tr>
<tr>
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<td>57</td>
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<td>18</td>
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<td>7</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>438</td>
<td>175</td>
<td>821</td>
</tr>
</tbody>
</table>
titers of antirabies antibody (above 10 IU/mL) can only be attained through booster dose vaccination at least every 2 years.

Application of these recommendations could resolve the problems due to diminished levels of antibody titers resulted from the cases of denied vaccine injection.

References

6. Fayaz A. Reports on activities for the year 2000. WHO Collaborating Center for Reference and Research on Rabies, 2000; Pasteur Institute of Iran, Tehran, Iran.