Rabies is a zoonotic disease, transmitted by animal bites, mainly dogs. About 99% of all human deaths from rabies occur in the developing nations. Rabies, which is globally endemic, poses a risk to international travelers. To improve recommendations for travelers, we assessed the global availability of rabies immune globulin (RIG). In Asia, it is still controversial whether it is safe to inject a contaminated animal bite wound with a foreign protein such as equine or human rabies immune globulin, even though this is recommended by the World Health Organization. A prospective study of 114 severe animal bite wounds which were injected with equine or human rabies immune globulin revealed an overall incidence of gross infection of 11.4%. Rabies post exposure prophylaxis in routine practice in view of the new Centers for Disease Control and Prevention and World Health Organization recommendations.

**Key Words:** DALYS, Mouse, Protein

**Introduction**

Rabies, present on all continents and endemic in most African and Asian countries, is a fatal zoonotic viral disease, transmitted to humans through contact (mainly bites and scratches) with infected animals, both domestic and wild. Rabies is estimated to cause at least 55,000 deaths per year worldwide, about 56% of which occur in Asia and 44% in Africa, particularly in rural areas on both continents. In Africa and Asia, these deaths are responsible for 1.74 million disability-adjusted life years (DALYs) lost each year. There is no specific treatment for rabies, which is a fatal disease.

1) Human rabies-

More than 15 million post-exposure prophylaxes every year. In most countries of Africa and Asia dogs continue to be the main hosts and are responsible for most of the human rabies deaths. Although all age groups are susceptible, rabies is most common in people younger than 15 years; post-exposure prophylaxis is given on average to 40% of children in Asia and Africa aged 5–14 years.

2) Animal Rabies-

Many dogs throughout the world receive rabies shots and some wildlife species are immunized orally using vaccine-loaded baits. It is estimated that at least 50 million dogs are vaccinated each year against rabies either in private practices or during national campaigns organized by ministries of health or agriculture.
Rabies is an entirely preventable disease. Prevention includes:
- Pre-exposure immunization of all persons at risk e.g. vets, animal handlers
- Immunization of dogs
- Correct post-exposure prophylaxis for all non-immune persons.

Rabies Virus

- Classification
  Family Rhabdoviridae – ‘bullet’ shaped
  Genus Lyssavirus
    - Rabies
    - Lagos bat strain
    - MO kola
    - Duvenhage
    - EBL-1
    - EBL-2
    - ABLV
- Rabies Virus
  Bullet Shaped Morphology
  Helical RNP Core
  RNA Structure and Organization

✓ Five proteins
  - Rib nucleoprotein (RNP) Core:
    o Nucleocapsid protein (N)
    o Nucleocapsid phosphor protein (NS or P)
    o RNA polymerase (L)
  - Matrix protein (M)
  - Glycoprotein (G)

Symptoms
- Headache, fever, sore throat
- Nervousness, confusion
- Pain or tingling at the site of the bite
- Hallucinations
- “Fear of water” due to spasms in the throat
- Paralysis
- Unable to move parts of the body
- Coma and death

Risk Animals
1) High Risk Animals
   - Raccoon
   - Dog
   - Skunk
   - Groundhog
   - Fox
   - Bat
   - “free-roaming” cats
2) Intermediate Risk Animals
   - Dogs
   - Cats – vaccinated or non-roaming
   - Livestock – horses, cattle, pigs
   - Other non-rodent wild animal species
     i.e. opossum, bear, deer, coyote, etc.
3) Low Risk Animal
   - Squirrels, chipmunks
   - Rats
   - Mice, voles
   - Indoor small caged pet rodents
   - Lagomorphs

Rabies Infection
Virus-laden saliva or other infectious material from the rabid animal must be introduced through a break in skin (bite) or onto mucous membranes.
Virus binds to a nerve cell & migrates to spinal cord to brain (centripetal spread), then viral replication occurs & produces encephalitis.

Pathogenesis
1. Viral particles travel out from brain (centrifugal spread) via nerve cells to salivary glands, where further replication occurs & secretion in saliva, rendering the person or animal to be infectious
2. At the time it gets to the salivary glands, this is the end stage of the disease, and death usually occurs shortly thereafter – within several days
3. Incubation period: Usually 4 weeks.

Rabies Virus Survival
1. If saliva or other material potentially containing the rabies virus is dry to the touch, the virus can be considered noninfectious.
2. Stability of the virus in the environment
   a) Strong soaps, detergents, acids and alkalis all inactivate the virus
   b) Heat inactivates the virus
   c) Radiation destroys the virus
   d) Lipid solvents inactivate the virus

Clinical Features of Rabies Infection
The incubation period is highly variable, ranging from 7 days to several years. It depends on several factors such as;
1. Dose of inoculum
2. The severity of the wound
3. The length of the neural path from the wound to the brain e.g. wounds on the face have a shorter incubation period than wounds in the leg.

Laboratory Diagnosis
The diagnosis of animal and human rabies can be made by 4 methods:
(1) Histopathology
(2) Virus cultivation
(3) Serology
(4) Virus antigen detection.
Although each of the first 3 methods has distinct advantages, none provide a rapid definitive diagnosis.
1) Histopathology - Negril bodies are pathognomonic of rabies. However, Negril bodies are only present in 71% of cases.
2) Virus cultivation - The most definitive means of diagnosis is by virus cultivation from infected tissue. Tissue culture lines, such as WI-38, BHK-21, or CER. Since rabies virus induce minimal CPE, IF is routinely used to detect the presence of rabies virus Ag in the tissue culture. The more commonly used method for virus isolation is by the inoculation of saliva, salivary gland tissue and brain tissue intracerebrally into infant mice. The mice should develop paralysis and death within 28 days. Upon death, the brains are examined for the presence of the virus by immunofluorescence.
3) Serology - circulating antibodies appear slowly in the course of infection but they are usually present by the time of onset of clinical symptoms. The most commonly used serological tests were the mouse infection neutralization test (MNT) or the rapid
fluorescent focus inhibition test (RFFIT). These tests have now been largely superseded by EIAs. Serology had been reported to be the most useful method for the diagnosis of rabies.

4) Rapid virus antigen detection - in recent years, virus antigen detection by IF had become widely used. The potentially infected tissue is incubated with fluorescein-labeled antibody. The cells are examined by fluorescent microscopy for the presence of fluorescent intracytoplasmic inclusions. The specimens which are usually used are corneal impressions (obtained by gently abrading the cornea with a microscopic slide) or neck skin biopsy (the cells examined are the sensory nerves).

Laboratory techniques in the diagnosis of rabies

Human Rabies Pre & Post-Exposure Prophylaxis

<table>
<thead>
<tr>
<th>Category</th>
<th>Type of contact</th>
<th>Type of exposure</th>
<th>Recommended treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Touching or feeding of animals; Licks on intact skin</td>
<td>None</td>
<td>None if reliable history is taken</td>
</tr>
<tr>
<td>II</td>
<td>Touching or feeding of animals; Licks on intact skin</td>
<td>Minor</td>
<td>Administer vaccine immediately; Stop treatment if animal remains healthy for 10 days or if animal is proven to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques</td>
</tr>
<tr>
<td>III</td>
<td>Single or multiple transdermal bites or scratches, licks on broken skin; Contamination of mucous membrane with saliva (i.e. licks); Exposures to bats</td>
<td>Severe</td>
<td>Administer RIG and vaccine immediately. Stop if animal remains healthy for 10 days or if animal is negative for rabies</td>
</tr>
</tbody>
</table>

1) Pre-Exposure Prophylaxis (PrEP)-
PrEP may be performed with any of the modern cell-derived vaccines and is recommended for anyone at increased risk of exposure to rabies virus. Traditionally, PrEP is recommended for anyone who is at continual, frequent or increased risk of exposure to the rabies virus either as a result of their residence or occupation (for example laboratory workers dealing with rabies virus and other Lyssaviruses, veterinarians and animal handlers).
Pre-exposure Vaccination
1) Recommended for veterinarians, veterinary technicians, animal control officers, animal shelter workers, rabies lab personnel and person working with wildlife.
2) Provides protection from unapparent exposures and when treatment is delayed
3) Also recommended for persons spending 1 month or more in countries with endemic dog rabies and in which PEP would likely be significantly delayed to geographic distances/ lack of medical infrastructure.

Pre-exposure Vaccination Protocol
1) Three doses of vaccine administered on days 0, 7 and 21 or 28
2) Dosage: 1.0 ml administered IM in the upper deltoid
3) Test serum every 2 years to determine if an adequate antibody level persists. If absent, administer booster

Recommended Pre-exposure

Exposure: No Rabies immunoglobulin needed

2) Post-Exposure prophylaxis (PEP)-
PEP which consists of local treatment of the wound, followed by vaccine therapy (with or without rabies immunoglobulin) should be initiated immediately following a transdermal bite or scratch by an animal suspected of being rabid or when possibly infectious material, usually saliva comes into direct contact with the victim’s mucosa or with fresh skin wounds. Prompt post-exposure use of CCVs combined with proper wound management and simultaneous administration of rabies immunoglobulin is almost invariably effective in preventing rabies, even following high-risk exposure.

Rabies Post-exposure (PEP)-
Two biologics are administered:
   a) Human Rabies Immunoglobulin (HRIG) – confers immediate protection with antibodies vs. rabies
b) Rabies Vaccine - patient develops antibodies over a 2 to 4 week period.

3) Recommended post-exposure prophylaxis for rabies infection -

**Recommended Post-exposure prophylaxis**
1. Immediate flushing and washing of the wound with soap and water, or other detergent. If soap or detergent are not available, flush extensively with water.
3. Active immunization: Administration of tissue culture vaccine according to one of WHO regimens.

Rabies Immunoglobulin
Rabies Immunoglobulin - Rabies Immunoglobulin (RIG) is a lifesaving drug in all category III exposures. WHO-APCRI Indian Rabies Survey (2004) revealed that the use of RIGs was as low as 2% in our country & one of the reasons for nonuse of RIGs by medical profession is the fear of anaphylaxis. However anaphylaxis is quite rare with currently available RIG preparations, as they are highly purified. Till 2001, the Equine RIG was manufactured in only one Government facility at Central Research Institute, Kanauji, Himachal Pradesh and the Human RIGs were imported and very expensive.

Note:
RIGs are always to be used along with rabies vaccine as early as possible & are never to be used alone to treat animal bite victims.
History of RIGs/ARS-
Use of rabies immunoglobulin for prevention of rabies dates back to 1890, when Babes for the first time demonstrated its utility in experimental animals. The usefulness of rabies immunoglobulin was conclusively demonstrated in 1945 by Hubel and his colleagues after a series of carefully controlled animal experiments. These studies proved that post exposure treatment with anti-rabies serum (ARS) given at the site of the bite soon after virus injection, along with vaccine, was much more effective than vaccine alone. In 1955, Koprowsky and others could reproduce similar results. For production of RIG, several animals were used, but RIG produced in horses became more popular because large quantities could be obtained. In late 1960s, highly purified and enzyme digested ERIG became available. Use of human serum for production of RIG was initiated by History as early as 1959. In 1971 Cubase standardized the production of human rabies immunoglobulin (HRIG) and determined the optimal dosage.

Indications for RIGs-
According to WHO, all transdermal bites or scratches viz. wounds that bleed, irrespective of site, number and severity are Category III exposures. It is a common misbelief that only severe, multiple wounds & bites on head and neck are category III exposures.

The following situations need rabies immunoglobulin-
I. All Category III exposures.
II. Bites by all wild animals viz. by mongoose, jackal, fox etc.
III. Even Category II exposures in immunocompromised/immunosuppressed individuals including HIV infected people & AIDS patients.

Note:
i) RIGs should also be administered in Category III exposures even by vaccinated pet animals.

Dosage of RIGs-
Unlike modern rabies vaccines, which are independent of body weight of the patient for their dosage, the dosage for administration of RIGs is decided on the basis of body weight.

For HRIGs, the dosage is 20 IU per kg body weight subject to a maximum of 1500 IU.
For ERIGs, the dosage is 40 IU per kg body weight subject to a maximum of 3000 IU.

Note:
i) The dosage of RIG should not exceed the recommended dose calculated as per the body weight of the patient.
ii) RIGs should be given as a single dose and should not be repeated.

Administer RIGs-
RIG is more effective if infiltrated immediately or within 24 hours of animal bite along with the first dose of vaccine. If vaccine alone was started, then RIG can be given up to 7 days after starting first dose of vaccine (3 doses of vaccine given on days 0, 3 & 7) as this will not interfere with the antibody production induced by the vaccine. However, RIG can be administered even a week or later after exposure to an animal, if the person has not received any vaccine.
However it should be remembered that RIGs should be administered at the earliest, after local wound treatment, to get the maximum benefit.

- Precautions to be taken while administering RIGs-
I. The patient should not be on an empty stomach.
II. RIG vial(s) taken out from refrigerator should be kept outside for a few minutes before administration to the patient (to warm it to room/body temperature).
III. While infiltrating RIG into bite wounds, care must be taken to avoid injecting into blood vessels and nerves. Sufficient care must also
be taken while infiltrating RIG into bite wounds near the eyes and genital region. Anatomical feasibility must always be kept in mind while injecting RIG.

IV. While injecting into finger tips, care must be taken to avoid compartment syndrome.

V. All emergency drugs and facilities for managing any adverse reactions must be available.

VI. If ERIG is being administered: Carefully elicit the history of any previous administration of horse sera viz. anti-tetanus, anti-diphtheria, anti-gas gangrene, anti-snake venom serum & even anti-rabies sera (ERIG).

VII. Keep the patient under observation for at least one hour after ERIG administration and then send home.

Types of RIGs-
(a) Human Rabies Immunoglobulin’s (HRIG):
These are imported and expensive. These are available as 2ml vials with a potency of 150 IU/ml. These are homologous in origin & have a longer half-life when compared to ERIG & are hence given at half the dose of ERIG. HRIG infiltration doesn’t require prior skin testing. Since HRIG has slower clearance than F (ab’2) 2 fragments from the body, it is advisable to use HRIG in multiple/severe exposures.

(b) Equine Rabies Immunoglobulin’s (ERIG):
These are indigenously produced both in Government and Private sectors. These are available in adequate quantities on a continual basis at an affordable price. These are available as 5 ml vials (Potency of 300 IU /ml). These are heterologous in origin & produced from hyper immunized horses. Most of the Equine Rabies Immunoglobulin’s available now have F (ab’)2 fragments. F (ab’) 2 is a specific part of the immunoglobulin which neutralizes the rabies virus and which has been freed from the react genic Fc fragment. Thus, the occurrence of adverse events has been significantly reduced.

Immunoglobulin Preparations
Introduction-
Passive immunity can be provided by administration of human immunoglobulin.1-3 the protection afforded is immediate, but is transient and lasts for only a few weeks, as the half-life of IgG, the major constituent, is between 3 and 4 weeks.

There are 2 types of immunoglobulin-
1) Normal immunoglobulin
2) Specific Immunoglobulin’s-
It is important to recognize that separate immunoglobulin preparations are provided for intramuscular (IM) use and for intravenous (IV) use. These have different properties, and the preparations should be given only by the recommended route. Administration of IM immunoglobulin by the IV route will lead to severe reactions.

1) Normal human immunoglobulin (NHIG)-
This is derived from the pooled plasma of blood donors. It contains antibody to microbial agents which are prevalent in the general population.

2) Specific Immunoglobulin’s-
Specific immunoglobulin preparations are obtained from pooled blood donations from patients convalescing from the relevant infection, donors recently vaccinated with the relevant vaccine, or those who, on screening, have been found to have sufficiently high antibody concentrations. This blood-derived specific Immunoglobulin’s therefore contain concentrations of antibody to an individual organism or toxin at a higher titer than would be present in normal immunoglobulin.

HRIG- Dosage Determination and Interference with the Active Immune Response-
Dosage of rabies immune globulin was calculated from the victim’s body weight, and
then the amount of rabies immune globulin would be injected as much as possible to all of the wounds. Increase dosage of rabies immune globulin was needed in situation of multiple severe bite-wounds especially among children whose had lower body weight than adults.

1) **Primary Outcome Measures:**

   Rabies neutralizing antibody titers in volunteers who receive HRIG 40 IU/kg [Time Frame: Change from baseline of Rabies Neutralizing Antibody Titers at 3 - month period]

   Rabies neutralizing antibody titers in volunteers who receive HRIG 40 IU/kg would be determined on day 0, 14, 28 and 90. Rant titers above 0.5 IU/ml would be considered as protective levels as WHO recommendation.

2) **Secondary Outcome Measures:**

   Number of participants who have Rabies Neutralizing antibody titers above protective levels. [Time Frame: Number of participants who have Rabies Neutralizing antibody titers above protective levels at 3-month period.]

   Number of participants who have Rabies Neutralizing antibody titers above protective levels (> 0.5 IU/mL as recommended by WHO) at 3-month period.

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Condition</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rabies Biological:</td>
<td>Human Rabies Immune Globulin</td>
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<tr>
<td>2</td>
<td>Study Type:</td>
<td>Interventional</td>
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<tr>
<td>3</td>
<td>Study Design:</td>
<td>Allocation: Non-Randomized</td>
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<td>4</td>
<td>Endpoint Classification:</td>
<td>Safety Study</td>
</tr>
<tr>
<td>5</td>
<td>Intervention Model:</td>
<td>Parallel Assignment</td>
</tr>
<tr>
<td>6</td>
<td>Primary Purpose:</td>
<td>Treatment</td>
</tr>
</tbody>
</table>

**HRIG (500 unit solution for injection vials)**-

Rabies immunoglobulin human is a medicine which is used in immunization against or treatment of rabies.

**Suitable medicine**-

Rabies immunoglobulin human is not suitable for everyone and some people should never use it. Other people should only use it with special care. It is important that the person prescribing this medicine knows your full medical history.

The prescriber may only prescribe this medicine with special care or may not prescribe it at all if :- are allergic or sensitive to or have had a reaction to any of the ingredients in the medicine have bleeding problems have problems with your immune system have recently had a vaccination or are having a vaccination soon

Over time it is possible that Rabies immunoglobulin human can become unsuitable for some people, or they may become
unsuitable for it. If at any time it appears that Rabies immunoglobulin human has become unsuitable, it is important that the prescriber is contacted immediately.

Global reservoirs of rabies virus are as follows:-

- Europe - Foxes, bats
- Middle East - Wolves, dogs
- Asia - Dogs
- Africa - Dogs, mongooses, antelopes
- North America - Foxes, skunks, raccoons, insectivorous bats
- South America - Dogs, vampire bats

Conclusion

Rabies Immunoglobulin (RIGs) is essential and lifesaving in all Category III exposures. The currently available Equine Rabies Immunoglobulin (ERIGs) is purified, safe, economical and effective. HRIGs are to be used when they are available and can be afforded by the patients.

RIGs are more effective than Rabies Vaccines and HRIGs are very less side effects compared to Rabies vaccines.

References