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CASE REPORT

Diagnostic difficulties in human rabies: A case report and review of the literature

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ABSTRACT

Rabies is a zoonotic disease with the highest fatality rate of any infectious disease. The clinical features of rabies encephalopathy are highly nonspecific at the onset and clinicians from low endemic areas usually face difficulties in recognizing cases during the early stages. The need for establishing a rapid and accurate test to identify rabies during the ante-mortem period is important. However, in actual clinical practice, the latter may remain difficult for various reasons.
In human rabies, positively identifying the antigen, antibody or genetic material by various diagnostic methods during the symptomatic period is affected by the unpredictable nature of viremia, levels of antibody immune response of the host, and the virulence of the infecting strain. Also, more advanced testing with greater sensitivity may not be readily available at all centers. Here we describe a case of a young male who was bitten by a rabid dog and developed progressive encephalopathy with a fatal outcome, with negative antibodies in the cerebrospinal fluid (CSF). A review of the literature on the clinical features, diagnostic tests, treatment and prevention of rabies is also presented.

Keywords: rabies, human rabies, rabies diagnosis

INTRODUCTION

Human rabies is a fatal disease produced by the rabies virus that belongs to the Rhabdoviridae family. The other members of the family include *Vesiculovirus*, *Lyssavirus*, the plant rhabdovirus group, and other unclassified rhabdoviruses. It produces a fatal encephalopathy or a paralytic syndrome in humans with only very few reported survivors, several of

them remaining alive for only a short period of time after the acute crisis. More than 99% of deaths occur in the developing world.[1] Various terrestrial mammals and bats are reported to be the main natural reservoirs for rabies.

The syndrome, due to its nonspecific nature at onset, is difficult to diagnose in the early stages, especially without history of an animal bite. The initial clinical features can be very nonspecific and varies from a flu-like illness to the paralytic form. The specific test for rabies is also plagued by a lack of sensitivity during the early stages of the disease. This may be due to the variability of the virus in different samples, the timing of sample collection, and the antibody response of the host. The predictive value of tests in atypical cases is yet to be determined.[2] Some advanced tests with high sensitivity are not routinely available. Here, we describe a case of a young male with history of a bite by a rabid dog. He later developed encephalopathy with no detectable antibodies in the CSF, thereby stressing the importance of clinical diagnosis.

CASE REPORT

A 26-year-old otherwise healthy Indian male was admitted to Hamad General Hospital, Qatar, with history of breathlessness, left thigh pain and dysuria for three days. There was no history of fever, cough, chest pain, diarrhea or any risk factors for pulmonary embolism. On arrival, the patient appeared anxious, alert and co-operative. His pulse rate was 150/min, regular, blood pressure 124/70 mmHg, and he was tachypneic. There was no pallor, jaundice, clubbing, lymphadenopathy or rash. Pupils were normal and reacting to light, muscle power and tendon reflexes were normal, and neck stiffness was absent. Chest was clear to auscultation, and the abdomen was soft without any organomegaly. His hemoglobin was 127 g/l, total leukocyte count 10.7×10^6/l and platelet count 257×10^6/l. Arterial blood gases were suggestive of metabolic acidosis. Other blood parameters and chest X-ray were normal. The patient was treated with a provisional diagnosis of sepsis and blood cultures were taken. He underwent a diagnostic bronchoalveolar lavage which was negative for Pneumocystis *jiroveci* by staining and PCR. He was treated with intravenous piperacillin and tazobactam combination (4.5 g) q6 hourly IV and oral azithromycin (500 mg) q24 hourly. The following day the patient's relatives were telephonically interviewed, which revealed a history of an unprovoked bite by a dog one month prior in India. The dog had also bitten several others and appeared frantic and died within a few days. The patient had decided not to undergo treatment due to the long waiting period at a nearby hospital.

Considering the clinical picture of mild encephalopathy and the close proximity of a bite by a rabid dog, diagnosis of rabies was considered and the patient was administered human rabies immunoglobulin intravenously along with Ribavirin 1000 mg q6h IV. Droplet and contact isolation precautions were taken. Lumbar puncture was performed (on the third day of symptoms) and this revealed a protein level of 0.25 g/l, glucose level of 5.5 mmol/l, total leukocyte count of 31/mm^3 with 98% lymphocytes and 2% neutrophils. CSF rabies antibodies were negative. The patient's encephalopathy steadily worsened. He required ventilatory and other supportive care and died on the twenty-eighth day of symptoms. Autopsy was not performed.

DISCUSSION

The endemic canine form of rabies kills approximately 55,000 humans per year, which mainly occurs in the rural areas of Asia and Africa (actual estimates are expected to be higher due to under-reporting).[1] Rabies remains endemic among animals in the Arabian Peninsula. In Saudi Arabia, most bites to humans come from rabid feral dogs however, no confirmed cases of rabies in humans have been reported in the last ten years.[3]

Rabies virus is an enveloped RNA virus and contains single-stranded negative-sense RNA, which contains five genes for the viral components. For rabies in wildlife, the virus circulates among various carnivorous animals, which function as reservoirs. In various parts of the world, red fox, raccoon, dog, mongoose, skunks, coyotes and some bat species maintain the reservoir pool, and "spill over" cases to animals and humans can occur from contact with these animals. Urban rabies is transmitted and maintained mainly by dogs and is responsible for 99% of human deaths due to rabies. Potentially, any mammal can develop rabies and there is one case report of a hen (*Gallus domesticus*) developing rabies after being bitten by a rabid dog.[4] Modes of inoculation of the virus other than bites include exposure through licks on broken skin or mucous membranes, aerosol transmission (bat-infested caves), and organ transplant. Following the introduction of the virus through the muscles as a

result of a bite, the virus enters the nerve endings and travels through the axoplasmic flow towards the central nervous system (CNS). Once it reaches the CNS, viral replication and further dissemination to the periphery occurs clinically manifesting as diffuse encephalopathy.

Clinical features

As the symptoms of rabies depend on the speed at which it reaches the CNS, which in-turn depends on the size of the inoculum, virulence of the strain, its proximity to the CNS and host immune status, incubation periods can vary greatly and range from a few weeks to one year. The earliest symptom is paresthesia developing at the bite site. The initial symptoms of rabies are nonspecific, such as fever, body aches, sore throat, and insomnia. The disease is very difficult to suspect at this stage unless there is history of an animal bite. The features of encephalopathy can develop over a period of days to weeks and is characterized by anxiety, agitation, disorientation, and breathlessness. It can mimic a panic attack or an anxiety disorder. During the course of encephalopathy, laryngeal spasms result on attempting to drink. In a large series from the Philippines, the characteristic hydrophobia symptom was seen in all cases, and aerophobia in 95.5% of patients.[5] Hydrophobia and aerophobia occur as a result of brainstem encephalitis and the dysfunction of the neurons around the nucleus ambiguous, which inhibit respiratory muscles as a protective mechanism against aspiration, leading to spasms of laryngeal and pharyngeal muscles.[6] Once the features of encephalitis appear, death follows within a few days.

Clinical diagnosis

Clinical diagnosis of rabies may be straightforward if the history of a bite by a rabid animal is present. Often, lack of experience of the treating physician in non-endemic areas, atypical presentation, and the forgotten history of an animal bite or lick make diagnosis a difficult task. In one series,[7] the differential diagnoses that were considered before a definitive diagnosis was made included panic disorder, tetanus, viral encephalitis, drug toxicities or withdrawal, CNS vasculitis, bronchitis with pleurisy, sepsis, viral encephalitis, cervical radiculopathy, muscle strain, lower back strain, anxiety, aseptic meningitis, Lyme meningoencephalitis with peripheral neuropathy, sinusitis, pneumonia, severe hyponatremia and atypical chest pain. Also, several patients had more than one hospital visit for various complaints like lower back pain, neck pain, headache, tremors, tingling of the hands, abdominal pain, fatigue, arm numbness and weakness. Severe abdominal pain[7] can be present and even reported as the first disease manifestation.[8]

It is possible that 20% of patients can present with a neuroparalytic syndrome that is indistinguishable from the Guillain – Barre syndrome (GBS). The unexplained appearance of encephalopathy should raise the possibility of rabies in patients with GBS. Also in rabies, multi-organ involvement can occur, leading to renal failure, acute respiratory distress syndrome, pericarditis, and myocarditis with complete heart block.[9]

Laboratory diagnosis

Despite several advances made in understanding the behavior of the virus, an accurate laboratory test to diagnose rabies during the ante-mortem period may not be routinely available in clinical practice. The accuracy of a postmortem diagnosis remains high, probably due to the larger amount of tissue and organs that may be sampled. Also, the need for an accurate and rapid test to diagnose and rule out rabies is great in the era of organ donation. The greatest difficulty faced is in differentiating between the paralytic form of rabies versus the neuroparalytic complications of old-fashioned neural tissue-based vaccines still being used in some developing countries.

In general, all tissue specimens collected for diagnosis should be refrigerated or kept in a 50% glycerine saline solution, especially those which require detection of nucleic acid elements, and should be transported following all standard precautions.[1] Factors influencing the test results in rabies are as follows: the levels of viral antigens and antibodies in the specimens, which in turn depend on the stage of disease, the virulence of the virus, and host immune response. The cross-reactivity with other viruses can influence the outcome of the test during the ante-mortem period. In postmortem salivary gland samples obtained from a dog, the virus concentration determined by immunofluorescence showed a varying distribution in different areas.[10]

The available tests are summarized in Table 1. Detection of rabies by fluorescent antibody technique (FAT) is limited by the unpredictable nature of the distribution of viral antigens in samples during the early stages. Antigens may also be absent in saliva or

Table 1. Laboratory tests for rabies.

Method	Specimen	Target	Sensitivity (%)	Specificity (%)
Ante-mortem				
Conventional RT-PCR[15]	Saliva	RNA	37	100
Real-time PCR (TaqMan)[15]	Saliva	RNA	75	100
Polymerase chain reaction (RT-hnPCR)* [11]	Skin biopsy (non-neural)	RNA	98	100
Direct dot-blot enzyme immunoassay (EIA)[16]	Saliva	Viral antigen	83.3	100
	CSF		91.6	100
Post-mortem				
Direct rapid immunohistochemical test (dRIT)[17]	Neural	Viral antigen	100	100
Direct microscopy**	Neural	Negri bodies	Low	low
Fluorescent antibody technique (FAT)	Neural	Virus nucleo-protein antigen	High	High
RT-PCR[18]	Neural	Viral RNA	100	100

*Not routinely used.
**No longer recommended.

CSF samples due to the presence of neutralizing antibodies. Though not routinely available, the best test for ante-mortem detection may be the hemi-nested polymerase chain reaction (RT-hnPCR), which shows high accuracy irrespective of the time duration of clinical symptoms.[11] There is one case report that demonstrated the success of hnRT-PCR where all other tests including FAT were negative.[12] Serum-neutralizing antibodies against the virus usually appear after the tenth day of symptoms,[13] and therefore serological techniques are reserved for assessing antibody response following vaccination rather than for making a laboratory diagnosis. The antibodies in the CSF appear two to seven days after their appearance in the serum. CSF antibodies are usually absent in patients with serum antibodies. In one series, it was detected in less than 50% of patients after the ninth day of symptoms.[14]

Imaging

The specific finding for rabies in imaging lacks good quality evidence and statistical association when compared with other viral encephalitides. However, MRI imaging may be more sensitive and specific for rabies and the suggestive features may be fronto-temporal hyperintense signals,[19] and non-enhancing T2 hyperintensities in the spinal cord, brainstem, thalamus and limbic cortex.[20] In Japanese encephalitis, characteristic bilateral thalamic hyperintense lesions are seen, a feature not seen in rabies. Gray matter involvement more towards the midline in rabies patients may help to differentiate it from acute disseminated encephalomyelitis (ADEM), which mainly involves the white matter.[21]

Treatment of acute case and outcomes

Rabies carries the highest fatality rate among all infectious diseases, and the treatment remains symptomatic with no specific antivirals that are proven to be effective. Less than ten survivors have been reported to date. The first reported case of survival involved a 15-year-old girl who did not receive post-exposure prophylaxis after being bitten by a bat. The treatment strategy used for this case was later known as the "Milwaukee Protocol," which involves inducing coma and treating with antiviral drugs.[22,23] The idea behind this was to keep the patient in a coma until the native immune response matured. The drugs used were ketamine, midazolam, and barbiturates for inducing coma. The antivirals used were ribavirin and amantadine. At present, the utility of this treatment protocol is debated as there are various factors that determine survival, such as the virulence of the virus (which varies from various

sources), proximity of the bite, and immune response and hence this protocol is no longer recommended.[24]

Prevention

Vaccinating pet animals is a good option, but it requires good quality control standards to make sure that adequate antibody titers are maintained in the serum of canines by booster doses. Evidence has shown that antibody levels gradually decrease over time after vaccination, which also depends on the dog's age and quality of the vaccine given.[25]

For humans, the vaccination schedule for pre-exposure prophylaxis (PrEP) consists of three doses of vaccine administered on days 0, 7, and 21 or 28; these doses are usually reserved for veterinarians and laboratory personnel.[26] The role of PrEP for the community in endemic areas is not clear, although small trials have shown good immunogenicity and safety.[27]

In areas where the occurrence of rabies is very rare, there are no standard guidelines for managing animal exposures and the treating physician is expected to make a reasonable decision regarding the administration of post-exposure prophylaxis (PEP).[28] If the animal is observable, especially in a low endemic area, it may be observed for ten days for any ill health before initiating the vaccine. The commonly used vaccines are human diploid cell vaccine (HDCV) and purified chick embryo cell vaccine (PCECV). The current Advisory Committee on Immunization Practices (AICP) recommendation[26] is to give four doses of vaccine – 1 ml of either HDCV or PCECV along with rabies immunoglobulin (RIG) – with exposure for previously unvaccinated patients. The vaccines are given intramuscularly on days 0, 3, 7, and 14. The previously practiced fifth dose of vaccine was abandoned following lack of evidence of any benefit, and is now administered only in states of reduced immunocompetence. Persons with previous vaccination, both pre- and post-exposure prophylaxis, with documented adequate rabies virus-neutralizing antibody response require only the first two doses of vaccination without RIG.

It may be noted that although no specific antivirals exist at present, the combination of prompt rabies PEP, wound care, infiltration of RIG into and around the wound, and multiple doses of rabies cell-culture vaccine is 100% effective in preventing human rabies.[26] Also, PCECV appears to be safe during pregnancy.[29]

Intradermal administration of a lower dose of vaccine (0.1 ml) seems to be equally effective in producing antibodies in the serum, a good option in resource-poor countries and recommended by the WHO.[30] The slightly increased adverse reaction associated with the intradermal route is linked to the route of administration. An even shorter course, with a booster dose at one year, was found to produce adequate anti-rabies antibodies in serum with an excellent safety profile.[31]

CONCLUSION

Rabies still remains the infectious disease with the highest fatality rate, and there is no proven specific therapy for the encephalopathy. Anti-mortem diagnosis can be difficult as discussed, and the best strategy for a definitive diagnosis would be to send multiple tissue samples for analysis. As negative tests do not exclude rabies, the physician should maintain a high degree of suspicion in cases of unexplained encephalopathies. The only method for preventing fatality remains in the combination of timely wound care, vaccine administration, and immunoglobulin infiltration which is highly efficacious. Due to the highly variable incubation period, the vaccine should not be withheld for delayed presentations. Methods to increase awareness among local endemic populations for seeking medical advice soon after animal exposure are needed, with good preventive programs at the government level to decrease the incidence of human rabies.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

KC cared for the patient. KC and RTP wrote the first draft. All other authors contributed to the editing process of the final draft. MM approved the final version of the manuscript. All authors read and approved the final manuscript.

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