

The Corneal Test in the Antemortem Diagnosis of Human Rabies

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OBJECTIVE: To evaluate the usefulness of the corneal test for the antemortem diagnosis of rabies in man.

BACKGROUND: In a high percentage of animals infected with rabies, the virus can be demonstrated in superficial corneal epithelial cells by immunofluorescent methods (1, 2). In experimentally infected mice, virus has been demonstrated in the cornea even before the onset of clinical signs of disease (1). A number of human cases have been reported in which an antemortem diagnosis of rabies has been confirmed using the corneal test, but no systematic effort has been made to determine the reliability of this diagnostic method in man.

PROGRESS: To date, we have studied four human cases of rabies. One was an American soldier hospitalized in Saigon, Vietnam, and the other three were Thai Nationals hospitalized at the Infectious Disease Hospital near Bangkok, Thailand. In each case a tentative clinical diagnosis of rabies had been made based upon clinical symptomatology and history before sampling was initiated.

At the earliest opportunity after a suspected rabies patient was admitted to the hospital, samples were taken for laboratory examination. Corneal impression smears were made by firmly pressing a clean fluoro-slide against the cornea, causing superficial corneal epithelial cells to adhere to the glass. This caused little discomfort to the patient, and usually required no anesthesia or sedation. In the laboratory the corneal impressions were stained with fluorescent antibody in the same manner as is used for brain impressions, except that the time-consuming acetone fixation step is unnecessary and was eliminated (3).

In addition to corneal impressions, samples of serum and saliva were obtained initially, and at frequent intervals during the clinical course of disease. Serum neutralizing antibody titers were determined in mice, and saliva was inoculated into mice intracranially for attempted virus isolation. After death, the diagnosis of rabies was confirmed by applying the standard fluorescent antibody test and mouse inoculation test to the brain. The presence of virus in the saliva, salivary gland, and cornea after death was also determined by mouse inoculation.

The results of studies in four patients are summarized in Table 1. In three of the four cases specific fluorescence was found in corneal impression smears by the fluorescent antibody technique. In each instance the corneal impressions were positive on the first occasion in which samples were taken, and the cornea remained positive throughout the clinical course of disease. In one of the four cases, the corneal impressions were negative. This woman presented clinical signs and symptoms highly suggestive of rabies, including dysphagic, hydrophobia and paralysis. Permission for autopsy was not granted and post mortem examination of the brain could not be performed. Virus was not found in a sample of saliva taken before

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Table 1 Laboratory Findings in Four Human Rabies Cases

Patient Description	Day of Disease ^e	Corneal Test	Serum Anti-rabies Titer	Saliva MI ^b	Brain	
					FAT ^c	MI ^b
Case 1. American, male age 22	8	+	1:5			
	12	+	1:30 ^d			
	20	+	1:125 ^d			
	21 ^a	+			+	+
Case 2. Thai, female age 43	2	+	<1:5			
	3 ^a	+			+	+
Case 3. Thai, female age 57	4	+	<1:5	+		
	6	+	<1:5	+		
	8	+	<1:5	+		
	9 ^a	+			+	+
Case 4. Thai, female age 57	4	-	<1:5	-	Autopsy not authorized	
	5 ^a	-				

^a Day of death

^b MI = Mouse inoculation test

^c FAT = Fluorescent antibody test

^d Titer determined after administration of hyperimmune serum

^e The days indicated are the number of days after initial appearance of clinical symptoms as reported by the patient

death, and the serum neutralizing antibody titer was negative. Although the symptomatology was highly suggestive of rabies, no pre or post mortem laboratory confirmation was possible, and the diagnosis is questionable.

The serum neutralizing antibody titer was not useful in corroborating the diagnosis of rabies in any of the 4 cases either before or after death. In three of the cases, the titer was negative (below the level of sensitivity of the method), and in the fourth case interpretation was confounded by the therapeutic administration of hyperimmune serum. In one case virus was isolated from saliva taken before death, but these laboratory results could not be made available to the attending physician until after the death of the patient. In short, the results of the corneal tests, taken during the life of the patient, and

processed and interpreted in the laboratory within two to three hours, were the only definitive laboratory data which could be provided to the physician to confirm his clinical diagnosis and to guide the clinical management of the case.

Saliva was sampled in two of the four cases, and in both cases the corneal test and saliva test were in agreement. Saliva will be sampled in all future cases in an attempt to determine the degree of correlation. If a high degree of agreement is found, the corneal test might be recommended as an indirect method of indicating whether the saliva might be expected to be infectious.

SUMMARY: An early laboratory diagnosis of rabies has been made in three human cases by the immunofluorescent demonstration of rabies antigen in corneal impression smears. The diagnosis in a fourth case in which corneal impressions were negative is uncertain. These studies are continuing. Further cases will be evaluated, and, in particular, the correlation between the presence of virus in cornea and in saliva will be evaluated.

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