Asian Journal of Biological and Life Sciences | Short Communication

Ante mortem molecular diagnosis of rabies from saliva and skin

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Submitted : 23.05.2012 Accepted : 02.06.2012 Published : 10.09.2012

Abstract

With the advent of molecular approaches, antemortem diagnosis of rabies has become more productive and meaningful. For antemortem diagnosis of rabies two most significant samples that have been projected by various workers are saliva and skin. The present review gathers the research studies carried out on saliva and skin by employing various molecular approaches. Molecular approaches reported by various workers for diagnosis of rabies virus are compared.

INTRODUCTION

Diagnosis of rabies is often mistaken for other disorders. Differentiation from other neurological diseases may require extensive investigations. Therefore diagnosis is often confirmed late in the course of the disease or postmortem. Delay in diagnosis greatly increases the number of contacts that require post exposure prophylaxis. The early detection of this dreaded disease is essential to eliminate the expenses and discomfort of unnecessary diagnostic tests and inappropriate therapy. With the advent of molecular approaches, it is now possible to detect rabies ante-mortem with the advent of molecular approaches. Since rabies virus appears in the two most non-invasive tissue/secretion viz. saliva and skin of infected animals, thus molecular approaches can be employed for reliable ante-mortem diagnosis. Ante-mortem diagnosis of rabies by molecular techniques on saliva and skin based on detecting viral RNA has been discussed in the present review.

Detection from Saliva

An optimized reverse transcription (RT)-PCR protocol for the intravitam detection of rabies virus genomic RNA in clinical samples using saliva [1] combined with an immunofluorescence assay performed with skin biopsy samples allowed detection of rabies in nine patients. Higher sensitivity of >98% was obtained [2] by using RT-PCR for ante-mortem diagnosis of rabies in human saliva samples. Isolation of rabies from saliva was attempted in 15 of the 20 cases of rabies diagnosed before death, and in 9 cases virus was found in 1 or more samples.

The utility of conventional RT-PCR and SYBR Green Real time PCR was evaluated [3] in the ante mortem diagnosis of rabies using saliva samples. Saliva samples collected from twenty-four patients presenting with typical clinical manifestations of rabies were tested in the two assays. Real time PCR assay was found to be more sensitive than conventional RT-PCR assay (sensitivity 75% versus 37%, $p = 0.0189$).

A study was conducted [4] in order to look for evidence of rabies virus in saliva of suspected live rabid dogs at the time of quarantine by using a SYBR Green real-time RT-PCR based assay for the detection of rabies virus RNA.

A new reverse-transcription was standardized [5] wherein Heminested polymerase chain reaction (hnRT-PCR) protocol was standardized by participation of 3 centres from Cambodia, Madagascar, and France. In this study, saliva samples provided the second-best results for sensitivity testing (70.2%). A sensitivity of 100% was obtained with the saliva sample when analyzed at least 3 successive samples per patient. RT-PCR on saliva for viral nucleic acid detection yielded a sensitivity of 50-70% and a specificity of 100% [6]

A sensitivity of 75.8% (47/62 samples) was obtained [7] by applying nucleic acid-amplification test methods with saliva samples for ante-mortem detection in human patients.

Detection from skin

Nuchal skin biopsy specimen were tested [2] by RT-PCR in 15 cases out of the 20 cases before death and rabies viral antigen was detected in 10 (66.6%) human patients. Likewise, RT-PCR was used [8] for ante-mortem diagnosis of rabies from neck skin biopsy samples and out of all the samples collected first positive result was obtained by RT-PCR on punch biopsy of neck skin sample.

RT-PCR was employed with primers targeted to the 3' terminal portion of the nucleoprotein gene (N), to test neck-skin samples of nine patients who had rabies in order to validate a diagnostic method that could serve as an additional tool for rabies diagnosis, particularly in ante-mortem samples and obtained a sensitivity of 70% per sample and 77.7% per patient [9].

Accuracy of the diagnosis by comparing the results obtained with use of biological fluid specimens and skin biopsy specimens with the results obtained with use of the standard rabies diagnostic procedure performed with a postmortem brain biopsy specimen were studied [5].

It may be concluded that in the times to come, molecular detection of rabies by non-invasive approaches is bound to bring in paradigm shift in the way rabies will be diagnosed in a future that is expected to be quite near.

REFERENCES

1. Crepin P, Audry L, Rotivel Y, Gacoin A, Caroff C and Bourhy H. Intravitam diagnosis of human rabies by PCR using saliva and cerebrospinal fluid. J. Clin. Microbiol. 1998: 36: 11171121.

2. Noah D L, Drenzek C L, Smith J S, Krebs J W, Orciari L, Shaddock J, Sanderlin D, Whitfield S, Fekadu M, Olson J G, Rupprecht C E and Childs J E. The epidemiology of human rabies in the United States, 19801996. Annals Intern. Med. 1998: 11: 922930.

3. Nagaraj T, Joel P V, Desai A, Kamat A, Madhusudana S N and Ravi V. Ante mortem diagnosis of human rabies using saliva samples: Comparison of real time and conventional RT-PCR techniques. J. Clin. Virol. 2006 : 36: 1723.

4. Saengseesom W, Mitmoonpitak C, Kasempimolporn S and Sitprija V. Real-time PCR analysis of dog cerebrospinal fluid and saliva samples for ante-mortem diagnosis of rabies. Southeast Asian J. Trop. Med. Pub. Health 2007: 38(1): 53-57.

5. Dacheux L, Reynes J M, Buchy P, Sivuth O, Diop B M, Rousset D, Rathat C, Jolly N, Dufourcq J B, Nareth C, Diop S, Rajerison I C R, Sadorge C, and Bourhy H. A Reliable Diagnosis of Human Rabies Based on Analysis of Skin Biopsy Specimens. Clin. Infect. Dis. 2008 : 47: 14101417.

6. Madhusudana S N and Sukumaran S M. Antemortem diagnosis and prevention of human rabies. Annals Indian Acad. Neurol. 2008 : 11(1): 312.

7. Wacharapluesadee S and Hemachudha T. Ante-mortem and post-mortem diagnosis of rabies using nucleic acid-amplification tests. Exp. Rev. Mol. Diag. 2010 : 10(2): 1-12.

8. Strauss R, Granz A, Wassermann-Neuhold M, Krause R, Bago Z, Revilla-Fernandez S, Simon-Soria F, Echevarria J E, Popow-Kraupp T, Allerberger F, Schonbauer M and Hrabcik H. A human case of travel-related rabies in Austria, September 2004. European Surveillance. 2005: 10(11).

9. Macedo C I, Carnieli Jr P, Brandao P E, Travassos da Rosa E S, Oliveira Rde N, Castilho J G, Medeiros R, Machado R R, Oliveira R C, Carrieri M L and Kotait I. Diagnosis of human rabies cases by polymerase chain reaction of neck-skin samples. Brazilian J. Infect. Dis. 2006 : 10(5): 341-345.