AIIMS-led team develops highly sensitive, portable test for TB meningitis

A diagnostic test for TB meningitis with nearly 100% sensitivity and about 91% specificity has been developed. From left: Abhijeet, Neera Sharma, Jaya Sivaswami Tyagi and Tarun Sharma.

A diagnostic test for TB meningitis (the most severe form of TB) with nearly 100% sensitivity and about 91% specificity has been developed by a multi-institutional team led by Prof. Jaya Sivaswami Tyagi from the Department of Biotechnology at AIIMS. The performance of the diagnostic test was evaluated in 87 cerebrospinal fluid samples obtained from paediatric subjects (39 TB meningitis patients and 48 controls). The results of the study were published recently in the journal *Tuberculosis.*

The diagnostic test is based on a derivative of a DNA aptamer (a small single-stranded DNA molecule that binds to a specific target molecule and is a chemical rival of antibodies) that shows high binding affinity in nanomolar range and high specificity to a TB antigen (HspX). Besides higher binding affinity, there is significantly higher load of the HspX antigen in cerebrospinal fluid samples, leading to higher sensitivity.

A rapid, point-of-care diagnostic test for TB meningitis that uses the DNA aptamer has already been adapted to a sensor format and is being evaluated on clinical samples. “It takes all of 30 minutes to get the result as we are using an electrochemical sensing platform,” says Prof. Tyagi.

“While antibodies have to be generated in animals and so will not be of uniform quality, aptamers can be produced in the lab,” says Dr. Tarun Kumar Sharma from the Centre for Biodesign and Diagnostics at Translational Health Science and Technology Institute (THSTI), Faridabad and the other corresponding author of the paper.

The aptamer-based diagnostic test for TB meningitis has been patented by AIIMS and THSTI and licensed to AptaBharat Innovation Pvt Limited, a THSTI spinoff founded by Dr. Sharma.

The currently used diagnostic methods — microscopy and culture of cerebrospinal fluid — suffer from huge limitations in terms of poor sensitivity and long turnaround time of up to eight weeks. Even GeneXpert has only 55% sensitivity. In settings such as India, where the prevalence of TB meningitis is high, one in six patients tested by Xpert will be missed.

Ten-year journey

It has taken the team about 10 years to reach this stage of developing a diagnostic test. Since there are very few bacteria in TB meningitis samples, the researchers wanted to evaluate the utility of using TB DNA for the diagnosis using PCR. In a paper published in 2009 in the *Journal of Medical Microbiology,* the researchers filtered 167 cerebrospinal fluid samples using a filter paper and studied the filtrate that contains the DNA of TB bacteria. DNA and other components of TB bacteria are present in the filtrate due to disintegration of the bacteria.

“We studied TB bacteria present on the filter paper and the TB DNA present in the filtrate and found TB DNA yielded significantly higher sensitivity of detection (88%) than the whole bacteria (53%). When we saw the filtrate had more DNA we wanted to check for antigens of TB bacteria,” recalls Dr. Sagarika Haldar from the Centre for Biodesign and Diagnostics at THSTI and first author in two papers. She is currently at the Department of Experimental Medicine and Biotechnology,

PGIMER, Chandigarh.

In a paper published in 2012 in *PLOS ONE,* the team studied 532 cerebrospinal fluid samples collected from children and looked for five TB antigens. “Though DNA and antigen were significantly higher in the filtrate compared with TB bacteria, the amount of TB antigens was far higher than DNA,” Dr. Haldar says. Using DNA for diagnosis would involve sophisticated instrument and amplification while antigen detection will be straight forward.

The team found the sensitivity of both tests — DNA and antigen — was similar. Of the five antigens, two were found to be excellent in terms of sensitivity and specificity. “We would need an ELISA reader to detect the antigens. Since we wanted to make a point-of-care diagnostic test for TB meningitis, we turned out attention to DNA aptamers,” says Dr. Haldar.

Using a DNA aptamer to bind to the TB antigen makes ELISA reader redundant in a portable assay format. The aptamer is also more sensitive, specific and stable compared with the antibody. Since the antigen is directly detected, only 5 microlitre of the sample is required.

Twenty-one aptamers were selected from the aptamer libarary and based on specificity one was chosen. Since all the 44 nucleotides of the aptamer don’t interact with the HspX TB antigen, the size of the aptamer was reduced to 28 nucleotides. Reducing the size of the aptamer will in turn reduce the cost of the diagnostic test.

“Once the mutation was done to reduce the size we found the binding improved. The mutant aptamer showed 2.5-fold higher binding than the parent aptamer,” says Abhijeet Dhiman from the Department of Biotechnology, AIIMS and first author of the 2018 paper. The optimised aptamer was tested on 87 cerebrospinal fluid samples and found to have nearly 100% sensitivity and about 91% specificity.

The device was tested in the open ocean at a depth of 1,600 to 2,300 ft.

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