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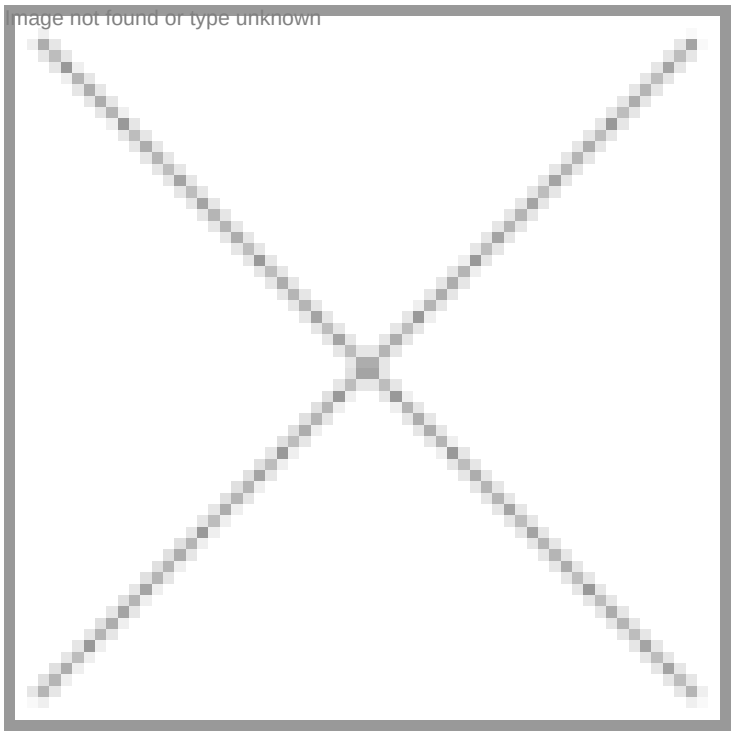

Rapid diagnosis of non-tubercular TB on the cards

AIIMS scientist develops a novel molecular method for the detection of non- tubercular mycobacterial infections.

With the resurgence of tuberculosis, especially in HIV infected patients, there has been increased focus on accurate diagnosis and treatment of TB, which is still rampant in the developing countries. Currently there exists a reliable methodology to detect pulmonary tuberculosis caused by the dreaded Mycobacterium tuberculosis, but there is still a lacuna in the detection of non-tubercular mycobacterial infections.

Now, Dr Sarman Singh, head, clinical microbiology division, department of laboratory medicine at the All India Institute of Medical Sciences (AIIMS) in New Delhi, along with his team has successfully developed novel multiplex PCR primers for the rapid and accurate diagnosis of infections caused by Non-Tubercular Mycobacteria (NTM).

Although the Mycobacterium genus represents a complex phenotypic and genotypic diversity amongst the more than 100 odd species, the most important human pathogenic species remains Mycobacterium tuberculosis. But, now with the AIDS epidemic, the significance of Non-Tubercular Mycobacteria also known as Mycobacteria Other Than Tuberculosis (NTM or MOTT) has increased. Most of these infections do not respond to the conventional anti-tubercular treatment and are misdiagnosed as infection with multi-drug resistant strains of M. tuberculosis due to lack of species identification, particularly in developing countries.

Conventionally, the identification of mycobacteria upto the species level is very slow, labor intensive, hazardous and not always reproducible in the labs. To overcome the shortcomings of conventional methods, Dr Singh, who has been focusing on intracellular pathogens, zeroed on molecular techniques that are being commonly used in recent times. "The advantage with molecular methods is that they are rapid, highly sensitive and specific and can be used on a large number of samples", he said.

"We have been working on the rapid detection of the extra pulmonary tuberculosis infection in HIV patients. In this direction,16S rRNA gene analysis is the most promising molecular method. The 16S rRNA gene is conserved in all the species of the Mycobacterium genus. Therefore this gene can be amplified by PCR in all species of Mycobacterium and the species can be identified by species specific probes or PCR primers," explained Dr Singh. But rather than developing species specific primers for each non-tubercular species of mycobacteria, the researchers took advantage of the fact that a cluster of 5 genes (esat-6) is conserved only in the Mycobacterium tuberculosis (MTB) complex species but is deleted from most non-tubercular mycobacterial (NTM) species. This gene cluster codes for an early secreted antigen and has been used as a target for molecular diagnosis of TB by Dr Singh's team.

In this way a novel method for differentiating MTB complex from NTM was developed, that uses a pair of PCR primers targeting 16S rRNA and esat-6 genes. These PCR primers have been patented. Dr Singh's group did a study based on this methodology at AIIMS (Published in the Japanese Journal of Infectious Diseases, Feb 2007) and found that the prevalence of non-tubercular mycobacteria was significantly high in the AIDS patients than in HIV negative cases (24 percent Vs 4.3 percent).

"The rapid and accurate diagnosis of mycobacteriosis by this combination of genus specific PCR primers with novel esat-6 primer set gains significance in the light of the fact that most non-tubercular mycobateria are not susceptible to conventional anti-tubercular treatment, it becomes desirable to identify these isolates up to species level, particularly from AIDS and clinically unresponsive cases, which are often considered as drug resistant cases," stated Dr Singh.

"By using this multiplex PCR directly on clinical samples, with the help of genus and species specific primers, the causative agent can be identified in a single run. The technique has already been standardized. The gel-based technology is ready for commercialization and for developing a rapid, non-gel based technique, it will take us another year or so. We ultimately want to have a strip or a dot kind of simple diagnostic test," he added. In addition, Dr Singh is also working with the National Physical Laboratory, New Delhi, on the concept of biosensors for TB diagnosis.

Diagnostic kit for leishmaniasis

Dr Sarman Singh had earlier developed a diagnostic system (Signal-KA), first of its kind in the world, for the early detection of visceral leishmaniasis. More than 90 percent of the world's cases of visceral leishmaniasis are found in India, Bangladesh, Nepal, Sudan, and Brazil. This is a parasitic disease spread by the bite of infected sand flies.

Signal-KA diagnoses the deadly visceral leishmaniasis (VL), commonly known as Kala-azar (KA), with 100 percent sensitivity and specificity. It is based on a novel recombinant antigen indigenously developed by Dr Singh's group at the department of laboratory medicine, AIIMS, through DBT support. The test is based on detection of antibodies in the patient's blood, against a novel recombinant antigen cloned and expressed from a clinical isolate of Leishmania donovani. The process and the product have been internationally patented in 2003 and the technology has been transferred to Surat-based Span Diagnostics in 2005. The company then has developed an immunofiltration rapid test using this patented product and process, thereby reducing the test time to less than 10 minutes. This has made this rapid flow-through test field friendly that can be used in the remotest areas of the country without the requirement of refrigeration, electricity or any other paraphernalia. Priced at Rs 100, the test system produces results that can be seen with naked eyes. Span did the clinical and field trials in various Kala-azar endemic and non-endemic areas with highest possible accuracy and reproducibility. This kit was dedicated to the nation by the Union minister for science and technology and earth sciences, Kapil Sibal in February last. It is learnt that the kit has received good response and has made an impact on the sale of its competitive brands.

In recognition of his efforts, the biotech product and process development and commercialization award 2006 was conferred on Dr Sarman Singh for the indigenous rapid test system.

Now the kit is being exported for its evaluation in Sudan, Ethiopia, Kenya and other countries through WHO, Geneva. Early and rapid detection of the disease is extremely important to initiate specific treatment and curtail the transmission of this vector borne disease. The disease is fortunately treatable if diagnosed at an early stage. Therefore, this development is a landmark development in the direction of Kala-azar Elimination Program, which envisages elimination of this disease from India and Nepal by 2012.

"With Signal KA already in the market, now we have further worked on the Post Kala-azar Dermal Leishmaniasis (PKDL) strains and conclusively proved that the strains causing PKDL and VL are genetically different strains. This negates the earlier premise that the strains causing PKDL are same as the VL causing strains," shared Dr Singh. His lab has already created PKDL specific recombinant antigen, which could form the basis of a two dot test for differentiating between the two kinds of leishmaniasis (VL and PKDL). Dr. Singh has also designed novel set of PCR primer to differentiate between VL and PKDL strains of leishmania. These primers have also been patented. "Now the privates sector has to come forward for commercializing this technology but since the number of PKDL patients are very few so the interest of the private parties has been lukewarm," he said.

Hopefully, some private entrepreneur will soon take this technology also to the masses.

Rolly Dureha