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DIAGNOSIS OF RHEUMATIC INFECTIONS CAUSED BY GROUP A *STREPTOCOCCUS PYOGENES*: FUTURE INVESTIGATION BY NANOTECHNOLOGY

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Group A Streptococcal (GAS) infections can lead to the development of severe post infectious sequelae such as rheumatic fever (RF) and rheumatic heart disease (RHD). Streptococcal infections are the major health concern in developing countries and in indigenous populations of developed nations. Historically a throat culture was used to confirm GAS infections. Different, rapid methods are developed for the diagnosis of the infections of *Streptococcus pyogenes* to immunologically detect the bacteria using the procedures such as latex agglutination assay, co-agglutination assay; enzyme immunoassay, gold particle assay and liposomes immunoassay have been developed. Recently nucleotide probes methods are also used for the diagnosis of GAS infections. Advancement in nanotechnology can be use to develop nanosensor and micro-electro-mechanical system for diagnosing streptococcal infections.

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1. Introduction

Group A streptococcus (GAS) causes illness ranging from uncomplicated pharyngitis to life-threatening necrotizing fasciitis, toxic shock, and rheumatic fever. The term rheumatic refers to any aches or pains in bones, joints and muscless. Rheumatic fever is named after the complaints it causes, i.e. fever accompanied with rheumatism. Many time cramps or growing pains in children is often misinterpreted with rheumatic fever and feared due to the death and disability it causes. Rheumatic fever is a disease occurring mostly among the children's of 5 to 15 years. This disease follows an episode of upper respiratory tract infection. Symptoms of rheumatic fever vary greatly, depending on which parts of the body become inflamed. Typically, symptoms being several weeks after the disappearance of throat symptoms. The most common symptoms of rheumatic infection are joint pain, fever, chest pain or palpitations caused by heart inflammation (carditis), jerky uncontrollable movements (sydenham's chorea), a rash and small bumps (nodules) under the skin. A child may have one symptom or several. In the first half of the twentieth century, the group A S*treptococcus* (GAS) was established as the sole etiologic agent of acute rheumatic fever (ARF) [1]. In the century's latter half, the clinical importance of variation in the virulence of strains of GAS has become clearer. Although still obscure, the pathogenesis of ARF requires primary infection of the throat by highly virulent GAS strains. These contain very large hyaluronate capsules and M protein molecules. The latter contain epitopes that are cross-reactive with host tissues and also contain superantigenic toxic moieties. In settings where ARF has become rare, GAS pharyngitis continues to be common, although it is caused by GAS strains of relatively lower virulence. These strains, however, colonize the throat avidly and stubbornly. Molecularly distinct pyoderma strains may cause acute glomerulonephritis, but they are not rheumatogenic, even though they may secondarily colonize and infect the throat [2]. A GAS continued in the rheumatogenecity for approximately 20% of sore throat. GAS pharyngitis in the developed

countries has been accumulating for decades, but the critical importance of GAS strain virulence in the pathogenesis of RF is still not widely appreciated [3]. Recent studies of the genetic control of the expression of various virulence factors of group A streptococci are beginning to explain the wide spectrum of group A streptococcal diseases and their striking epidemiological variation [4-5-6]. Since world war II , the treatment of GAS pharyngitis has been strongly directed towards the primary prevention of rheumatic fever and suppurative complications.

2. Problems in diagnosis of GAS infections

It is difficult to differentiate GAS pharyngitis with much more common viral throat infections. The presence of fever , exudative pharyngitis , tender enlarged cervical lymph nodes and the absence of cough , coryza and hoarseness have at best a better predictive value no better than 70% [7]. On the other hand for non-exudative, sporadic GAS pharyngitis , the clinical diagnosis is hardly better than an even guess [8]. To complicate matters, some viral infections, notably infectious mononucleosis and adenovirus, may also produce exudative pharyngitis. Laboratory confirmation is therefore required for precise diagnosis, particularly in settings where RF is still present, and certainly when RF is prevalent. A clear diagnosis of RF, and particularly rheumatic carditis, is important since it commits an individual to prolonged prophylactic antibiotic therapy. As RF becomes rare in developed countries, its familiarity to younger physicians also wanes. Moreover, the diagnosis of RF may be particularly difficult when it presents as an isolated major manifestation. Unfortunately, RF remains a clinical syndrome without a single pathognomic feature. The Jones criteria became particularly useful in clinical investigation to ensure admission to clinical studies of a uniform cohort of clear-cut cases of ARF [9]. Thus, these guidelines avoid over diagnosis but do not always capture the more subtle manifestations of the disease. The major manifestations are *Polyarthritis, carditis, and chorea,* and less frequently, but no less characteristically, *subcutaneous nodules and erythema marginatum*. In the 1960s, when antistreptolysin O and other GAS antibody titers generally became available to clinical laboratories a committee of American Heart Association revised the Jones criteria suggesting that, particularly those of polyarthritis, could be strengthened by including evidence of antecedent GAS infection [10]. Some limitations were emphasized; circumstances in which a diagnosis of ARF may be made without strict adherence to the Jones criteria [11]. Most patients with recurrent ARF also fulfill the Jones criteria, but in some the diagnosis of a recurrence is less obvious. For example, when rheumatic valvular disease preexists, clear recognition of a new bout of carditis requires evidence of fresh cardiac injury such as pericarditis, acute cardiac enlargement or congestive heart failure, or a newly detected murmur from a valve not previously affected. The Jones Criteria, therefore, apply more readily to initial attacks, and more diagnostic latitude is sometimes needed to interpret recurrent carditis in patients with pre-existing rheumatic heart disease. The steps in the evolution of the modification of the Jones Criteria have been reviewed in detail [12].

3. Diagnosis of rheumatic infections

RF and RHD are a major health concern worldwide but especially in indigenous community. Streptococcal M-protein was identified over 60 years ago by Rebecca Lancefield [13]. M-protein is major virulence factor due to its antiphagocytic property [14]. It is essentially important to perform the accurate diagnosis at an early stage of infection and select the optimal antibacterial agents.

In general biological procedures it is mandatory to (i) analyse the clinical symptoms (ii) culture the specimen and (iii) isolate and identify *Streptococcus pyogenes* from the culture and then the therapeutic strategy is determined after these items are sufficiently examined.

3.1 Classical methods

Historically a throat culture was used to confirm GAS infections [15]. In routine throat swab analysis clinical samples are streaked on blood agar plates and incubated at 37^0C for 24 hrs in the presence of 5% CO_2. Plates are analyzed for presence of characteristics appearance of the hemolytic ring around the colonies grown on the plate (β-hemolysis) and further identify *Streptococcus pyogenes* by direct smear and staining by Gram stain. Throat cultures, however, have the advantage of revealing the presence of mucoid colonies on blood agar to alert clinical laboratories. Early detection of clusters of large mucoid GAS colonies in throat cultures signal danger. However it is always accompanied by the difficulties in the identification of the causative bacteria. Actual identification of the causative bacteria is quite difficult because of a variety of shapes of the colonies which are formed dependent upon the culture conditions, thus the identification is avoided. Also the bacteria from the specimen have to be proliferate for along time in the appropriate medium to the number large enough for applying drug sensitivity and then at least 3 to 4 days of incubation period is required to attain the result of test. Thus rapid diagnosis cannot be achieved in accordance with the classical method. Additionally in case of the diagnosis of the patients who had already been treated with a large dose of antibiotics when the possible infection was suspected, the growth and proliferation of the bacteria may be prevented

even if the bacteria are present in the specimen. Accordingly the feasibility of successful culture of the bacteria from these specimens may become extremely low.

3.2 Molecular Methods

3.2.1 Rapid Antigen Detection Tests (RADT)

In 1978, El Kholy *et al* published the results of a study describing the identification of GAS directly from tonsillar scrapings within 30 min, by using a modified nitrous extraction procedure coupled with an immune precipitin reaction [16]. This study proved to have a profound impact on the diagnosis of streptococcal pharyngitis infection, paving the way for the introduction of commercially available rapid antigen kits for GAS. The first rapid antigen kit for GAS, which was introduced in the early 1980s, utilized a latex agglutination method. In addition to methodological differences in rapid antigen tests for GAS, commercial kits can be also divided into two groups based upon the complexity classification of the Clinical Laboratory Improvement Act of 1988: those with a waived status and those without a waived status. A current list of rapid antigen tests for GAS, waived and not waived, can be found at the U.S. Food and Drug Administration website. Improvements in ease of performance and the proliferation of test depend upon significant improvement in test sensitivity. There are numerous published studies to demonstrate that the 90 to 95% sensitivity often claimed in packages inserts is often not attained [17-18-19]. The package insert for the Abbott TestPack Strep A kit claimed a sensitivity of 95.7%. In contrast, however, the specificity of all of the rapid antigen tests for GAS was generally >97% [20]. Laboratory confirmation of the presence or absence of GAS requires rapid antigen detection tests (RADTs) as well as throat culture. Although in the absence of an immune response, the presence of GAS by either test is only a presumptive diagnosis, a negative test by either of these methods is a powerful negative predictor of GAS pharyngitis (>95%). RADTs are currently quite popular in microbiological laboratories because they can be processed from fresh throat swabs and reported in very less time [21]. When greater precision of diagnosis is critical and the RADT is negative, a throat culture is still recommended. In current clinical practice, the practical value of throat cultures, especially for adults, has become controversial, especially where RF is no longer prevalent [22-23]. Guidelines by expert committees of the American Academy of Pediatrics, the Infectious Disease Society of America and American Heart Association [24] favor greater precision in diagnosis by the use of throat cultures. In the interest of reducing excessive antibiotic usage that promotes emergence of resistant organisms, the American College of Physicians' published its own guidelines for diagnosis of GAS in adults. These guidelines eschew throat cultures in favor of RADTs, and suggest that even the latter may be unnecessary in the presence of

clearly expressed clinical manifestations of GAS pharyngitis. It is apparent that the threat of rheumatic fever, or other severe complications, and the economic resources available to a given population will influence the practicality of the use of laboratory tests for support of the diagnosis of GAS pharyngitis. Moreover, annual and seasonal variation in the severity of GAS disease may be another factor influencing the local perceived need for precision in the diagnosis of GAS pharyngitis.

In 1987 A novel color test for the rapid detection of group A streptococci has been developed [25].The test, designed to be suitable for use in clinical laboratories as well as by less experienced personnel, incorporates the simplicity of latex tests with a color change to indicate the presence of group A streptococcal antigen. The test, which takes 5 min, was evaluated with 646 throat swabs, with a 15.6% incidence of group A streptococci; for swabs which yielded 10 or more group A streptococcal colonies in cultures, the sensitivity was 96.8%, and the specificity was 99.1%. In addition, the color test was 100% sensitive and specific when used to detect group A streptococcal antigen in beta-hemolytic colonies from culture plates. Recently, rapid and advance methods for the diagnosis of infections of *Streptococcus pyogenes* has been developed such as co-enzyme agglutination, enzyme immune assay, gold particle assay and liposome immunoassay have been developed. All of these methods are carried out by extracting C-polysaccharide on the surface of the bacterial bodies of *Streptococcus pyogenes* with nitrous acid or enzyme and detecting the presence of the bacteria using the polysaccharide as an antigen. RADT is also based on this principle. However, the above immunological methods are problematic because the results there of are often inconsistent with the results obtained by the culture method and because the manipulation for carrying out the methods are complicated. Further species specificity of these immunological methods is not satisfactory due to the properties of this diagnosis method in which antigen-antibody reactions are utilized, namely, detection of the bacteria except for *Streptococcus pyogenes* which carry Group A antigen. Although the infectious diseases caused by *Streptococcus pyogenes* are diseases of which rapid and accurate diagnosis has been required, the conventional and enzyme mediated diagnosis method could not have complied with such demand.

3.3 Nucleic Acid Based Identification Method

Nucleic acid amplification techniques take tiny amounts of DNA or RNA and replicate them many times, which can detect minute traces of an organism in a specimen, avoiding the need for culture. These techniques are particularly useful for organisms that are difficult to culture or identify using other methods (eg, viruses, obligate intracellular pathogens, fungi, mycobacteria, some other bacteria), or are present in low numbers. Over the past two decades , polymerase chain reaction (PCR) has been used extensively as a diagnostic tool in various fields , such as genetic screening , infectious disease diagnosis. PCR is a very sensitive method which is currently being used in many laboratories. With advancement of PCR and its application in modern diagnosis the diagnosis of streptococcal infection has been improved. Different workers have developed PCR methods alone or in association with other methods for diagnosis of GAS infections. PCR base diagnosis depends upon e*mm gene* which is associated with isolates of group A streptococci, confirming the involvement of the M-protein as an epidemiological marker [26-27]. A major limitation of PCR based diagnosis is the inhibition of DNA polymerase reaction by many substance found in clinical materials [28].

3.3.1 Probe base diagnosis

Probe base diagnosis is based on the fact that probe has a specific reactivity towards DNA or RNA derived from causative bacteria of infectious diseases, specifically *Streptococcus pyogenes* and to elucidation of the nucleotide sequences of the portion of the gene essentially derived from *Streptococcus pyogenes* which should be comprised in the probe.

3.3.2 Oligonucleotide Microarrays for Identification of Streptococcus pyogenes

DNA microarray is a high-throughput technology used in molecular biology and in medicine. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles of a specific DNA sequence. The ideal assay for GAS identification not only would provide quick and accurate diagnostic results but also would reveal antibiotic resistance patterns and genotype information, aiding not only in treatment but in epidemiologic assessment as well. The oligonucleotide microarray is a promising new technology which could potentially address this need [29]. Now a day's oligonucleotide resequencing microarrays is used for identifying GAS and its associated antibiotic resistance markers. It is widely accepted technology for GAS diagnosis. Combine use of resequencing DNA microarrays with either random nucleic acid amplification or multiplex PCR for GAS detection are most advance technology these days. In detecting *Streptococcus pyogenes* from coded clinical samples, this approach demonstrated an excellent concordance with a more established culture method. This technology showed the potential of resequencing microarrays for efficient and accurate detection of GAS and its associated antibiotic resistance markers with the benefit of sequencing information from microarray analysis.

3.3.3 Future diagnosis by Nanotechnology

Nanotechnology is an upcoming technology widely applicable in molecular diagnostics. Now days Nanoparticles and carbon nano tubes are of great research interest. There are many drawback in diagnosis of rheumatic infections by above described methods. To increase the sensitivity and selectivity of diagnosis more diagnostic protocol has to be proposed.

Scanometric DNA Array Detection with Nanoparticle Probes method has also been developed to detect combinatorial DNA arrays. In this method oligonucleotide targets are lebelled with nanoparticle rather than fluorophore probes [30] .Nanotechnology integrated with electrochemistry could be a boon in diagnosis. Many workers diagnosed human disease by using Nanoparticles [31] and Mini sensing chip for point-of-care diagnosis utilizing micro-electro-mechanical system and nano-technology has also been developed [32].

These type of advance technology using interdisciplinary research area can develop more sensitive, reliable , rapid, cost effective and miniaturized lab on chip method for diagnosis of rheumatic infect infections.

4. Conclusions

Due to advances in microbiological analysis and PCR invention by kerry mullis in 1983 detection of infectious agents has gone easy. Among all the diagnostics methods throat culture methods is still applied in the microbiological laboratory for preliminary detection of bacterium in the clinical specimens. However, RADT are currently quite popular with clinicians because they can be processed from fresh throat swabs and reported within hours. They are available in convenient commercial kits. The specificity of RADT has been reported to be high as 95%, their sensitivity may be considerably less. So if RADT is negative a throat culture is still applied. Fast discovery of nanotechnology is also providing a new pace in the area of molecular diagnostics. Several Fast laboratory confirmations are therefore required for precise diagnosis particularly in setting where RF is still present, and certainly when RF is prevalent. In recent years, there has been considerable interest in the development of Nanosensors and DNA Sensors due to their numerous applications in diagnosis of infectious agents. To achieve maximal sensitivity and minimal assay time nanotechnology are the current interest in the area of molecular diagnostics.

In conclusion nanotechnology can be integrated with existing technologies to enhance disease diagnosis.

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