MICROBIOLOGICAL DIAGNOSIS OF STREPTOCOCCAL PHARYNGITIS: LACUNAE AND THEIR IMPLICATIONS

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Abstract

Post-streptococcal sequelae, especially acute rheumatic fever/rheumatic heart disease continue to occur in significant proportions in many parts of the world. Despite several attempts with various intervention strategies, little success has been achieved in the control of acute rheumatic fever/rheumatic heart disease in India. The success of the control programmes depends upon timely primary prophylaxis with benzathine penicillin for which a microbiological confirmation of group A streptococcal pharyngitis is essential. Isolation of beta hemolytic streptococci from throat cultures and their identification as GAS in the laboratory, clinches the microbiological diagnosis while demonstration of a ‘significant rise’ in antibody titers such as Anti-streptolysin O and Anti-deoxyribonuclease B differentiates it from a group A streptococcal carrier state or pharyngitis of a viral etiology. Despite the easiness with which these can be achieved, many laboratories in India are not equipped to do so. Enhancing bacteriological and serological facilities in laboratories across the country will drastically improve the clinician’s ability to diagnose bona fide GAS pharyngitis and help to institute penicillin prophylaxis at the appropriate time. This will go a long way in enhancing the compliance to penicillin prophylaxis which is the cornerstone of any RF/RHD control program.

Key words: Group A Streptococci (GAS), Pharyngitis, Post-streptococcal sequelae, RF/RHD, ASO, ADNB

Rheumatic fever (RF) and rheumatic heart disease (RHD) continue to be a problem of major concern in most developing countries including India.\(^1\) Given a prevalence rate of 4 to 6 per 1000 children per year,\(^7\) there is approximately 1 to 1.25 million cases of RF/RHD in India today. Despite sporadic reports of a declining trend in the incidence of RF/RHD,\(^8\) these two complications continue to occur in endemic proportions and engage cardiologists and cardiac surgeons alike. Apart from the increased morbidity and mortality due to these conditions, the high cost of surgical intervention for the treatment of later complications of RF/RHD puts heavy economic burden on the families of affected children. Timely diagnosis and treatment of streptococcal pharyngitis with benzathine penicillin therefore acquires special significance in the prevention of RF/RHD in endemic communities.

A microbiological diagnosis of clinically suspected pharyngitis is essential for the confirmation of a bona fide group A streptococcal (GAS) infection.\(^9,10\) About two-thirds of all clinical pharyngitis is caused by viruses, often self-limiting and for which no effective treatment exists. On the other hand, adequate treatment of streptococcal pharyngitis with benzathine penicillin is the method of choice for the prevention of post-streptococcal sequelae. Therefore, differentiating streptococcal pharyngitis from that of a viral etiology will help the clinician to administer penicillin to the affected child at the appropriate time.

Clinical Features

In general, the clinical features of GAS pharyngitis are not specific and cannot be easily differentiated from that of non-streptococcal pharyngitis; therefore, sole diagnosis on clinical grounds is highly improbable. However, various clinical and epidemiological factors may narrow down the diagnosis, which may be further confirmed by laboratory methods.\(^11,12\) Epidemiologically, GAS pharyngitis is often seen in children between 5 and 15 years of age and tends to occur in colder months of the year. Patients with GAS pharyngitis often complain of pain while swallowing, fever, enlarged cervical lymph nodes and fatigue. Headache, nausea, vomiting and abdominal pain may be seen especially in children. Tonsils are reddened and swollen. In acute cases, the roof of the mouth may have fine petechial lesions.\(^13\) Although none of these are specific for GAS pharyngitis, absence of fever or presence of clinical features such as cough, hoarseness and running nose are common symptoms of viral upper respiratory infections.\(^13\)

Clinical Specimens

The success in isolating GAS in culture or getting a positive result with a rapid antigen detection test lies in collection of a well-taken throat swab. Two swabs may be rubbed well over both the tonsils and posterior pharyngeal wall, taking care not to touch the oral cavity or any other oropharyngeal region.\(^9,13\) Specimens should be collected prior to any antibiotic treatment and processed in the laboratory.
without any delay. Blood sample may be collected for antibody tests.

Isolation and identification of Group A β-hemolytic streptococci (GAS)

Laboratory diagnosis of streptococcal pharyngitis depends upon the successful isolation of β-hemolytic streptococci (BHS) and its identification as GAS. In itself this is not a difficult task, provided one has easy access to quality sheep blood agar plates (BA). Pre-sterilized quality BA plates are either not easily available in the Indian market or they are expensive; therefore most laboratories prepare them in their own laboratory. Maintaining a sheep pen for the purpose of collecting sterile sheep blood for preparation of BA plates can be both expensive and exasperating. Collecting sheep blood from the local slaughterhouses is an eminently viable proposition, provided one takes extra precautions to ensure absolute sterility of blood during collection. Many laboratories circumvent this problem by using outdated human blood obtained from blood bank. This is neither scientifically accepted nor satisfactory since human blood often contains antibiotics and other anti bacterial substances including anti-streptococcal antibodies that inhibit the growth of GAS. Using horse or ox blood is also recommended; but in Indian situation, they are more difficult to obtain than sheep blood, which therefore is recommended for the isolation and characterization of BHS/GAS.

Identification of GAS

Bacitracin susceptibility

In many laboratories bacitracin susceptibility test is the method of choice to identify GAS. This test has a sensitivity of >95%, is only a presumptive test and is not recommended since group G and C streptococci can give false positive results. Batch to batch variation may occur in the commercial discs and therefore it is essential to test each batch for quality control with known GAS strains. If this is done regularly, one can resort to GAS identification by this method.

Group identification

The recommended method of GAS identification is by testing β-hemolytic colonies on BA for group A specific carbohydrate antigen (ACHO). Identification of GAS by grouping is a specific method although there are isolated cases where group A has been identified as group G or vice-versa, due to antigenic cross-reactions. Grouping can be done on the organism isolated from throat cultures or extracts prepared directly from throat cultures. Numerous methods are available in the laboratory for this, of which the time tested Lancefield’s hot-acid extraction technique and Fuller’s formamide extraction method are the most widely used. The micro-nitrous acid extraction procedure of El Kholy is a rapid method that can be standardized in any routine diagnostic laboratory with ease. When performed together with the co agglutination reagents, this technique can identify a GAS strain from a BA plate in about 30-45 minutes. In general GAS can be identified on the same day if BHS are well isolated on the BA plate. Otherwise it takes an additional 24 hours for the final identification. The rapidity with which GAS is identified in a throat culture isolate is important because delay in diagnosis may mean delay in treatment and this could unnecessarily predispose the child to post-streptococcal sequelae.

Unavailability of grouping antisera used for grouping of BHS is a deterrent for many laboratories to identify GAS. Commercially available antisera are exorbitantly costly, while producing them in routine diagnostic laboratories requires certain expertise and is labor intensive. More recently, use of animals for antisera production has been restricted for ethical considerations. In our laboratory, grouping antisera were first prepared in early 1960’s, a practice that has been followed till date. Intravenous immunization of locally available rabbits with heat-killed formalin inactivated group A cells result in the production of high potency antisera. If properly preserved at +4°C with added merthiolate, these antisera will remain potent for many years. In our experience such homemade antisera are of high quality and highly cost-effective. Once produced, these antisera can easily be modified into cost-effective co agglutination or latex particle based reagents.

We first produced such co agglutination reagents in our laboratory to test groups A, B, C, F and G in 1977 and have used them for group identification since then. Major advantage of this system is that highly potent antisera are diluted and therefore used in small quantities during preparation of the co-agglutination reagent. For example, 100 µl of group A antiserum is added to 1 ml of 10% Staph. aureus Cowan 1 cells and then diluted to 10 mL with phosphate buffer. Since we use only 50 µl of the reagent for identification of each strain, one can test approximately 200 strains with 10 ml reagent. Therefore, as compared to commercial products, the homemade reagents work out to be extremely cheap and can be used to detect GAS from BA or broth cultures. If properly preserved, they keep well for several weeks under ordinary refrigeration.

Rapid Antigen Detection Tests (RADTs)

Introduced in the 1980s, RADTs have a basis of extracting carbohydrate antigen from BHS and identify them by immunological methods. Despite their higher cost, they can provide a result within a few hours even when done directly on throat cultures. This enhances the patient compliance to treatment and has been shown to significantly increase the number of patients appropriately treated for GAS pharyngitis. Although tests such as latex agglutination, co-agglutination, enzyme immunoassays, liposomal and optical immunoassays have been employed for this purpose, most of them have sensitivity between 70% and 90%, but with a specificity of >95% with culture as a gold standard. Therefore a negative
RADT should always be confirmed with a throat culture. Though molecular techniques have been standardized recently with improved sensitivity, factors such as cost and feasibility has hindered their use in smaller laboratories.

Use of commercially available kits to identify GAS directly from throat cultures has not become popular in India because of their cost. Evaluation of such kits in countries where group C and G are also endemic has shown high degree of false positivity (personal observations). Since penicillin treatment depends upon a positive test, such cases may result in over treatment with penicillin.

Interpretation of Cultures

In countries where streptococcal infections are endemic, pharyngeal carriage of GAS is a common event. Therefore isolation of GAS from throat cultures should be interpreted with caution, especially in a child with viral pharyngitis. In such instances, determination of four-fold rise in titers of anti-streptococcal antibodies can differentiate bona-fide GAS pharyngitis from GAS carrier state.

Serological Diagnosis

Historically, determination of anti-streptolysin O antibodies (ASO) had been the mainstay of confirming a diagnosis of GAS pharyngitis as well as RF. Demonstration of a significant or four-fold rise in titer on paired serum samples taken at an interval of 7 to 14 days apart will indicate an ongoing or an acute infection. On the other hand, presence of GAS in throat in the absence of a significant rise in antibodies indicates a carrier state and no GAS infection. Practical difficulties in getting two serum samples from children and the time taken to demonstrate a four-fold rise in titer make this unfeasible on a routine basis. Alternately, titer obtained with a single serum sample can be interpreted based on a cut-off value defined as the upper limit of normal (ULN). ULN represents the highest level of antibodies that can be observed in 20% of normal individuals who have demonstrable antibodies in them. It is to be emphasized that ULN titers should be determined in different geographical areas because such titers are distinctly higher in endemic regions as compared to non-endemic areas. Any ASO titer above these cut-off values will indicate a GAS infection. This method is generally convenient and reliable although an antibody response from an earlier GAS infection may confuse the final interpretation of the current ASO titer.

Therefore, isolation of BHS and its identification as GAS in the presence of clinical symptoms of pharyngitis confirms its streptococcal etiology and needs no further confirmation by antibody tests. ASO and ADNB are often used to: (1) confirm GAS infections where facilities for culture do not exist (2) to confirm doubtful positive rapid antigen detection test or (3) confirm a diagnosis of post-streptococcal etiology. Thus, culture techniques, which give direct evidence of GAS etiology and takes less time than antibody detection, are recommended in a clinical setting. Often this can be achieved within 24 hours of processing throat culture which will help the clinician to institute penicillin prophylaxis at the earliest.

One major disadvantage of ASO test is its inability to demonstrate an antibody response in many cases of impetigo. In such cases, determination of antideoxyribonuclease B antibody (ADNB) is a valuable method of diagnosing GAS infection. The ADNB test is more sensitive and the magnitude of antibody response is higher than that of ASO both in cases pharyngitis and impetigo as well as the non suppurative sequelae.

For many years, we were determining ASO and ADNB titers by the conventional micro-titer technique using SLO and DNase B enzyme produced and standardized in our laboratory. Though these tests performed well in our hands for many years, they are labor intensive, cumbersome and time consuming. Therefore, recently we changed over to nephelometric titration, which is an automated, simple and rapid method that does not require much technical expertise. However, the equipment is expensive and therefore, many laboratories may find it difficult to establish this technique for antibody titrations.

Many laboratories in India now use commercial latex agglutination-based ASO kits for routine diagnostic purpose. Caution need be exercised while interpreting the titers for two reasons. Firstly, there is no quality control on these kits after they are imported to India so that batch to batch variations do occur while using them in diagnostic laboratories. Secondly, the cut-off titers recommended in these kits are based on evaluation done in countries where GAS infections are less endemic than India. Laboratories using these kits should therefore test the quality of each batch of the kit using appropriate controls, on a routine basis. They should also determine their own cut-off titers for the kit by determining ULN for their population using the same brand of kit.

Antibiotic Susceptibility Testing

Despite reported penicillin tolerance, GAS continues to be exquisitely susceptible to penicillin which therefore is the drug of choice to treat the infections caused by them. Thus in practice it is not necessary to test their susceptibility on a routine basis; however it is imperative to look for emergence of resistance to penicillin in our locality. More importantly, resistance to other antibiotics such as macrolides is on the increase which can be monitored only by regular testing of GAS strains to such antibiotics. In our laboratory, we have been monitoring antibiotic resistance in GAS since 1970 and this has helped us to detect a significant increase in erythromycin resistance since 1993.

Empirical treatment of clinical pharyngitis with various antibiotics including penicillins is a common practice in general practice. However this cannot be recommended in highly endemic situations, for several reasons. Firstly, oral antibiotics...
do not give as much coverage as benzathine penicillin, to patients who may later go on to develop RF/RHD due to lack of adequate antibiotic protection. Secondly, over treatment with penicillin may pave way to increased penicillin tolerance and subsequent penicillin resistance. Finally, one may encounter larger number penicillin anaphylaxis which can significantly reduce patient’s compliance to antibiotic treatment. Thus scientifically, it is better to institute the treatment after confirming a GAS etiology which would overcome the above problems.

Epidemiological Typing

Typing of GAS strains based on T and M proteins are of epidemiological interest. With a GAS vaccine becoming a reality now than ever before, data on the prevalence of M types in a community has acquired great significance. Conventionally, M typing was a tedious and almost impossible task and workers used to generate M type data extrapolated from the data obtained by T typing. With the advent of gene sequencing technology, M typing technique has been revolutionized and almost 100% typing can be done with the modern gene sequencing method. We have recently standardized this method in our laboratory and result on typing of 227 GAS strains show high heterogeneity among GAS strains circulating in the community. This is quite different from what one sees in non endemic temperate countries where a few M types are responsible for most of the invasive GAS infections. While most diagnostic laboratories will find it difficult to adopt this technology, it will be important for us to generate such data on the prevalence of M types in our community. This will help us to assess whether a vaccine designed in the context of a non endemic population will be equally effective in an endemic situation.

Dos and Don’ts

Physicians should identify the suspected cases of GAS pharyngitis with the given clinical and epidemiological features and send appropriate specimens for laboratory confirmation. Throat cultures should be collected with care as the success in getting reliable results relies on their proper collection. Sheep blood and not human blood should be used for preparing blood agar. GAS should be identified by grouping techniques with either in-house or commercially prepared antisera. Bacitracin susceptibility could be used for routine identification of GAS if appropriate quality control is carried out with every batch of bacitracin discs. RADTs can be used for rapid diagnosis but all negative tests should be confirmed with throat culture. ASO and ADNB may be used in patients for the confirmation of diagnosis, but should not be used to interpret when throat culture is negative. Interpretation should be made in comparison with ULN as standardized for a particular population. Antibiotic susceptibility testing though not mandatory may be very useful to detect erythromycin resistance and penicillin tolerance if any. If possible epidemiological typing may be undertaken to determine the M types circulating in the community which may be useful in assessing or developing a vaccine at a later stage.

Conclusions

In the final analysis, microbiological diagnosis of GAS pharyngitis can be a challenging task at every stage of its execution. Emphasis on basic bacteriological techniques, quality of the reagents and techniques as well as proper interpretation of results based on local conditions is the secret of its successful implementation. Given the magnitude of the problem of RF/RHD in India and the need for its prevention, there is no doubt that bacteriology laboratories in India will have to play a much bigger role than they do now. For this, we have to upgrade our expertise to diagnose GAS infections in the laboratory. Until such times, RF/RHD will continue to cause much morbidity and mortality because microbiological techniques form an integral part of their diagnosis.

References

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