RESEARCH PAPER

Utility of Detecting *sof* Gene as Evidence of *Streptococcus pyogenes* Infection in Acute Rheumatic Fever

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Objectives: To determine the diagnostic accuracy of polymerase chain reaction-based detection of *sof* gene compared to throat swab culture for *S. pyogenes* infection in patients with acute rheumatic fever and those with recurrence of rheumatic activity. **Methods**: 40 patients between 3 to 18 years of age, with clinical diagnosis of acute rheumatic fever or new activity in established rheumatic heart disease were included. The amplicon of 228bp of *sof* gene was detected using a polymerase chain reaction-based technique and the results were compared with throat swab culture for *Streptococcus pyogenes*. **Results**: 10 patients had a positive throat swab culture and 11 had *sof* gene detected. The sensitivity and specificity of the test was 100% and 96.7%, respectively compared to throat swab culture (P=0.001). The positive predictive value and the negative predictive value was 90.9% and 100% respectively. **Conclusions:** Polymerase chain reaction-based detection of *sof* gene provides an alternative to throat swab culture in diagnosing activity in Acute Rheumatic Fever or established Rheumatic heart disease.

Keywords: *Diagnosis, Rheumatic heart disease, Streptococcal infection*.

Evidence of Group A β-hemolytic streptococci (GABHS) infection is an essential criterion for the diagnosis of Acute Rheumatic fever (ARF) [1]. A positive throat swab culture for GABHS is considered the gold standard for evidence of previous streptococcal infection. However, it has been observed that only 10-20% patients with ARF have positive throat swab culture [2,3]. In the last two decades, a few polymerase chain reaction (PCR) based tests have been developed with sensitivity and specificity above 90% and reporting times that range from 15 minutes to 3 hours, depending on the technique [4-6]. One of these detects the amplicon of 228 base pair (bp) of *serum opacity factor* (*sof)* gene, unique to invasive species of *S. pyogenes* [7]. This test has been entirely developed in India with a turn-around time comparable to other PCR-based tests [4]. This study was conducted to determine the diagnostic accuracy of PCR based technique of detecting *sof* gene in comparison with throat swab culture.

METHODS

This cross-sectional, observational hospital-based pilot study was conducted between August 2012 and January 2014. Patients between the ages of 3 to 18 years, with a clinical diagnosis of ARF or recurrence of activity in previously diagnosed RHD (Modified Jones criteria) [1] were consecutively enrolled. All patients with congenital heart disease (CHD) were excluded. The protocol was approved by the institutional ethics committee.

Carditis was defined clinically, by the presence of a murmur in the aortic or mitral area, or development of a new murmur in an established patient of RHD. High C-Reactive protein (CRP) levels was defined as values above 6 mg/dL. Anti-streptolysin O (ASO) titers above 200 U/L were considered high as the hospital laboratory uses this cut off for positive results and does not quantify further. Two throat swabs were taken from the posterior pharynx and tonsillar surfaces. The first was sent for culture on blood agar (reference test) and the second was evaluated for the *sof* gene by the PCR based technique (index test). A Chest *X*-Ray, 12-lead electrocardiogram and 2-Dimensional Echocardiogram were also obtained.

The results of PCR-based *sof* gene detection was assessed for performance against throat swab culture using the diagnostic statistics: sensitivity, specificity, positive predictive value (PPV) and negative predictive

WHAT THIS STUDY ADDS?

- A novel PCR-based test can detect Group A β hemolytic streptococcus with reasonable accuracy as compared to throat swab culture.

value (NPV). Statistical significance was accepted at $P<0.05$, two tailed.

RESULTS

Forty patients met the eligibility criteria. Eleven patients had recurrent ARF and 29 patients were new cases (***Table* I**). Throat swab culture grew GABHS in 10 patients. The *sof* gene was detected in 11 patients. One patient presenting with isolated chorea had a negative TSC but was positive for the *sof* gene. The test demonstrated a specificity of 96.7%, and sensitivity of 100%, PPV of 90.9% and NPV of 100.0%. The diagnostic accuracy was 97.5%.

DISCUSSION

Many studies have reported that the inherent quality of the PCR and superior extraction techniques makes them superior to throat swab culture [4]. The PCR-based *sof* gene test had parameters of diagnostic accuracy that were comparable to the existing PCR based tests [4-7]. This test detects the *sof* gene, which is a bi-functional protein capable of binding the host-extracellular matrix components of fibronectin and fibrinogen [7]. The amplicon of 228 bp is a unique characteristic of the *sof* gene which is found in the invasive species of *S. pyogenes* and does not share homology with other species of streptococcus (including Group C and G streptococci) [7]. The main advantage of this test is the ability to give results within an hour, which could help in making clinical decisions in real time [7]. In addition, a positive *sof* gene test in chorea demonstrates its clinical relevance in symptoms with long latent periods, where throat swab culture are expected to be negative [8]. Another Indian PCR based test using the amplicon of 398 bp of *mga* gene to detect GABHS has been developed; however, it has not been tested in clinical studies [9].

TABLE I DISTRIBUTION OF THE DEFINING CRITERIA IN THE STUDY POPULATION (*N*=40)

Defining Criteria	*Number (%)*
New cases of ARF	29 (72.5)
No previous history of ARF	29 (72.5)
Presence of 2 major criteria	4 (10)
Presence of 1 major and 2 minor criteria	25 (62.5)
Recurrence of ARF	11 (27.5)
Previous history of ARF without residual heart disease	3 (7. 5)
Previous history of ARF with residual heart disease	8 (20)
Presence of 2 major criteria	2 (5)
Presence of 1 major and 2 minor criteria	1 (2.5)

ARF: Acute rheumatic fever.

We acknowledge a few limitations in this study. We did not conduct this study in patients presenting with sore throat. However, our aim was to determine the diagnostic accuracy of an experimental test *versus* the gold standard in patients with ARF/recurrence of rheumatic activity. Since the sample size was small it is impossible to generalize these results to the community. However, the high parameters of diagnostic accuracy, and short 'turn-around' time (<60 minutes) of this PCR-based test, makes a compelling argument to conduct large prospective community trials.

To conclude, our study shows that the PCR based detection of the amplicon of the 228 bp of *sof* gene offers an alternative way to detect *S. pyogenes* activity in patients suspected of ARF or recurrence of activity in those with RHD.

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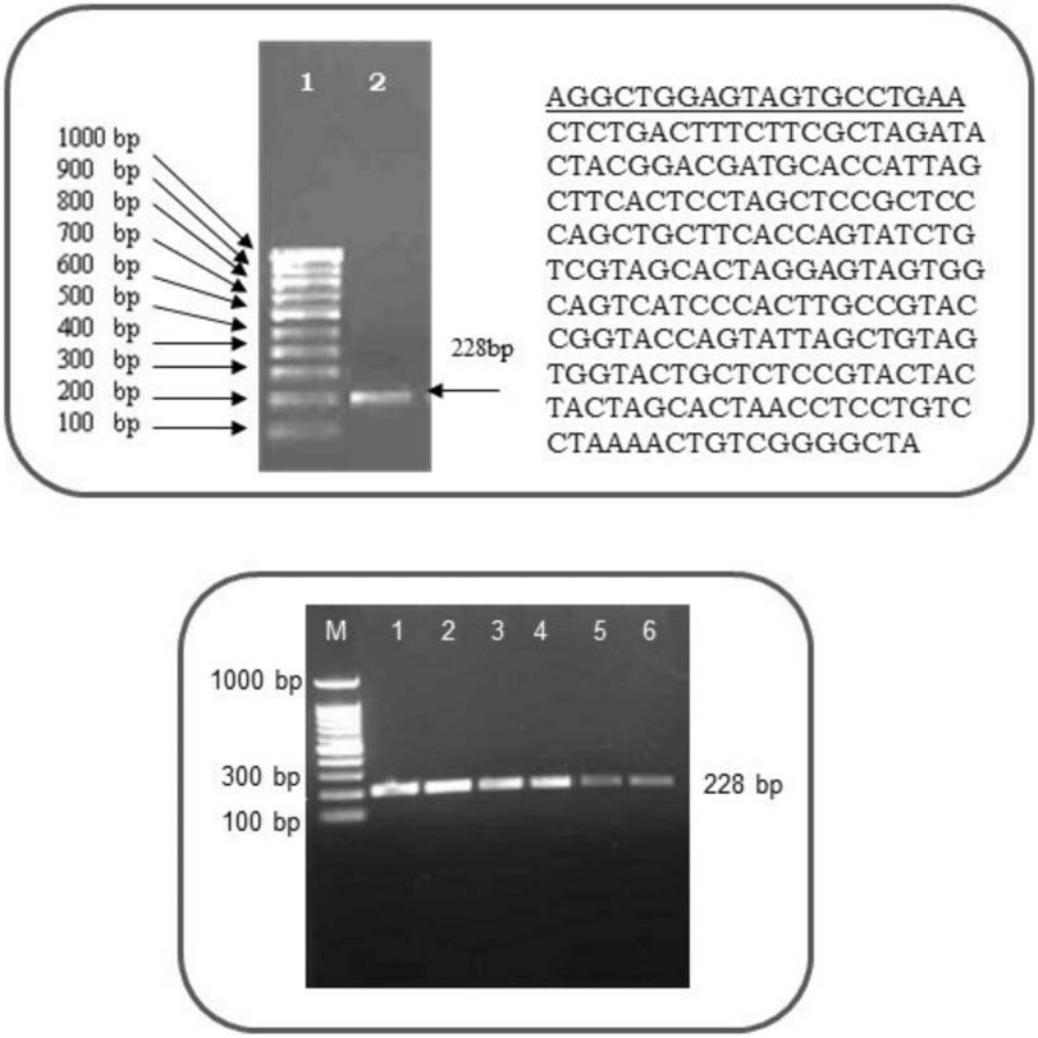

***Web Fig.* 1** *Top panel: Lane 1: DNA Ladder 100bp; Lane 2: purified PCR product (228 bp). Bottom panel: sof gene as a specific genetic marker for detection of Streptococcus pyogenes.*