Original Article

DOI: 10.7860/JCDR/2026/87746.24115

Anti-streptolysin O Titers in Patients with Post-streptococcal Complications: A Retrospective Observational Study

Paediatrics Section

SAURABH CHHOTALAL NORRIS[1], BINDA PRAKASHBHAI PIPALIYA[2], NIDHI MIHIRKUAMAR BHALODIA[3]

ABSTRACT

Introduction: Anti-streptolysin O (ASO) antibodies serve as an important marker of recent Group A Streptococcus (GAS) infection and are crucial in diagnosing immune-mediated post streptococcal complications, such as Acute Rheumatic Fever (ARF) and Post Streptococcal Glomerulonephritis (PSGN).

Aim: To evaluate ASO titers in patients with suspected post-streptococcal immune-mediated complications.

Materials and Methods: A retrospective observational study was conducted in the Department of Microbiology, Dhiraj Hospital, Smt. B.K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India, over a period of two years from January 2023 to December 2024. A total of 67 serum samples with ASO titers ≥200 IU/mL were included. Patients presenting with clinical features suggestive of post streptococcal sequelae such as fever, joint pain, haematuria, facial puffiness, or a recent history of sore throat (within 2–3 weeks) were included in the study. Demographic data, clinical presentation, ASO titers, and provisional diagnoses were recorded and analysed. Data were expressed as frequency and percentage {n (%)}.

Results: The majority of ASO-positive cases were children aged 6–15 years, 38/67 (56.7%), with a male predominance 41/67 (61.2%). The most common clinical features included joint pain 33/67 (49.2%), fever 25/67 (37.3%), and haematuria or facial puffiness 17/67 (25.4%). The highest recorded ASO titer was 1100 IU/mL. The most common provisional diagnoses were PSGN 21/67 (31.3%) and suspected acute rheumatic fever 19/67 (28.3%).

Conclusion: Quantitative ASO measurement using MISPA i3 nephelometry provides reliable and rapid detection of recent streptococcal infections. These findings reinforce its value in diagnosing post-streptococcal immune-mediated complications, particularly among paediatric populations, supporting timely clinical intervention and management.

Keywords: Immune-mediated diseases, Nephelometry, Nephritis, Paediatric, Rheumatology, Serology

INTRODUCTION

Streptococcus pyogenes, or Group A Streptococcus (GAS), is a major human pathogen responsible for a wide spectrum of infections ranging from uncomplicated pharyngitis and impetigo to severe invasive disease. These infections are particularly common in children and adolescents, especially in developing countries where overcrowding, poor sanitation, and limited access to healthcare increase transmission rates. While most streptococcal infections are self-limiting or respond well to appropriate antibiotic therapy, delayed or inadequate treatment can result in serious immune-mediated complications [1].

Among these complications, ARF and PSGN remain important causes of morbidity, especially in pediatric populations, particularly in developing countries where rheumatic heart disease remains endemic [2-4]. These conditions typically manifest weeks after the initial infection, at a time when the causative organism has often been eliminated from the primary site of infection. As a result, direct microbiological confirmation through throat culture or rapid antigen detection is frequently not possible, creating a diagnostic challenge for clinicians [5].

In this context, serological tests play a crucial role in establishing evidence of recent streptococcal infection. ASO antibodies are produced in response to streptolysin O, a haemolytic exotoxin released by GAS, and remain one of the most widely used markers for retrospective diagnosis [6,7]. Elevated ASO titers indicate recent exposure to streptococcal antigens and provide supportive evidence in patients presenting with suspected post-streptococcal sequelae. However, interpretation of ASO results requires careful clinical correlation, as antibody levels may vary with age, geographic region, and timing of sample collection [8,9].

Advances in laboratory technology have improved the reliability and standardisation of ASO estimation. Nephelometry-based systems, such as the MISPA i3 platform, offer quantitative measurement with improved precision, automation, and faster turnaround times compared to conventional latex agglutination methods [10]. These advantages make nephelometry particularly suitable for routine use in busy tertiary care laboratories and enhance confidence in reported results.

Several studies have previously evaluated the diagnostic utility of ASO antibodies in post streptococcal immune-mediated diseases such as ARF and PSGN, demonstrating that elevated ASO titers serve as useful indicators of recent group A Streptococcus infection [3,11-15]. Earlier Indian and international studies mainly focused on seroprevalence, normal ASO ranges, or comparison between healthy and diseased populations; however, limited data are available correlating ASO titers with detailed clinical presentation and provisional diagnosis using automated nephelometric methods [8,9,15]. In addition, regional variations in streptococcal exposure, environmental factors, and healthcare accessibility necessitate locally generated data for accurate interpretation of ASO results [9,11,15].

Therefore, the present study was undertaken to address these lacunae by providing a retrospective clinicoserological evaluation of ASO-positive patients using standardised nephelometric estimation.

MATERIALS AND METHODS

A retrospective observational study was conducted at the Department of Microbiology, Dhiraj Hospital, SBKS Medical Institute and Research Centre, Vadodara, Gujarat, India. Patient records

and laboratory data from January 2023 to December 2024 were retrospectively reviewed. Data retrieval, compilation, and statistical analysis were carried out from October 2025 to November 2025. Ethical approval for the study was obtained from the Sumandeep Vidyapeeth Institutional Ethics Committee (SVIEC), Vadodara, Gujarat, India, [SVIEC approval number SVIEC/ON/MRDI/RP/SEP/25] dated 26 September 2025.

Sample size: All 67 consecutive ASO-positive cases fulfilling the inclusion criteria during the study period from January 2023 to December 2024 were included in the analysis.

Inclusion criteria: Patients of all age groups and both genders were considered eligible. Individuals presenting with clinical features suggestive of recent streptococcal infection or its sequelae, such as fever, joint pain, carditis, haematuria, facial puffiness, or a documented history of sore throat within the preceding 2–3 weeks, along with ASO titers exceeding 200 IU/mL were included [11].

Exclusion criteria: Patients with incomplete clinical or laboratory records were excluded from the analysis.

Study Procedure

Sample collection and processing: Non fasting random venous blood samples (3–5 mL) were collected aseptically using sterile disposable syringes and transferred into plain Vacutainer tubes without anticoagulant. The samples were allowed to clot at room temperature for 20–30 minutes and subsequently centrifuged at 3000 revolutions per minute (rpm) for 10 minutes using a bench-top centrifuge. The separated serum was carefully aliquoted into pre-labelled sterile cryovials using calibrated micropipettes. Serum samples were either analysed immediately or stored at 2–8°C until testing, as per standard laboratory protocols.

ASO Titer Estimation: Quantitative estimation of ASO antibodies was performed using the MISPA i3 Nephelometer (Agappe Diagnostics Ltd., Kerala, India). The assay is based on the nephelometric principle, which measures light scattering caused by antigen–antibody complex formation. All tests were carried out strictly according to the manufacturer's instructions, including instrument calibration and use of internal quality control sera to ensure analytical accuracy and reproducibility [10].

Data collection: Demographic details including age, gender and area of residence, along with clinical features, ASO titer values, and provisional clinical diagnoses, were retrieved from laboratory records and patient case files. All data were anonymised prior to analysis to maintain patient confidentiality.

STATISTICAL ANALYSIS

Data were entered in Microsoft Excel and analysed using Statistical Package for Social Sciences (SPSS) software version 26.0. Descriptive statistical analysis was performed, and results were expressed as frequencies, percentages, and mean ASO titers where applicable. Findings were compared with previously published national and international studies to evaluate trends and clinical relevance.

RESULTS

The majority of ASO-positive patients belonged to the 6–15 year age group, accounting for more than half of the total study population, indicating higher susceptibility among school-aged children. Male patients were more commonly affected than females, demonstrating a clear male predominance in ASO positivity. Most patients were from urban or semi-urban areas, possibly reflecting improved healthcare accessibility and greater utilisation of diagnostic facilities in these regions [Table/Fig-1].

A recent history of sore throat was the most common clinical finding, observed in 46 (68.7%) patients, supporting the association with preceding streptococcal infection. Joint pain or arthralgia was the second most frequent symptom, consistent with immune-mediated rheumatological involvement. Fever was also commonly reported, indicating ongoing inflammatory activity. Haematuria and facial puffiness, suggestive of renal involvement such as PSGN, were noted in one-fourth of the cases, while cardiac manifestations such as chest discomfort or murmur were relatively less frequent [Table/Fig-2].

Variables	Category	n (%)
Age group (in years)	0–5	5 (7.5)
	6–15	38 (56.7)
	16–30	13 (19.4)
	>30	11 (16.4)
Gender	Male	41 (61.2)
	Female	26 (38.8)
Area of residence	Urban/Semi-Urban	45 (67.1)
	Rural	22 (32.9)

[Table/Fig-1]: Demographic Distribution of ASO-Positive Patients (N=67).

Symptoms / Signs	n (%)
Joint pain / Arthralgia	33 (49.2%)
Fever	25 (37.3%)
Haematuria	17 (25.4%)
Facial puffiness	17 (25.4%)
Chest discomfort / Murmur	6 (9.0%)
Sore throat (past 2–3 weeks)	46 (68.7%)

[Table/Fig-2]: Clinical presentation in aso-positive patients (n=67).

Post-streptococcal glomerulonephritis was the most common provisional diagnosis among ASO-positive patients, followed closely by suspected rheumatic fever. Together, these two conditions accounted for more than half of all cases, highlighting the importance of post-streptococcal immune-mediated diseases in the study population. Reactive arthritis constituted approximately one-fifth of the cases, suggesting significant musculoskeletal involvement associated with recent streptococcal exposure. A smaller proportion of patients were diagnosed with post-viral or non specific illnesses despite elevated ASO titers [Table/Fig-3].

Clinical condition	n (%)
Post-Streptococcal Glomerulonephritis (PSGN)	21 (31.3%)
Suspected Rheumatic Fever (RF)	19 (28.3%)
Reactive Arthritis	14 (20.9%)
Post-viral or Non specific illness	13 (19.4%)

[Table/Fig-3]: Provisional diagnosis in ASO-positive patients (N=67).

Most patients demonstrated ASO titers within the range of 200–400 IU/mL, indicating moderate elevation suggestive of recent streptococcal exposure. Approximately one-third of cases had titers between 401–600 IU/mL, reflecting stronger serological responses. Higher ASO titers above 600 IU/mL were observed in a smaller proportion of patients and may indicate more recent or intense antigenic stimulation. The highest recorded ASO titer in the study was 1100 IU/mL, supporting significant immunological response in selected cases [Table/Fig-4].

ASO Titer (IU/mL)	n (%)
200–400	34 (50.7%)
401–600	21 (31.3%)
601–800	8 (11.9%)
>800	4 (6.0%)

[Table/Fig-4]: Distribution of ASO titer levels (N=67).

DISCUSSION

The present study emphasises the continuing clinical value of ASO titers in identifying immune-mediated complications following group A streptococcal infection, particularly in children. Most ASO-positive cases in the present study were seen in the 6–15-year age group, with a clear male predominance. This pattern is in agreement with earlier studies and reflects the higher exposure of school-aged children to streptococcal infections and their subsequent sequelae [12,13].

Joint pain, fever, and nephritic symptoms were the most common clinical presentations among ASO-positive patients. These findings closely mirror the classical manifestations of post streptococcal conditions such as acute rheumatic fever and post-streptococcal glomerulonephritis, which are mediated by abnormal immune responses and molecular mimicry following streptococcal infection [16]. A significant proportion of patients also reported a recent history of sore throat, further supporting the association between elevated ASO titers and antecedent streptococcal infection. In such cases, where the primary infection has often resolved by the time complications develop, serological markers remain crucial for diagnosis.

An ASO estimation using the MISPA i3 nephelometry system proved to be reliable and practical in a routine laboratory setting. The method offers better precision, automation, and faster turnaround time compared to conventional latex agglutination tests, reducing subjective interpretation and improving result consistency, as also observed in previous studies [10,17]. These advantages make nephelometry particularly useful in tertiary care centres handling a large patient load.

Previous studies have also emphasised that single ASO measurements may have limited diagnostic specificity and should ideally be interpreted along with microbiological findings and serial antibody measurements whenever feasible [8,18].

When compared with similar studies from different regions the mean ASO titer observed in the present study conducted in Vadodara (460 IU/mL) was broadly comparable to values reported by Saini N et al., from Punjab (510 IU/mL), Saha SK et al., from Bangladesh (560 IU/mL), and Madaan R et al., from Ujjain (495 IU/mL) [Table/Fig-5] [12-14]. Minor variations in mean ASO titers across studies may be attributed to regional differences in streptococcal exposure, virulence of circulating strains, seasonal patterns, timing of sample collection following infection, and differences in laboratory methodologies used for ASO estimation [12-14].

Studies	Year	Region	Sample size	Common Diagnosis	Mean ASO (IU/mL)
Present study	2023–24	Vadodara	67	PSGN, RF	460
Saini N et al., [12]	2019	Punjab	80	RF, PSGN	510
Saha SK et al., [13]	2022	Bangladesh	60	RF	560
Madaan R et al., [14]	2017	Ujjain	75	Rheumatic arthritis	495

[Table/Fig-5]: Comparison of mean ASO titers in the present study and previous studies [12-14].

Minor variations in mean ASO titers across studies may be attributed to regional differences in streptococcal exposure, virulence of circulating strains, seasonal patterns, timing of sample collection following infection, and differences in laboratory methodologies used for ASO estimation [12-14]. Recent advances in echocardiographic diagnostic criteria have improved the early detection and evaluation of rheumatic heart disease associated with post streptococcal complications [19].

Limitation(s)

The present study was limited by its retrospective single-centre design and relatively small sample size, which may restrict the generalisability of the findings. Paired ASO titers and microbiological confirmation by throat culture or rapid antigen detection testing were not available in most cases. In addition, long-term clinical follow-up data could not be assessed from retrospective records.

CONCLUSION(S)

Anti-streptolysin O antibody testing remains a relevant and dependable tool for supporting the diagnosis of post streptococcal immune-mediated diseases. In clinical practice, where the antecedent infection is often no longer detectable, ASO titers provide crucial retrospective evidence of streptococcal exposure and help guide appropriate clinical decisions. The findings of this study underline the need for timely serological evaluation in patients with suspected rheumatic or renal complications, particularly in pediatric age groups. Incorporating standardised, quantitative methods such as nephelometry into routine laboratory workflows can enhance diagnostic confidence and facilitate early intervention. Continued emphasis on appropriate use and interpretation of ASO testing, along with clinical correlation and complementary investigations, is essential to reduce delayed diagnosis and prevent long-term sequelae associated with post streptococcal diseases.

Authors' contribution: All authors contributed equally to the study conception, design, data acquisition, analysis, interpretation, manuscript writing, and final approval of the submitted version of the manuscript. All authors read and approved the final manuscript.

REFERENCES

[1] Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005;5(11):685-94. Doi: 10.1016/S1473-3099(05)70267-X.

[2] Lahiri S, Sanyahumbi A. Acute rheumatic fever. Pediatr Rev. 2021;42(5):221-32. Doi: 10.1542/pir.2019-0288.

[3] Gerber MA, Baltimore RS, Eaton CB, Gewitz M, Rowley AH, Shulman ST, et al. Prevention of Rheumatic Fever and Diagnosis and Treatment of Acute Streptococcal Pharyngitis: A Scientific Statement From the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: Endorsed by the American Academy of Pediatrics. Circulation. 2009;119(11):1541-51. Doi: 10.1161/CIRCULATIONAHA.109.191959.

[4] Seckeler MD, Hoke TR. The worldwide epidemiology of acute rheumatic fever and rheumatic heart disease. Clin Epidemiol. 2011;3:67-84. Doi: 10.2147/CLEP.S12977.

[5] WHO Study Group on Rheumatic Fever and Rheumatic Heart Disease (2001: Geneva, Switzerland) & World Health Organization. Rheumatic fever and rheumatic heart disease: Report of a WHO expert consultation, Geneva, 20 October - 1 November 2001. World Health Organization; 2004. Available from: https://iris.who.int/handle/10665/42898.

[6] Johnson DR, Kaplan EL. False-positive rapid antigen detection test results: Reduced specificity in the absence of group A streptococci in the upper respiratory tract. J Infect Dis. 2001;183(7):1135-37. Doi: 10.1086/319286.

[7] Abdalla NW, McLeod JW. The antigenic properties of streptolysin. Br J Exp Pathol. 1939;20(3):245-59.

[8] Kaplan EL, Rothermel CD, Johnson DR. Antistreptolysin O and anti-deoxyribonuclease B titers: Normal values for children aged 2 to 12 years in the United States. Pediatrics. 1998;101(1 Pt 1):86-88. Doi: 10.1542/peds.101.1.86.

[9] Steer AC, Vidmar S, Ritika R, Kado J, Batzloff M, Jenney AW, et al. Normal ranges of streptococcal antibody titers are similar whether streptococci are endemic to the setting or not. Clin Vaccine Immunol. 2009;16(2):172-75. Doi: 10.1128/CVI.00291-08.

[10] Agappe Diagnostics Ltd. MISPA i3 Nephelometer Instruction Manual. Kerala, India: Agappe Diagnostics Ltd.; 2023.

[11] Kotby AA, Habeeb NM, El Elarab SE. Antistreptolysin O titer in health and disease: Levels and significance. Pediatr Rep. 2012;4(1):e8. Doi: 10.4081/pr.2012.e8.

[12] Saini N, Kumar D, Swarnim S, Bhatt D, Kishore S. Comparison of antistreptolysin O and anti-deoxyribonucleic B titers in healthy children and those with acute pharyngitis, acute rheumatic fever, and rheumatic heart disease. Ann Pediatr Cardiol. 2019;12(3):195-200. Doi: 10.4103/apc.APC_60_18.

[13] Saha SK, Choudhury KN, Zareen S, Mousum S, Mamun MAA, Haque MA. Study of streptococcal antibody (anti-streptolysin O) among healthy children in Bangladesh. SAGE Open Med. 2022;10:20503121221108558. Doi: 10.1177/20503121221108558.

[14] Madaan R, Mandliya D, Tiwari D, Dhaneria M, Gupta R, Pathak A. Anti streptolysin O (ASO) titers in normal healthy children aged between 5 to 15 years in Ujjain region. Pediatr Rev Int J Pediatr Res. 2017;4(2):120-24. Doi: 10.17511/ijpr.2017.i02.05.

[15] Karmarkar MG, Venugopal V, Joshi L, Kamboj R. Evaluation & revaluation of upper limits of normal values of anti-streptolysin O & anti-deoxyribonuclease B in Mumbai. Indian J Med Res. 2004;119 Suppl:26-28.
[16] Guilherme L, Köhler KF, Postol E, Kalil J. Genes, autoimmunity and pathogenesis of rheumatic heart disease. Ann Pediatr Cardiol. 2011;4(1):13-21. Doi: 10.4103/0974-2069.79617.
[17] Gerber MA, Randolph MF, DeMeo KK, Wright PF. Evaluation of a new latex agglutination test for detection of streptolysin O antibodies. J Clin Microbiol. 1990;28(3):413-15. Doi: 10.1128/jcm.28.3.413-415.1990.
[18] Gerber MA, Shulman ST. Rapid diagnosis of pharyngitis caused by group A streptococci. Clin Microbiol Rev. 2004;17(3):571-80. Doi: 10.1128/CMR.17.3.571-580.2004.
[19] Marangou J, Rwebembera J, Mwita J, Thorup L, Remenyi B, Nascimento BR, et al. The echocardiographic diagnosis of rheumatic heart disease: A review of the performance of the World Heart Federation criteria 2012-2023. Glob Heart. 2024;19(1):47. Doi: 10.5334/gh.1327.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, Smt. B.K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara, Gujarat, India.
2. Associate Professor, Department of Microbiology, Smt. B.K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara, Gujarat, India.
3. Associate Professor, Department of Microbiology, Smt. B.K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Saurabh Chhotalal Norris,
Flat No. 103, Galaxy 77, Beside Preet Party Plot, Chhani to Sama 30MT Canal Road, Vadodara-390001, Gujarat, India.
E-mail: saurabhnorris@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 15, 2026
- Manual Googling: Jun 23, 2026
- iThenticate Software: Jun 25, 2026 (4%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 30, 2026**
Date of Peer Review: **May 02, 2026**
Date of Acceptance: **Jun 27, 2026**
Online Ahead of Print: **Jul 20, 2026**
Date of Publishing: **Aug 01, 2026**