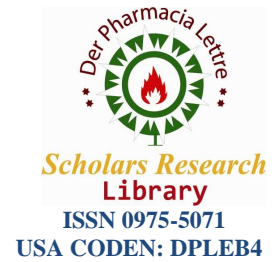




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Serum opacity factor as a tool in the detection of *Streptococcal pyoderma* cases in β - haomolytic streptococcal infection

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ABSTRACT

Streptococcal pyoderma is a matter of great concern, since the organism possess several virulence factors which are both anchored on the cell surface and are also secreted by the bacteria. Beta-hemolytic streptococci (BHS) are prevalent in pyodermal infections. *Streptococcus pyogenes* have several extracellular products and express a virulence factor- serum opacity factor (SOF) which opacifies mammalian serum by interacting with high-density lipoprotein. It is a large extracellular and surface bound protein of Group A streptococci (GAS), which is capable of binding fibronectin. The present study was carried out to determine the prevalence of (SOF) producing hemolytic streptococci in pyoderma cases and the commonly involve serogroups. 103 pyodermal patients in the age group of 1-50 years and with the clinical presentation of pyoderma comprised the study group. Paired swabs from the lesions were obtained by standard methods from each patient. One swab was subjected to Gram staining and observed microscopically for the presence of Gram positive cocci in chains. Other swab was inoculated on crystal violet blood agar for isolation of BHS. The blood agar plates were incubated overnight in an atmosphere containing 5-10% CO₂ in a candle jar. β - Hemolytic colonies obtained were identified as per standard methods. SOF in the culture supernatant was obtained by centrifugation of overnight cultures and addition of 10 μ l of culture supernatant to 100 μ l of horse serum (Hi Media) in a microtitre plate. Plates were sealed and incubated overnight in a moist chamber at 37°C. Following incubation 100 μ l of normal saline was added to each well and results were read visually and opacity was scored as 0 to 4+. Serogrouping of β -hemolytic colonies isolated was performed by rapid latex agglutination test (Remel Streptex kit) as per the procedure. Among the BHS isolates the predominant group identified belong to Group A streptococci. Among those Group A isolates 83% of them were able to produce serum opacity factor.

Key words: Streptococci, pyoderma, SOF

INTRODUCTION

Isolation of Group A streptococci from skin infection is always a matter of concern as the nephritogenic GAS express pathogenic factors that have high affinity for kidney cells and can lead to post streptococcal glomerulonephritis in untreated cases¹. In addition to several extracellular products, *Streptococcus pyogenes* express a virulence factor-serum opacity factor, which opacifies mammalian serum by interacting with high-density lipoprotein² and is a large extracellular and surface bound protein of GAS, which is capable of binding fibronectin³. Hence the present study was carried out to determine the prevalent group of streptococci in pyoderma cases and their ability to produce SOF, so that it may be used as a rapid diagnostic tool.

MATERIALS AND METHODS

A total of 103 patients attended Urban Health Centre, Rajah Muthiah Medical College, Chidambaram in the age group of 1-50 years and with the clinical presentation of pyoderma comprised the study group. Patients who had treatment during the past two months were excluded from the study. Paired swabs from the lesions were obtained by standard collection methods from each of the patient.

Smear prepared from the swab was subjected to Gram staining and observed microscopically for the presence of Gram positive cocci in chains. Other swab was inoculated on to crystal violet blood agar for the isolation of β hemolytic streptococci. The blood agar plates were incubated overnight in an atmosphere containing 5-10% CO₂ in a candle jar. β -hemolytic colonies obtained were identified as per standard methods.

SERUM OPACITY REACTION

The ability of Serum Opacity factor of the culture isolates were determined by opacity formation with horse serum. SOF was obtained by centrifugation of overnight cultures of organism and the addition of 10 μ l of culture supernatant to 100 μ l of horse serum (HiMedia Laboratories, Bombay) in a microtitre plate. Plates were sealed and incubated overnight in a moist chamber at 37°C. Following incubation 100 μ L of normal saline was added to each well and results were read visually and opacity was scored as 0 to 4+. Known SOF positive and negative strains maintained in the laboratory were used as controls. Horse serum with no culture extract added was also included to avoid false positive reaction⁴.

LANCEFIELD GROUPING

Serogrouping of β -hemolytic colonies obtained was performed by rapid latex agglutination test (Remel Streptex kit) as per the instructions of the manufacturer. 400 μ L of reconstituted extraction enzyme was taken in a sterile dry test tube. About 4 - 5 colonies of BHS were emulsified in enzyme and the suspension incubated at 37°C in a water bath for 30 minutes with intermittent shaking. A drop of latex suspension (polystyrene latex coated with purified group specific rabbit antibody) was dispensed on a reaction card followed by the addition of a drop of the extracted antigen. Contents were mixed and the card was gently rocked for a maximum of one minute and observed for the presence of agglutination. In a negative result, milky appearance remained substantially unchanged throughout one minute without any agglutination.

RESULTS

Eighty two of the 103 patients were in the age group of less than 10 years. Infection in males was more common (83.3%) than among females (69.4%) (Table:1). BHS culture positive in 76.7% of patients with pyoderma (Table:1). Among BHS isolated the predominant serogroup was Group A followed by Group C streptococcus (Table:2). serum opacity factor was produced by 83% of GAS.

TABLE-I Isolation of β -hemolytic Streptococci in pyoderma cases Age and sex wise distribution

AGE	MALES		FEMALES		TOTAL	
	No of samples	No of BHS isolated (%)	No of samples	No of BHS isolated (%)	No of samples	No of BHS isolated (%)
1- 10	47	39 (82.9)	35	24 (68.6)	82	63 (76.8)
11-20	03	03 (100)	06	04 (66.6)	09	07 (77.8)
21-30	01	01 (100)	05	03 (60.0)	06	04 (66.6)
31-40	02	02 (100)	01	01 (100)	03	03 (100)
41-50	01	-	02	02 (100)	03	02 (66.7)
TOTAL	54	45 (83.3)	49	34 (69.4)	103	79 (76.7)

TABLE 2 Lancefield groups of β -hemolytic streptococci from pyoderma. (n = 79)

Lancefield group	No of isolates	Isolation rate
Group A	70	88.6
Group C	06	7.6
Group G	03	3.8

DISCUSSION

Group A streptococci was isolated in 70 cases (67.9%) out of 103 cases of pyoderma in this study which is similar to other studies^{5, 6, 7}. However, lower prevalence rates of 13 to 21% have been reported by other workers^{8, 9}. Factors

such as geographic location, lower socio-economic status coupled with illiterate rural and semi-rural subjects and inaccessibility to health care could be the reason for such higher prevalence.

Present study reveals a preponderance of isolation in children less than 10 years of age (63 cases out of 82 BHS isolated) (79.7%). It was confirmed by different authors in their studies¹⁰ Throat infection by GAS tends to cause rheumatic fever, whereas skin infection usually leads to glomerulonephritis because isolates from skin infections are not able to aggregate human collagen which is required for induction of rheumatic fever. Hence isolation of GAS from cases of pyoderma assumes greater significance as it can lead to post streptococcal glomerulonephritis in untreated cases.

SOF production was higher (83.3%) in this study than other reports which showed a variation of 34 -50%^{11,12}. This could be due to the M type variation. M types may be divided into opacity factor positive and opacity factor negative and hence it is used to subtype the GAS isolates. Furthermore,

CONCLUSION

It is proposed that the presence of SOF mediates bacterial binding with extracellular host protein fibronectin, thus facilitating better adherence to host epithelial cells. Hence, pyoderma caused by GAS appeared to be common. This fact supported by the high incidence of SOF producing GAS isolates shown in this study. If it is true then SOF may simply be used as a diagnostic tool in GAS produced pyoderma cases, commonly seen in children.

REFERENCES

- [1] Feldmeier H, Chhatwal GS, Guerra H. *Tropical Medicine and International Health* **2005**, 10, 713-716.
- [2] Courtney HS, Hasty DL, Dale JB. *Infect Immun* **2003**, 71, 5097-5.
- [3] Gillen CM, Towers P3, McMillan DJ, Delvecchio A, Sriprakash KS, Currie B et al. *Microbiology* **2002**, 148, 169-178.
- [4] Johnson DR, Kaplan EL, Sramek J, Bicova R, Havlick J, Havilckova H et al. World Health Organization **1996**, 46-52.
- [5] Anthony BF, Kaplan EL, Wannamaker LW, Chapm. *Am.J Epidemiol* **1976**, 104, 652-66.
- [6] Nelson KE, Bisno AL, Waytz P, Brunti Moses VK, Haque R. *Am.J.Epidemiol* **1976**, 103, 270-83.
- [7] GardinerDL. and SriPrakash K.S. *J Din Microbiol* **1996**, 34, 1448-1452.
- [8] Ohadage DP, Sali YA.. *J.Dermatol Venereol and Leprol* **1999**, 65, 177-181.
- [9] Mariette MS, Garg BR, Reba K. *Indian J Dermatol Venerol Leprol* **1992**, 58, 183-187.
- [10] Fail Mezger TC. *J.Dermatol.* **1978**, 7, 583.
- [11] Arthur Jeng, Varjs Sakota, Zhongya Li, Vivekananda Datta, Bernard Beall and Victor Nizet.. *Journal of Bacteriol.* **2003**, 1208-1217.
- [12] Prakash K,Dutta S.*Ind. J. Med.Resear.* **1991**, 93, 222-4.