

Prevalence of Anti-streptolysin O antibodies at Bhadrak Region, India

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Abstract

This study was carried out to detect the elevated serum Anti-streptolysin O (ASO) in patients which were coming in OPD of Regional Research Institute of Unani Medicine with various clinical conditions during the period of January 2011 to December 2012. The serum samples were tested for ASO antibodies by latex agglutination test. Total 205 patients including 30.24 % male and 69.7 % female were tested for ASO serum levels. 60 were found positive and 145 were negative. In 60 positive cases 16 were male and 44 were female. The prevalence of ASO antibody in total cases was 29.26 %. The prevalence in male was 25.8 % and in female was 30.76 %. The presence of elevated streptococcal antibody titers in such a population probably reflects a medium background prevalence of streptococcal infections.

Keywords: Anti-streptolysin O, Agglutination, Streptococcal infections, Serum

Introduction

Streptococcus pyogenes (Group A *Streptococcus*/GAS) is one of the most common and ubiquitous human pathogens. It causes a wide array of infections, the most frequent of which are acute pharyngitis ("strep throat") and impetigo (pyoderma). It is also associated with two main non-suppurative sequelae: acute rheumatic fever (ARF) and acute glomerulonephritis (AGN) (Bisno, 1991). Rheumatic fever causes inflammation of tissues and organs and can result in serious damage to the heart valves, joints, central nervous system and skin. Susceptibility to rheumatic fever in certain individuals has been ascribed to a number of factors. These include genetic determinants, for example, HLAs and the presence of certain markers such as the B-cell alloantigens. One of the factors originally considered in rheumatic fever susceptibility is an innate state of immune hyperresponsiveness, particularly to streptococcal antigens (Meiselas et al., 1961; Stollerman, 1972; Rejholec, 1957).

The detection of streptococcal infection is done by positive culture for group A *Streptococcus* from the throat or through anti-streptococcal antibody tests (Homer and Shulman, 1991). The throat cultures are spontaneously negative due to previous antibiotic treatment, or positive due to a carriage state. Hence streptococcal antibody tests have become a method commonly used to provide evidence of preceding streptococcal infection (Fink, 1991).

During infection, the host may produce antibodies to one or more extracellular products of group A streptococci, and these antibodies are useful markers of recent streptococcal infection. Tests that measure antibodies to the extra-

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cellular products are commercially available and more commonly used. The anti-streptolysin O (ASO) assay was the first such test to be developed and is still widely used. ASO is not only useful in the diagnosis of streptococcal infections or complications, but also in the follow-up process and in evaluating the effectiveness of treatments. It measures the ability of serum to neutralize streptolysin O.

Material and Methods

Serum Collection

Serum samples were collected from 205 patients between the periods of January 2011 to December 2012 at Regional Research Institute of Unani Medicine in Bhadrak, India. Blood samples from patients were obtained using a standard 2-mL syringe. Samples were allowed to coagulate for 30 minutes at room temperature in a serum-collecting tube. The sample was centrifuged at 3000 rpm for 5 minutes. The serum was then separated by using micropipette.

Procedure

All the serum samples were tested by ASO kit (Span Diagnostics P. Ltd. India). The instructions, reagents and accessories to follow were supplied with the kit.

Test serum and reagents were kept at room temperature before testing. 40µl patient's serum within the circled area was placed on the clean and dry special glass slide provided in the kit. One drop of well mixed ASO latex reagents was added to serum. The reagent and serum using the applicator stick were mixed. The slide was rotated and observed for agglutination macroscopically within two minutes.

Results

Total 205 patients were included in this study. From them, 30.24 % male and 69.7 % female tested for ASO serum levels (Figure 1), 60 were positive and 145 were negative.

In 60 positive cases 16 were male and 44 were female. In 145 negative cases 46 were male and 99 were female (Figure 2). Highest positive case (27 patients) was found in the age group of 21- 40 while 23 patients were positive in age group of 41-60 (Figure 3). The prevalence of total case was 29.26 %. The prevalence of total female was 30.76 % and the prevalence of total male was 25.8 % (Table 1).

The number of positive cases in different age group is given in table 2. The highest prevalence of male among positive cases found in the age group 41-

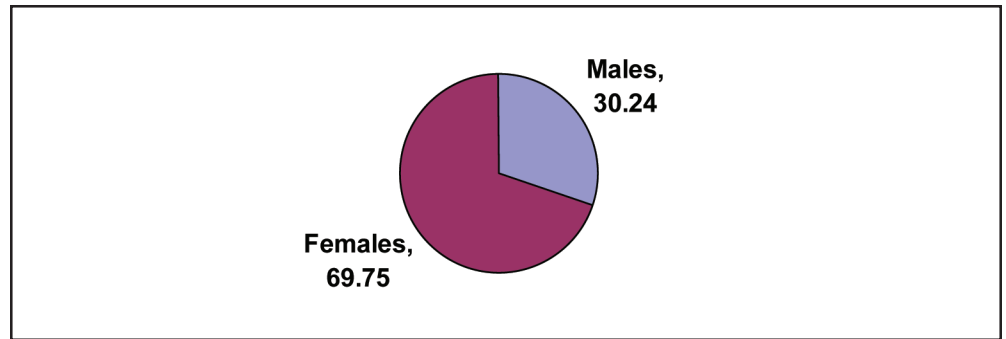


Figure 1: Blood sample collection in General OPD attendance at RRIUM, Bhadrak

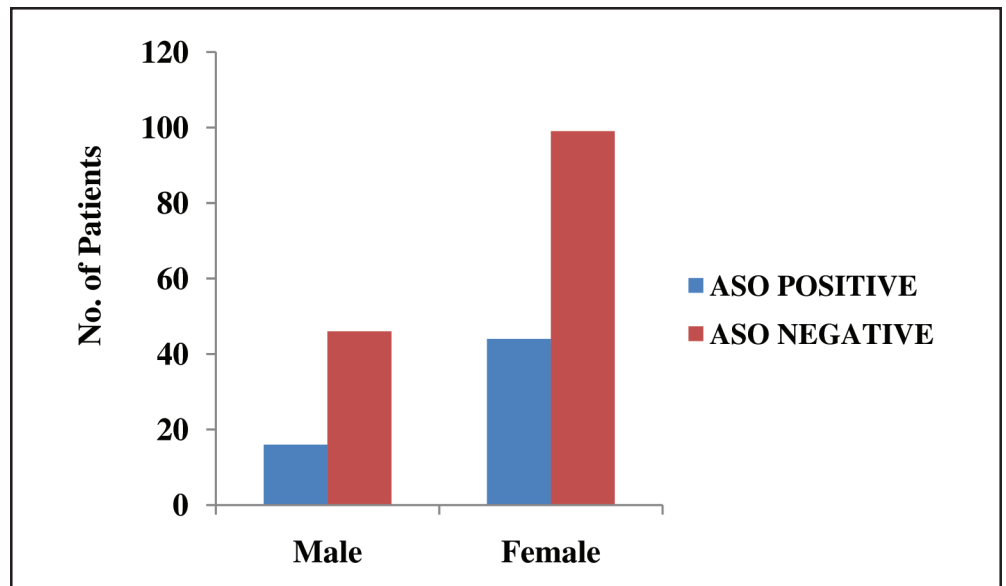


Figure 2: Sex wise distribution of ASO Positive and ASO Negative cases at RRIUM, Bhadrak

Table 1: Sex wise prevalence of all streptococcal infection among General OPD at RRIUM, Bhadrak

Sex	ASO Positive	ASO Negative	Total (%)	Prevalence Rate (%)
Male	16	46	62 (30.24)	25.84
Female	44	99	143 (69.75)	30.76
Total	60	145	205 (100)	29.6

60 (6/16, 37.5%) and the highest prevalence of female among positive cases found in the age group 21 – 40 (22/44, 50 %) (Figure 4, Figure 5). The prevalence rate of male and female in age group 21-40 was 31.25 % and 50 % respectively. This indicates that the prevalence rate is higher in females than male in this age group.

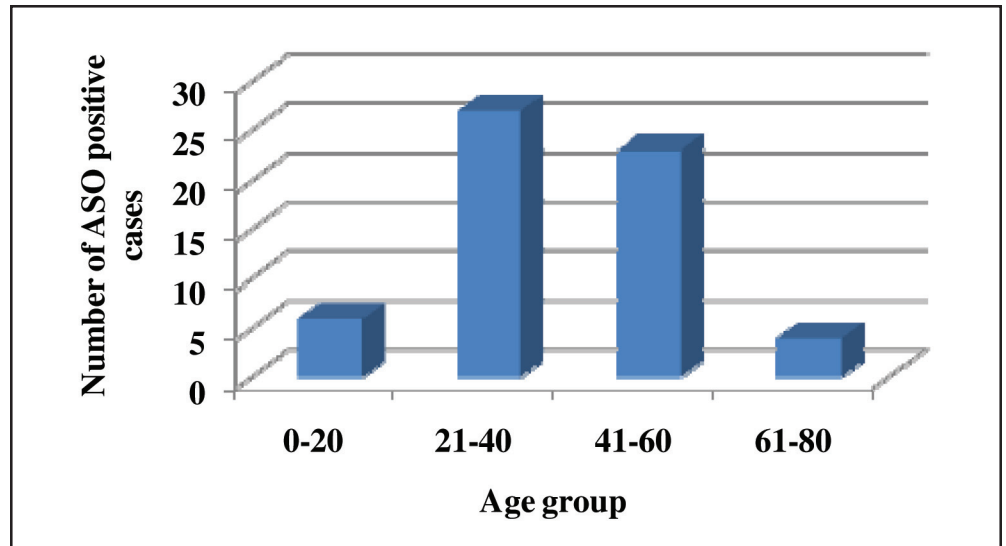


Figure 3: Age wise distribution of total Anti-streptolysin O (ASO) positive cases

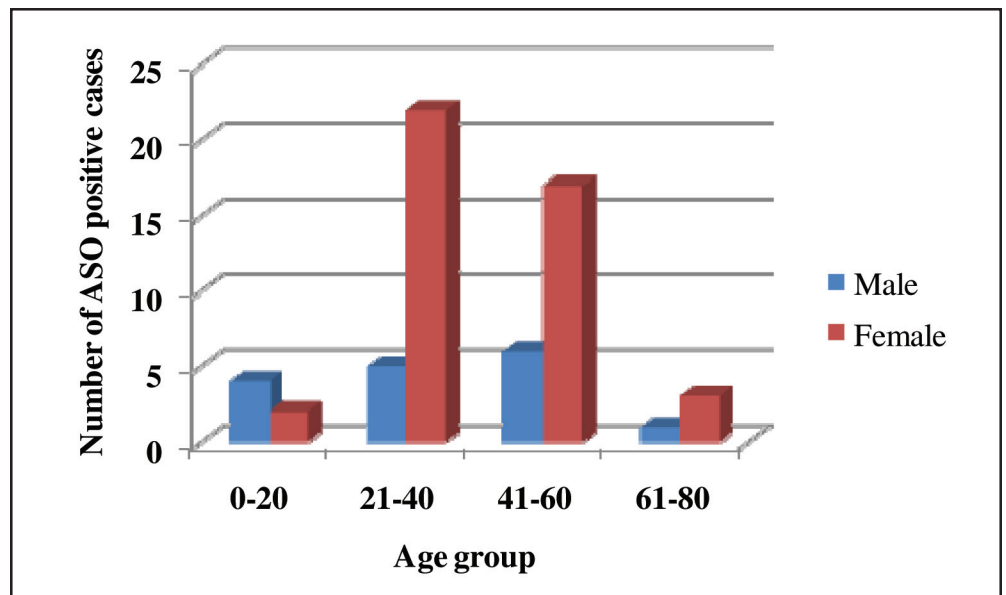


Figure 4: The comparative chart of ASO positive cases between Male and Female in different age group

The prevalence rate of male and female in age group 41-60 was 37.5 % and 38.6 % respectively which was almost similar. The overall data clearly indicates that the prevalence of ASO positive case is slightly higher in females than males.

Discussion

The serological test for ASO is commonly used to aid in the diagnosis of post-streptococcal non-suppurative sequelae such as ARF and glomerulonephritis (Kimoto *et al.*, 2005; Batsford *et al.*, 2002).

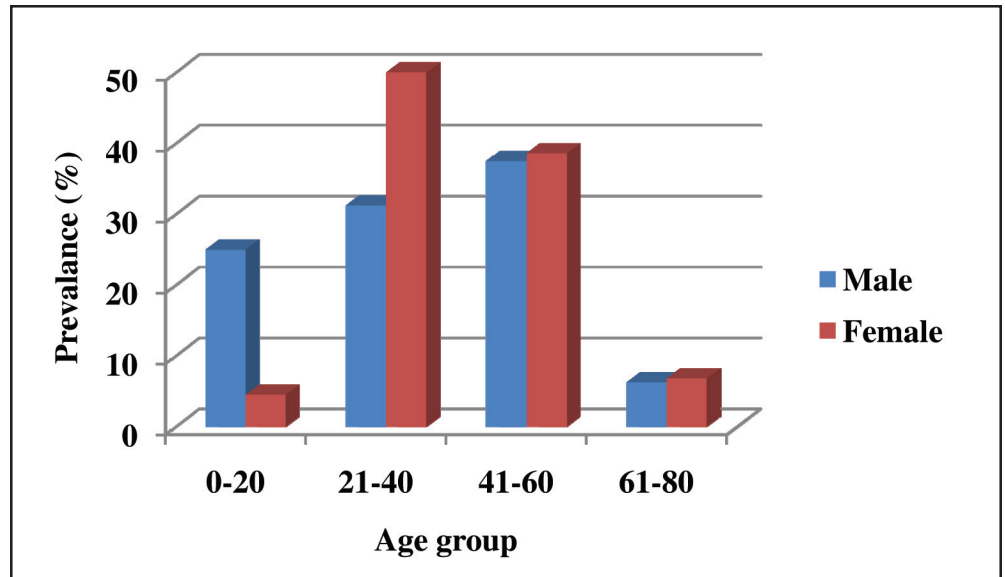


Figure 5: The prevalence rate of ASO among positive cases in different age group

Table 2: The prevalence rate of ASO among positive cases in different age group

Age group	Total No. of Positive cases	No. of Positive Male (%)	No. of Positive Female (%)
0-20	6	4 (25)	2 (4.5)
21-40	27	5 (31.25)	22 (50)
41-60	23	6 (37.5)	17 (38.6)
61-80	4	1 (6.25)	3 (6.8)
Total	60	16 (100)	44 (100)

Conventional laboratory practice is to measure levels of antibodies to various combinations of the extra-cellular Group A Streptococcus antigens. However, Blyth and Robertson (2006) showed that the addition of anti-streptokinase antibodies measurement did not increase the sensitivity and specificity of serological testing for the diagnosis of acute post-streptococcal disease (Blyth and Robertson, 2006). ASO test method is based on an immunologic reaction between streptococcal exotoxins bound to biologically inert latex particles and streptococcal antibodies in the test sample. Visible agglutination occurs when increased antibody level, are present in the test specimen. A positive ASO titer indicates nonspecific immune stimulation due to past streptococcal exposure resulting in polyclonal gammopathy (Sainani and Sainani, 2006).

Fujikawa and Okuni (1979) observed that ASO elevation occurs only in 60% of rheumatic fever. But after one more test is added either

antideoxyribonuclease-B titre or streptokinase test, they were able to diagnose rheumatic fever with 95% accuracy (Fujikawa and Okuni, 1979). In this study, 60 samples were positive out of 205 samples. Our study showed 29.26 % prevalence of ASO positive cases whereas the same study which was conducted in Nepal showed 45.45 % prevalence of ASO positive cases (Khan *et al.*, 2012). Similar study was also performed where 20.89 % prevalence was observed (Kandel *et al.*, 2007). Further in an another study conducted at CMS Teaching Hospital, Bharatpur, Nepal, 4230 serum samples of rheumatic fever, glomerulonephritis, rheumatic heart disease and rheumatic arthritis were tested for ASO antibodies by latex agglutination test during the period of January 2003 to December 2009. Among them 1944 samples were positive and 2286 samples were found negative with overall prevalence of 45.95 %. (Dewasy *et al.*, 2010)

This clearly indicates that the ASO levels vary with age group of the study population and geographical distribution. This study, together with data derived from the present study indicates that the levels of the streptococcal antibodies in healthy populations can vary substantially, depending on the frequency of streptococcal infections in those populations. The presence of elevated streptococcal antibody titers in such a population probably reflects a medium background prevalence of streptococcal infections.

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