ANTI-STREPTOLYSIN O TITERS IN NORMAL HEALTHY CHILDREN OF 5-15 YEARS IN CHENNAI CITY

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CERTIFICATE

Certified that this dissertation entitled **"Anti - Streptolysin O Titers In Normal Healthy Children Of 5-15 Years In Chennai City"** is a bonafide work done by Dr. S.Kesavan, Post Graduate Student of Pediatric Medicine, Institute of Child Health and Hospital for Children, Madras Medical College, during the academic years 2004 – 2007.

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DECLARATION

I declare that this dissertation entitled **"Anti-Streptolysin O Titers In Normal Healthy Children Of 5-15 Years In Chennai City**" has been conducted by me at the Institute of Child Health and Hospital for Children. It is submitted in part of fulfillment of the award of the degree of M.D (Pediatrics) for the March 2007 examination to be held under the Tamil Nadu Dr. M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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CONTENTS

INTRODUCTION

Group A Streptococcal infections and their late sequelae like rheumatic fever and rheumatic heart disease remain an important and major health problem in India. The incidence of rheumatic fever varies from 0.2 to 0.75 per 1000 children per year in school children of age 5 to 15 years. The prevalence of rheumatic heart disease varies from 1 to 5.4 per 1000 children [1]. Such data have emphasized the importance of accurate clinical diagnosis often requiring laboratory confirmation of preceding Group A streptococcal infection.

Group A streptococcus also known, as Streptococcus pyogenes is a common cause of infections of upper respiratory tact and the skin in the children. It is less common cause of perianal cellulitis, vaginitis, septicemia, pneumonia, endocarditis, pericarditis, osteomyelitis, suppurative arthritis, myositis, cellulitis, and omphalitis. These microorganisms are also the cause of two distinct clinical conditions scarlet fever and erysipelas, as well as toxic shock syndrome and necrotizing fascitis. Group A Streptococcus is also the cause of two potentially serious non-suppurative complications namely rheumatic fever and acute glomerulo nephritis.

STEPTOCOCCUS:

Streptococci are gram-positive cocci arranged in chains or pairs. They are part of normal flora of humans and animals. Some are pathogenic. Most important of them is Streptococcus pyogenes.

CLASSIFICATION:

Several systems of classification have been employed but in medical bacteriology the following method is useful.

Streptococci are first divided into obligate anaerobes and facultative anaerobes. The obligate anaerobe is peptostreptococcus. The aerobic and facultative anaerobe is classified on the basis of hemolysis on 5% horse blood agar pour plate cultures as

1. alpha hemolytic streptococci
2. beta hemolytic streptococci
3. gamma hemolytic streptococci

Alpha hemolytic streptococci produce a greenish discolouration with partial hemolysis around the colonies. The zone of hemolysis is small, (1 – 2 mm wide) with indefinite margins and unlysed erythrocytes can be made out microscopically within this zone. The alpha streptococci

are normal commensals in the throat, but may cause opportunistic infections rarely. Streptococcus viridans and pneumococci belong to this group.

A Beta hemolytic streptococci produce a sharply defined clear colourless zone of hemolysis, 2 – 4 mm wide, within which the red cells are completely lysed. The term “hemolytic streptococci” strictly applies only to beta lytic strains. Most pathogenic streptococci are belonging to this group.

Gama hemolytic streptococci are non hemolytic, producing no change in medium, so referred to as indifferent streptococci. They include fecal streptococci (enterococci, streptococcus faecalis) and related species. They are called the enterococcus group.

Beta hemolytic were classified by Lancefield (1933) into groups based on the carbohydrate antigen on the cell wall known as Lancefield groups, twenty of which have been identified. The great majority of hemolytic streptococci that produce human infections belong to group A. These are further subdivided into types based on protein antigens (M, T, and R) present on the cell surface (Griffith typing). About eighty types of streptococcus pyogenes have been identified so far.

STREPTOCOCCUS PYOGENES:

It is an aerobe and a facultative anaerobe growing best at a temperature of 37°C. It ferments several sugars, producing acid but no gas. It is catalase negative. Hydrolysis of pyrrolidonyl naphthylamide(PYR) , failure to ferment ribose and sensitivity to bacitracin are useful in differentiating streptococcus pyogenes from others .

ANTIGENIC STRUCTURE:

The capsule which inhibits phagocytosis is not antigenic in humans. The cell wall which is composed of an outer layer of protein and lipoteichoic acid , middle layer of group specific carbohydrate and an inner layer of peptidoglycan .

Several protein antigens have been identified in the outer part of cell wall. Streptococcus pyogenes can be typed based on the surface proteins M, T, R. The M protein is the most important of these. It acts as a virulence factor by inhibiting phagocytosis. Hair like pili projects through the capsule of group A streptococcus. The pili consist partly of M protein and are covered with lipoteichoic acid which is important in the attachment of streptococcus to epithelial cells.

Various structural component of streptococcus pyogenes exhibit cross reaction with different tissues of the human body. Antigenic relationships have been demonstrated between capsular hyaluronic acid and human synovial fluid, cell wall protein and myocardium, group A carbohydrate and cardiac valves, cytoplasmic membrane antigens and vascular intima, and peptidoglycans and skin antigens. It has been postulated that these antigenic cross reactions may account for some of the manifestations of rheumatic fever and other streptococcal diseases, the tissue damage being of immunological nature.

Streptococcal components	**Mammalian tissue constituent**
Streptococcal hyaluronic acid.	Mammalian hyaluronic acid and protein polysaccharide.
Group A carbohydrate.	Glycoproteins of heart valves.
Protein cell wall.	Sarcolemma of cardiac and skeletal muscle.
Protein of cell membrane.	Sarcolemma of cardiac and skeletal muscle.
Glycoprotein of cell membrane.	Glycoprotein of glomerular basement membrane.
Antigen of cell membrane.	Histocompatibility antigen.

TOXINS AND VIRULENCE FACTORS:

Streptococcus pyogenes forms several exotoxins and enzymes which constitute to its virulence. Besides these, M protein and C polysaccharide are also important.

Cellular components	**Extra cellular components**
Hyaluronic acid capsule	Streptolysin S,O
Group specific carbohydrate	Hyaluronidase
M T R proteins	Streptokinase
Mucopeptide	NADase
Poly glycerophosphate	DNase
ß glucoronidase	Erythrogenic toxin
Lipoproteinase	Proteinase
DNA	RNase
RNA	Amylase

HEMOLYSINS –

Group A streptococcus produce two hemolysins streptolysin O and S. streptolysin O is so called because it is oxygen labile and heat labile. It is antigenic in human and inhibited by cholesterol and toxic to variety of cell types including leucocytes monocytes and cultured cells. Because of its oxygen lability streptolysin O is primarily responsible for the beta hemolysis seen around sub surface colonies of group A streptococci in pour plates or in the stabbed regions of inoculated sheep blood agar plates. Streptolysin O is also produced by some group C and group G

streptococci. Antistreptolysin O appears in sera following streptococcal infection. Estimation of ASO is a standard serological procedure for the retrospective diagnosis of infection with streptococcus pyogenes.

Streptolysin S is an oxygen stable hemolysin. It is not antigenic in humans and also toxic to variety of cell types. Streptolysin S is active in both surface and subsurface hemolysis when the organisms are grown on sheep blood agar. Both streptolysin O and S can induce injury to non erythrocytic cell types causing the formation of slits and pores in the cell membrane and subsequent leakage of cellular contents.

PYROGENIC EXOTOXINS – (ERYTHROGENIC TOXINS):

It is also called as Dick, scarlatinal toxin. Three types of pyrogenic exotoxins are present. Type A, B and C. Type A has been most widely studied. It is produced by group A streptococci that carry a lysogenic phage and is a super antigen. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever. Streptococcal pyrogenic exotoxins C may also contribute to the syndrome, while the role of exotoxin B is unclear. The group A streptococci associated with toxic shock syndrome are primarily of M proteins type 1 and 3.

STREPTOKINASE – (FIBRINOLYSIN) :

This promotes lysis of human fibrin clots by activating plasminogen. It is an antigenic protein. Antistreptokinase antibody provides retrospective evidence of streptococcal infection. Streptokinase appears to play a biological role in streptococcal infections by breaking down the fibrin barrier around the lesions and facilitating the spread of infections. Streptokinase is given intravenously for the treatment of early myocardial infarction and other thromboembolic disorders.

DEOXYRIBONUCLEASE - (STREPTODORNASE):

This cause depolymerisation of DNA, which helps to liquefy the thick pus and may be responsible for the thin serous character of streptococcal exudates. This property has been applied therapeutically in liquefying localized collections of thick exudates as in empyema. A preparation containing streptokinase and streptodornase is available for this purpose.

Four antigenically distinct DNAase A, B, C and D have been recognized of which type B is the most antigenic. Demonstration of anti-DNAase antibody is useful in the retrospective diagnosis of Streptococcus pyogenes infection particularly in skin infection, where ASO titers may be low.

NICOTINAMIDE ADENINE DINUCLEOTIDASE (NADase) –

It is formerly known as diphosphopyridine nucleotidase (DPNase). This act on the coenzyme NAD and liberates nicotinamide from the molecule. It is antigenic and is neutralized by the antibody in the convalescent sera.

HYALURONIDASE –

This enzyme breaks down the hyaluronic acid of the tissues. It depolymerises the ground substance of connective tissue resulting in contiguous spread of organism.

It is antigenic and specific antibodies appear in the convalescent sera.

Many strains also produce serum opacity factor, proteinase, phosphatase, esterase, amylase, N-acetyl glucosaminidase, neuraminidase and other toxins or enzymes.

STREPTOCOCCUS PHARYNGITIS:

Group A Streptococcus pharyngitis is one of the common bacterial infections in children. It accounts for 20 to 40 % of exudative pharyngitis in children. They are uncommon before 2 to 3 years of age. The incidence increases among children and then declines in late adolescence. It occurs

throughout the year but is reported most often during winter and spring. Illness often spread among siblings and classmates.

Colonization of pharynx by group A Streptococcus may result in asymptomatic carriage or acute infection. The M protein is the major virulence factor.

CLINICAL FEATURES:

Onset of pharyngitis is often rapid with prominent sore throat and fever, headache and gastrointestinal symptoms are frequent. The pharynx is red and the tonsils are enlarged and classically covered with a yellow blood tinged exudate. The anterior cervical nodes are enlarged and tender.

DIAGNOSIS

THROAT SWAB CULTURE:

Throat swab culture remains the gold stranded for diagnosis of streptococcus pharyngitis. False positivity can occur if other organisms are misdiagnosed. False negative cultures are attributed to inadequate specimen and patients on prior antibiotics. It has 90 to 95% sensitivity of detection. The disadvantage of throat swab is delay in culture result.

RAPID ANTIGEN DETECTION TESTS:

Rapid antigen detection tests have been developed for identification of group A Streptococcus from throat swabs. These kits use enzymatic or chemical methods to extract the antigen from the swab, and then use enzyme immuno assay or agglutination tests to demonstrate the presence of the antigen. The tests can be completed minutes to hours after specimen is obtained.

Although they are expensive, the advantage is the rapidity of identification and treatment. The rapid antigen detection tests have excellent specificity of more than 95% when compared to culture. Unfortunately sensitivity of these tests is 80 to 90% possibly lower than culture.

TREATMENT:

Most untreated episodes of streptococcal pharyngitis resolve uneventfully in few days but early antibiotic therapy hastens clinical recovery by 12 -24 hours. The primary benefit of treatment is the prevention of acute rheumatic fever, which is almost completely successful if antibiotic treatment is instituted within 9 days of illness. Antibiotic therapy should be started immediately without culture for children with symptomatic pharyngitis and a positive streptococcal rapid

antigen test; clinical diagnosis of scarlet fever; household contact with documented streptococcal pharyngitis; a past history of acute rheumatic fever; or a recent history of acute rheumatic fever in a family member.

A variety of antimicrobial agents are effective. Group A streptococci remains universally susceptible to penicillin, which has a narrow spectrum and few adverse effects. Penicillin V in a dose of 250 mg BID or TID for 10 days for children and 250 to 500 mg per dose for adolescence and adults. Oral amoxicillin in a dose of 750 mg once daily for 10 days is also effective. Single intramuscular dose of benzathine penicillin (6, 00,000 IU for children less than 27 kg, 1.2 million units for larger children and adults.) or benzathine – Procaine penicillin G combination provides adequate blood level for more than 10 days. Erythromycin (erythromycin ethyl succinate 40 mg/kg/day divided TID or QID for 10 days or erythromycin esteolate 20 – 40 mg/kg/day divided BID, TID or QID for 10 days.) is recommended for patients allergic to beta lactam antibiotics. The treatment regimen most effective for eradicating streptococcal carriage is Clindamycin 20 mg/kg per day divided in 3 doses orally for 10 days.

ACUTE RHEUMATIC FEVER:

First clinical description of rheumatic heart disease was reported by Rene T. Laennec in 1818, when he introduced the stethoscope. But the full syndrome of rheumatic fever was described by Cheadle in 1886. The correlation between the history of streptococcal sore throat and rheumatic fever was strongly suspected in early 1900s.

Acute rheumatic fever is important non suppurative sequelae of groupA streptococcus infection of throat. Studies have shown that 2-3% of individuals with untreated group A Streptococcus pharyngitis will develop acute rheumatic fever .[2] Acute rheumatic fever most often occurs in children with peak age related incidence between 5 to 15 years.

Epidemiological risk factors classically associated with individual attacks and especially with out breaks of acute rheumatic fever, include lower standard of living, over crowding, under nutrition and poverty. It is also more common in urban centers than in rural communities. It occurs in all races and in all parts of world. Although it is traditionally considered to be a disease of temperate climate, it is now more commonly reported in tropical climates, particularly in developing countries.

The disease has been more common among socially and economically disadvantaged populations. However the outbreaks in US in late 1980s and early 1990s cannot be explained by these factors. The large Utagh outbreak of more than 600 cases during 17 years has affected mainly middle class people with ready to access medical care. Therefore one can conclude that the organism itself as well as the degree of host and herd immunity to prevalent M types in an affected community is equally important risk factors.

THE GENETIC SUSCEPTIBILITY:

Relatively few individuals (2 – 3%) will develop rheumatic fever after acute streptococcal pharyngitis [2]. However in those who have had previous episodes of rheumatic fever, recurrence will occur in approximately half [3]. Children who develop rheumatic fever demonstrate an exaggerated antibody response to toxins produced by streptococcus (such as ASO) compared with other patients who recovered from streptococcal pharyngitis and did not develop rheumatic fever [4].

Genetic predisposition is evidenced by high concordance (20%) among monozygotic twins and by high incidence among Maoris of New Zealand compared with their Caucasians neighbours irrespective of their economic and social status [5]. Further evidence is provided by the

association of the disease with inheritance of HLA antigen markers (HLA – DR4, 2, 3, 1, 78; DR – 53, Dw 10) among different population and ethnic groups [6], by the productive effect of HLA DR 5 and by the association with high level of expression of B cell alloantigen D 8/17. The genetic susceptibility to rheumatic fever is mediated by a single recessive gene [7].

RHEUMATOGENIC STREPTOCOCCI:

Among the group A beta hemolytic streptococci, there are rheumatogenic strains causing rheumatic fever (1, 3, 5, 6, 18, 24) [8]. Past and recent out breaks of both infections by group A streptococcus and rheumatic fever showed highly mucoid colonies in samples grown in blood agar [9]. The mucoid appearance in the culture is probably a result of hydrolysis of the capsule hyaluronidase that is produced during the growth of the organism.

PATHOGENISIS:

Several theories of pathogenesis of acute rheumatic fever and rheumatic heart disease have been proposed, but only two are seriously considered.

1) CYTOTOXIC THEORY:

Group A streptococcus toxin may be involved in pathogenesis of acute rheumatic fever and rheumatic heart disease. Group A streptococcus produce several enzymes that are cytotoxic for mammalian cells. For example antistreptolysin O has a direct cytotoxic effect on mammalian cells in tissue culture and most of the proponents of the cytotoxic theory have focused on this enzyme. However one of the major problems with the cytotoxicity hypothesis is its inability to explain the latent period between pharyngitis and onset of acute rheumatic fever.

2) IMMUNE MEDIATED THEORY:

This has been suggested by clinical similarity of acute rheumatic fever to other illness produced by immunopathogenetic processes and by the latent period between group A Streptococcus infection and acute rheumatic fever. The hypothesis of antigenic mimicry between human and group A Streptococcus antigens has been studied extensively and concentrated on two interactions. First is similarity between group specific carbohydrate of group A Streptococcus and glycoprotein of heart valves, and second involves molecular similarity among streptococcal cell membrane and M protein sarcolemma and other moieties of human myocardial cell.

DIAGNOSIS:

Because no clinical or laboratory finding is pathognomonic for acute rheumatic fever T.Dukett Jones in 1944 proposed guidelines to aid in diagnosis and limit over diagnosis. The most recent modification of Jones criteria (updated 1992) was published in 1992 by a special writing group of the American Heart Association [10].

JONES CRITERIA, UPDATED (1992).[10]

Major	**Minor**
1) Carditis. 2) Poly arthritis. 3) Chorea. 4) Erythema marginatum. 5) Subcutaneous nodules.	Clinical findings: Fever Arthralgia. Laboratory findings: Elevated acute phase reactants. ESR. CRP. Prolonged PR interval.
Supporting evidence for an antecedent group A streptococcal infection: Positive throat culture or rapid streptococcal antigen test. Elevated or rising streptococcal antibody titer.	

To fulfill the criteria either two major or one major and two minor plus evidence of an antecedent streptococcus infection are required. The latter may be provided by recovery of organism in culture or by evidence

of immune response to one of the commonly measured group A streptococcal antibodies like anti streptolysin O or anti DNAase B.

Acute rheumatic fever typically develops 2 to 4 weeks after an acute episode of group A streptococcus pharyngitis, at a time when clinical findings of pharyngitis are no longer present and when only 10 to 20 % of throat culture or rapid antigen test results are positive. In about one third of patients with rheumatic fever will not have a history of antecedent pharyngitis, moreover mere presence of organism can indicate carrier state which is seen in 2.5 to 35.4%. [11]. Therefore evidence of an antecedent group A streptococcus is usually based on elevated or increasing titers.

A slide agglutination test (streptozyme test) has been introduced and it is purposed to detect antibodies against five different group A streptococcal antigens. Although this test is rapid and simple to perform and widely available, it is less standardized and less reproducible than other tests and should not be used as a diagnostic test for evidence of group A Streptococcus infection. If only a single antibody is measured (usually ASO) only 80 to 85 % of patients with acute rheumatic fever have an elevated titer, however 95 to 100 % have an elevation of titer if three different antibodies (ASO, anti-DNase B, antihyaluranidase) are measured. Therefore when acute rheumatic fever is suspected clinically,

multiple antibody tests are performed.

Except for patients with chorea, clinical findings of acute rheumatic fever generally coincide with peak antistreptococcal antibody responses. The diagnosis of acute rheumatic fever should not be made in patients with elevated or increasing antibody titers, who do not fulfill the Jones criteria, because such titer changes maybe coincidental. This is most often true in younger school aged children, may be of who have group A Streptococcus pyoderma in summer or unrelated group A Streptococcus pharyngitis during winter and spring months.

Because acute rheumatic fever and post streptococcal glomerulonephritis are non-suppurative sequelae of group A Streptococcus infection and there is a latent period between the streptococcal infection and onset of disease, serum taken at the disease onset may really be convalescent; a rising titer may not be demonstrated. Hence an upper limit of normal value (ULN) is useful when acute and convalescent sera unavailable[12]. For the purpose of uniformity ULN is defined as that titer exceeded by 20% of normal population that is 80th percentile [13].

Clinical microbiological laboratories often use interpretative criteria suggested by manufacturers of commercial antibody test kits.

Because such ‘normal’ values may only reflect appropriate titer for adult, which is almost always lower than for children, can be problematic.

TREATMENT:

All patients with rheumatic fever should be placed on bed rest and monitor closely for evidence of Carditis.

ANTIBIOTIC THERAPY:

Once the diagnosis of the rheumatic fever has been established and regardless of throat culture results the patient should receive 10 days of oral penicillin or erythromycin or a single intramuscular injection of Benzathine penicillin to eradicate group A streptococci from the upper respiratory tract, after this initial course of antibiotic therapy the patient should be started on long term antibiotic prophylaxis.

ANTI INFLAMMATORY THERAPY:

Patients with typical migratory polyarthritis and those with carditis without cardiomegaly or congestive cardiac failure should be treated with oral salicylates. The usual dose of aspirin is 100mg/kg/24 hours divided QID per oral for 3 to 5 days followed by 75mg/kg/24hours divided QID per oral for 4 weeks.

Patients with carditis and cardiomegaly or congestive heart failure

should receive corticosteroids. The usual dose of prednisolone is 2 mg/ kg / 24 hours in four divided doses for 2 – 3 weeks followed by a tapering of the dose that reduces the dose by 5 mg/ 24 hours every 2 – 3 days. At the beginning of tapering of prednisolone dose, aspirin should be started at 75 mg/ kg / 24 hours in four divided doses for 6 weeks. The supportive therapy of patients with moderate to severe carditis includes digoxin, fluid and salt restriction, diuretics and oxygen. The cardio toxicity of digoxin is enhanced with myocarditis.

PREVENTION:

Prevention of both initial and recurrent episodes of acute rheumatic fever depends on controlling group A streptococcal infection of the upper respiratory tract.

PRIMARY PREVENTION:

The prevention of initial attacks (primary prevention) depends on identification and eradication of group A streptococcus that produces the episodes of acute pharyngitis. Appropriate antibiotic therapy instituted before the nineth day of symptoms of acute group A streptococcal pharyngitis is highly effective in preventing first attack of rheumatic fever from that episode.

SECONDARY PREVENTION:

Secondary prevention is directed at preventing acute group A streptococcal pharyngitis in patients at substantial risk of recurrent acute rheumatic fever. Individuals who have already suffered an attack of acute rheumatic fever are particularly susceptible to recurrence of rheumatic fever with any group A streptococcal upper respiratory tract infection, whether or not they are symptomatic. Therefore these patients should receive antibiotic prophylaxis to prevent recurrences.

Antibiotic	***Dose***	***Mode***
Benzathine penicillin	1,20,000 IU every 3 -4 weeks	Intra muscular
Penicillin V	250 mg BID	Oral
Sulphadiazine	500 – 1000 mg OD	**Oral**
Erythromycin (allergy to penicillin)	250 mg BID	Oral

Patients who did not have carditis with their initial episode of acute rheumatic fever have a relatively low risk of carditis with recurrences. Antibiotic prophylaxis may be discontinued in these patients when they reach their early twenties and after at least 5 years have elapsed since

their last episode of rheumatic fever. The decision to discontinue prophylactic antibiotic should be made only after careful consideration of potential risks and benefits and of epidemiological factors such as the risk of exposure to group A streptococcal infections.

Patients who have carditis with their initial episode of acute rheumatic fever are at relatively increased risk of having carditis with recurrences and of sustaining additional cardiac damage. So they should receive antibiotic prophylaxis well into adulthood and perhaps for life.

ANTISTREPTOLYSIN O:

Antistreptolysin O is an antibody produced by human against streptococcal extra cellular enzyme called streptolysin O. It starts appearing after 1 week of streptococcal infection especially pharyngitis. It rises rapidly and reaches maximum by about 3 to 4 weeks and then starts disappearing after 12 weeks. This is not specific for any type of post streptococcal disease, but indicates if a streptococcal infection was present or not. Serial ASO titer testing is often performed to differentiate between acute and convalescent sera.

False positives:

1) Fats mainly beta lipoproteins in blood may cause false high values of ASO by neutralizing streptolysin O.

2) Tuberculosis, liver disease mainly acute viral hepatitis and other bacterial contamination also can produce false high values.

3) Hemolysed blood samples also can produce false high values.

False negatives:

1) Antibiotic intake prior to sampling can reduce the number of streptococci, and there by reduce the ASO level.

2) Steroid intake also reduces ASO by causing immune suppression.

3) Streptococcal skin infection may not cause significant raise in ASO titer.

4) 20% of acute rheumatic fever itself can have normal ASO.

UNITS:

ASO can be measured in two units; they are Todd units and

International units per ml.

Todd unit;

Defined as amount of serum just neutralizing 2 ½ minimum hemolytic doses of a standardized streptolysin. One international unit equals to 1.04 Todd units [14] .

METHODS TO DETERMINE ASO TITER:

1) HEMOLYTIC OR RANTZ RANDALL METHOD:

It is based on the properties of streptolysin O to produce hemolysis to a standardized rabbit erythrocyte suspension. Serial dilutions of the patient serum are mixed to 1 IU/ml of streptolysin O solution and the residual hemolytic activity is measured through the hemoglobin released by erythrocyte suspension on standard conditions. It is a semi quantitative method that has been used for several years as reference method. It is long and tedious.

2) LATEX METHOD:

It is based on the ability of ASO anti bodies present in the patient's sera to agglutinate latex particles coated with streptolysin O molecules. It is a qualitative method important by its simplicity.

3) LIGHT SCATERING IMMUNO ASSAY

These Immuno assays are based on the measurement of scattered or absorbed light, are an extension of the basic principles underlying that Latex agglutination tests. The change in the light scattered or blocked by Antibody or Antigen in solutions, is used to measure the amount of antigen or antibody, when causes the immunological antigen - antibody precipitation or agglutination reactions.

TURBIDIMETRY;

Turbidimetry is the measurement of light scattering species in solution by means of a decrease in intensity of the incident beam after it has passed through the solution. For turbidimetry assays change in the amount of light absorbed (inverse of amount transmitted) can be related to the amount of agglutination, which occurs. Hence the amount of analyte (the species causing agglutination in the sample) can be easily determined.

Turbidity is measured at 180° from the incident beam, or more simply in the same manner as absorption measurement are made in spectrophotometer. The turbidity can be measured on most spectrophotometer and automated chemistry analyzers.

This has been designed to measure ASO concentration in the range of 50 – 800 IU/ml and it is linear between the measuring ranges. No

prozone effect was observed up to a concentration of 1250 IU/ml of ASO. No interference is observed with bilirubin up to 50 mg/dl glucose up to 500mg/dl, hemoglobin up to 500 mg/dl, triglycerides up to 1g/dl and albumin up to 10g/dl.

NEPHELOMETERY:

It is the technique for measuring the light scattering species in solution by means of the light intensity at an angle away from the incident light passing through the sample. Nephelometry assays present an indirect method of measurement of the amount of analyte in a sample by measuring the amount of lights scattered or reflected at a given angle (90°) from the origin. Some nephelometers are designed to measure scattered light at an angle other than 90° in order to take advantage of the increased forward scatter intensity caused by light scattering from larger particles (example immune complexes).

The choice between turbidimetry and nephelometry depends on the application and available instrumentation. Until recently the statement was often made that for relatively clear solutions, in which transmission of light in the forward direction is greater than 95% small changes in absorption due to turbidity were difficult to measure with precision, and nephelometry was the method of choice. However with advent of stable

high-resolution photometric systems, turbidimetric measurements have become competitive in sensitivity with nephelometric methods for immunological quantitations of serum protein.

ANTI-DNASE B:

This is an antibody against deoxyribonuclease B antigen produced by group A Streptococcus. It is also used to diagnose antecedent streptococcal infection. When ASO and anti-DNase B are done concurrently 95% of previous streptococcal infections are detected. If both are repeatedly negative the likelihood is that the symptoms are not due to Streptococcus.

When the diagnosis of acute rheumatic fever, American Heart Association recommends ASO rather than anti-DNase B, even though anti-DNase B is more sensitive than ASO, its results are too variable. It also should be noted that, while ASO is the recommended test, when ASO and anti-DNase B are done together, the combination is better than either of these alone.

STREPTOZYME TEST:

It is a screening test to find out antibodies against five antigens like streptolysin O, DNase B, streptokinase, NADase and hyaluronidase. It has advantages over ASO and anti-DNase B. it can detect several antibodies in single assay. It is technically quick and easy to perform, and is unaffected by factors that produce false positive ASO. It has disadvantages' also. As the test detects different antibodies, it does not determine which have has been detected, and it is not sensitive in children as compared to adults, the fact that borderline elevations, which could be significant in children, may not be detectable at all. As with ASO a serially rising titer is more significant than single determination.

LITERATURE REVIEW

Sunil sethi, et al from Chandigarh have done ASO titer on 200 healthy children of 5 to 15 years. They have included children with no history of any recent throat infection in their study. ASO determination was done by stranded neutralization test. Found to have geometric mean titer (GMT) of 111.63 IU and ULN of 238.59 IU for children of 5-15 years. They also observed that GMT of 113.72 IU in 5 to 10 years and 110.32 IU in 11 to 15 years age group. The ULN was found to be 230.62 IU and 242.87 IU in 5 to 10 years and 10 to 15 years respectively. Though there was a difference in ULN in both age groups it was not statistically significant [15].

M G Karmarkar, et al from Mumbai have done ASO and anti-DNase B for 40 children and 160 adults in 1991-92 and also in 2001-02 of age and sex matched population. The inclusion criteria were volunteers with no features suggestive of sore throat in the past three months and no history of joint pain and not taken any antibiotic for the same. The ASO determination was done by tube dilution method. Found to have ULN of ASO 244 IU during 1991 and 305 IU during 2001in children. The adults' value was 195 IU in both the years. This study showed an upward shift of ASO from 244 IU to 305 IU in 10 years in same population. No significant seasonal variation was noted in both the years [16].

Danchin M H, et al from Australia have done ASO titer on 60 children in 2002. The inclusion criteria: children with no history of recent streptococcal infection. This study showed higher values in age group of 6-9 years. The ULN was 120 IU in 4-5 years, 480 IU in 6-9 years and 320 IU in 10-14 years.[17]

Edward L Kaplan, et al from United States have estimated ASO titer on 1131 children of 2 to 12 years in 1994-99. Inclusion criteria: children presented with pharyngitis and sera were taken on initial visit. Found to have GMT of 89 IU and ULN of 240 IU. They concluded that this age specific ASO are increased with age and they were slightly higher than previously reported[18]

Zaman M M, et al from Bangladesh have estimated ASO titer on 361 apparently healthy rural school children of 5 to 14 years, and found to have GMT of 241 IU and ULN of 390 IU. The ASO titer was measured by quantitative method using an auto analyzer.[19]

Berrios X, et al have measured ASO on 135 healthy children during 1986-89, and found to have GMT of 62 Todds unit in age group of 5 to 9 years, 127 TU in 10 to 14 years and 114 TU in more than 15 years. There were no significant difference detected between these results and those from earlier 1978-81 studies.[20]

Renneberg J, et al from Sweden studied upper 95% confidence limits for ASO titer on 741 sera from general population, shown that titer from children were increased abruptly with increasing age, from a mean of 21 units in children 3years of age or less to 211 units in 7 to 8 years. In children of 9 to 12 years exhibited a plateau with mean values of 168 to 258 units. In age more than 20 years, the mean titer diminished with increasing age. Individuals with age more than 70 years showed a mean titer of 83 units. This study concluded that age related reference values for ASO increase the clinical relevance of these tests.[21]

Fujikawa S, et al have studied the annual changes of ASO titer in school children from 1975 to 1981. The ULN highest in 1975 when 19.7% of children showed more than 500 units, the lowest in 1980 when 20.1% of children showed more than 80 units. Thus there was a significant difference in the ULN between two years; we must consider annual changes of ULN to make accurate diagnosis.[22]

Ayroub E M, et al from United States have studied group A Streptococcal antibodies in subjects with or without rheumatic fever in areas with high or low incidence of rheumatic fever. The levels of streptococcal antibody titer in population with or with out rheumatic fever from area with relatively high or low incidence rheumatic fever were compared. This revealed absence of consistent difference in the

GMT between rheumatic and non rheumatic fever from high prevalent area. This contrasted with low prevalent area where titers were significantly higher in rheumatic than non rheumatic. Conclusion: the presence of raised ASO in such population which probably reflects a high back ground prevalence of streptococcal infection, should be taken into consideration when evaluating the role of the group A Streptococcus in non purulent complications of those infections.[23]

Gharagozloo R, et al from Tehran, Iran have done on 3129 healthy individuals during summer and winter period and compared the seasonal variation. During summer 6% showed high titer of more than 250 IU. The same individual when tested during winter showed 11% having more than 250 IU. The seasonal difference was statistically significant.[24]

Cengiz AT, et al from Turkey studied ASO titer on 120 normal persons from different age groups. The GMT was 160.37 ± 14.08 Todd units. Only 20% of normal persons have had a titer over 200 Todd units[25].

Naicker GW, et al from Auckland have studied on 12 year old school children and ASO was determined. The mean titer did not change over a period of 12 months in paired samples. This showed absence of seasonal variation [26.]

Shanthi Rajkumar, et al from Chennai screened 666 school girls for

repeated sore throat, joint pain and swelling, chest pain, epistaxis, breathlessness, palpitation and abdominal pain etc. out of 666 girls screened 124 showed one of those above symptoms, and they were screened for presence of group A Streptococcus, ASO, CRP. Of these, 89.5% of children indicated repeated sore throat, but only 4% were positive for culture. ASO, CRP levels were higher in 11 to 15 years than in 5 to 10 years. [27]

STUDY JUSTIFICATION

ASO titer level is dependent on the age group, geographical location, site of infection and socio economic class. Different studies from different areas showing different ULN of ASO in different age groups.

MG Karmarkar et al [16] concluded that we have to constantly and periodically recheck on those very same values. Such checks may yield us results, which may serve as a foundation stone for further research on changing antigenic stimuli. They also noticed that the ULN of ASO was increased from 244 IU in 1991-92 to 305 in 2001-02.

Berroix X, et al [20] concluded that no significant differences were detected between the results in 1986-89 and those from 1978-81. Also Fujikawa [22] noticed to have significant difference of ULN of ASO between 1975 and 1980.

Sunil sethi, et al (15) also said that ULN and GMT might vary for children living in other regions; establishment of values in other areas will require additional studies. They also noticed 11 to 15 years of age having higher ULN than 5 to 10 years, although the difference was not statistically significant.

Renneberg, et al [21] also noticed ASO in sera from children increased abruptly with increasing age.

Study from Australia by Dandin MH, et al [27] found that age group 6 to 9 years having higher ULN than 10 to 14 years.

Ghargozloo R, et al [24] showed there was a seasonal difference in ULN of ASO, while MG Karmarkar et al [16] and Naicker GW et al [26] observed no seasonal variation.

No study yet done in literature comparing the socio economic variation even though there is a significant increase in incidence of rheumatic fever in lower socio economic group.

So keeping all these variations from various studies in mind, and no studies yet done in Chennai to find the ULN of ASO in normal healthy children of 5 to 15 years, this study was chosen.

AIM OF THE STUDY

1. To estimate the upper limit of normal value (ULN) of antistreptolysin O in healthy children of 5 to 15 years in Chennai city.

2. To compare the variation in titer with reference to age and socioeconomic status.

SUBJECTS AND METHODS

METHODOLOGY:

1) STUDY DESIGN	:	Descriptive study
2) STUDY PERIOD	:	March 05 to September 06
3) STUDY PLACE	:	Corporation and Matriculation schools in Chennai City.
4) STUDY POPULATION	:	School children
Inclusion criteria	:	children of 5 to 15 years
		Male/ female
Exclusion criteria	:	H/o sore throat
		H/o fever
		H/o arthritis
		H/o antibiotic intake
		in the past three months.

5) SAMPLE SIZE : 200 children

The GMT in 5-10 years age group is 113.7 and that in 11- 15 years age group is 110.3. With alpha error of 5% and power of 80%, the required sample size is 100 in each group.

6) SAMPLING TECHNIQUE: Stratified random sampling

MANEUVER:

Two Corporation schools and two Matriculation schools were selected after getting permission from head of the institutions. Using stratified random sampling technique 50 children from each school were included in the study. For each age group 100 children were selected and for each socio economic group 100 children were selected. Written consent from each child's parents after explaining about the procedure and consequences obtained.

Because there was a difficulty in extracting monthly income from children and parents, the Corporation school children were taken as lower socio economic group and children from Matriculation schools were taken as higher socio economic group. The samples were collected from July 05 to June 06 to cover seasonal variation.

The children were selected using above said inclusion and exclusion criteria. There is no need for fasting before sampling. After cleaning the local area thoroughly with spirit and about 2 ml of blood was drawn using sterile syringes and needles. The blood samples were transported to microbiology laboratory at Institute of Child Health and Hospital for Children immediately. There they were centrifuged and sera were separated. These separated sera were kept under -20° C still further use.

The ASO titer was estimated from those stored sera by using new method called turbidimetric immuno assay. This is an internationally accepted method to determine ASO in human serum. This is an quantitative method to estimate ASO like nephelometric method. As we need to have an exact value of ASO in serum, we cannot use qualitative methods like latex agglutination, and the older methods like tube agglutination are very tedious and cumbersome, we have chosen this method to know what is the exact level of ASO in the serum. Figure (1) shows the ASO kit by which the determination was done. Figure (2) shows the semi auto analyzer, used for analysis.

Principle:

The turbidimetric immuno assay is based on the principle of agglutination. The test specimen is mixed with latex reagent and activation buffer, and allowed to react. Presence of antibody in the test specimen results in formation of an insoluble complex resulting in an increase in turbidity, which is measured at 505- 578 nm wavelength. The increase in turbidity corresponds to the concentration of ASO in the serum.

Figure 1 showing ASO Kit

Figure 2 showing Semi Auto Analyzer

Test procedure:

The stored sera were brought to room temperature before estimation. Sera were made sure that they are not hemolysed or lipemic to avoid false high values. The reagents also brought to room temperature before use.

The reagents are

Activation buffer (R1) - ready to use buffer.

Latex reagent (R2) - ready to use uniform suspension of polystyrene latex particles coated with stabilized streptolysin O.

Calibrator – a lyophilized preparation of ASO positive serum, which is equivalent to stated amount of ASO on IU/ml basis, when hydrated appropriately. The strength of calibrator after reconstitution will be mentioned in the packet.

Calibration curve:

The calibrator was reconstituted with 1 ml of distilled water and swirled to make solution homogenous after 10 minutes. Then the ASO calibration curve was prepared by diluting the calibrator serially as mentioned below.

Test tube no	1	2	3	4	5
Calib.dilut. no	D1	D2	D3	D4	D5
Normal saline	-	100µl	100µl	100µl	100µl
Calibrator	100µl	100µl	100µl	100µl	100µl
ASO IU/ml	800	400	200	100	50

Preparation of calibration curve was done by, mixing 500 µl of activation buffer (R1) and 50 µl of latex reagent (R2) in the measuring cuvette, and keeping for 5 minutes at 37ºC. Then 100 µl of calibrator D1 was added and mixed well. The absorbance (A1) was read exactly at ten seconds, and also at the end of four minutes (A2). The same steps were repeated for each diluted calibrator D2 to D5. ΔA (A2- A1) calculated for each calibrator (D1 to D5). Then a graph was plotted with ΔA versus concentration of ASO.

The procedure was done with specimen in the place of calibrator, ΔA was calculated for test specimen. ΔA of specimen was interpolated on the calibration curve, and the concentration of ASO of the specimen was obtained. ASO titer for each samples were calculated by the same method as described above.

STATISTICAL ANALYSIS:

The Geometric Mean Titer (GMT) was obtained for the titer values. 80^{th} percentile was taken as the upper limit (ULN) [13]. The significance between the groups was determined using the Mann – Whitney U test. The significance was determined at 5%.

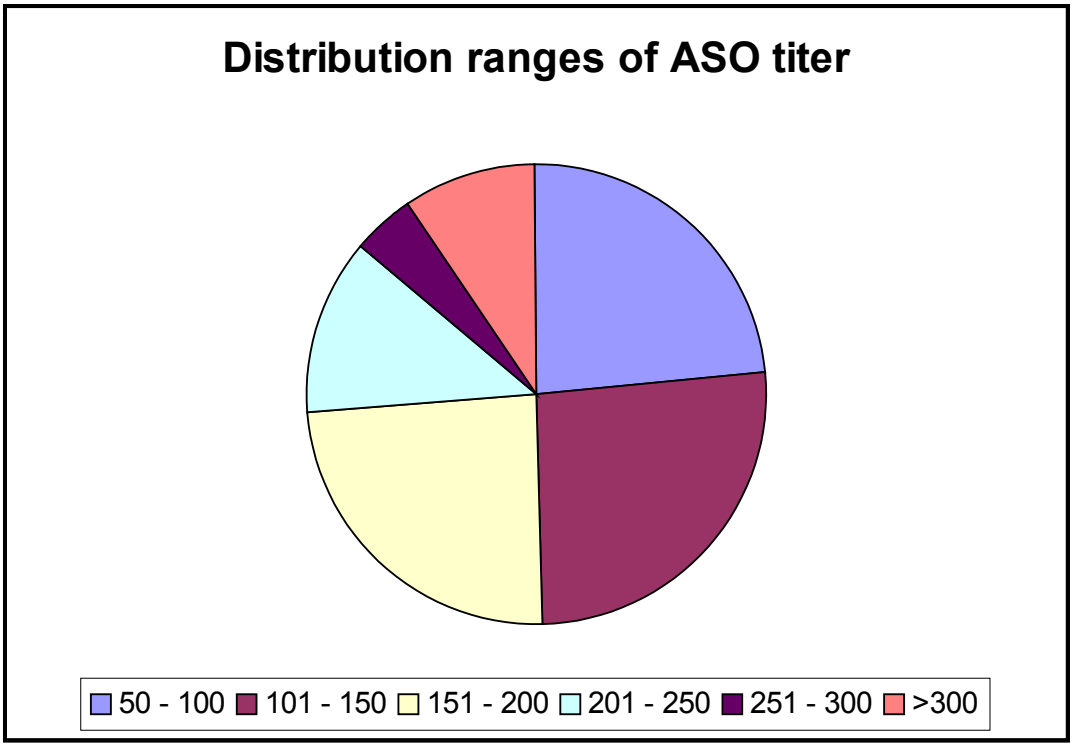
OBSERVATION

The upper limit of normal for whole sample is 227 IU/ml. The frequency distribution of ASO titer of these 200 children is given below in table (1). Of these, maximum number of children are between 101 to 150 IU/ml (26%). There are also 23.5% of children who had titer between 50 to 100 IU/ml. Only 4.5% children are between 251 to 300 IU/ml. But there are about 9.5% of children who had above 300 IU/ml.

Table (1)

Distribution ranges of ASO titer:

Titer	No of children	Percentage
50-100	47	23.5
101-150	52	26
151-200	48	24
201-250	25	12.5
251-300	9	4.5
› 300	19	9.5


Distribution ranges of ASO titer
50 - 100
101 - 150
151 - 200
201 - 250
251 - 300
>300

The upper limit of normal titer for children of age group of 5 to 15 years including both lower and upper socio economic classes is 227 IU/ml. The GMT for all children in study group is 147 IU/ml.

Table (2)

GMT and ULN of children 5- 15 years in both socio economic classes:

Group	GMT	ULN
Over all	147	227

The ULN for age group of 5 to 10 years is 233 IU/ml and for 11 to 15 years is 216 IU/ml. Though there is a difference between these groups, it is not statistically significant (p=0.67). The GMT for both groups is 149 IU/ml and 144 IU/ml respectively.

Table (3)

The comparison GMT and ULN among the age groups:

Age group	GMT	ULN	p-value
5- 10 years	149	233	0.67
11-15 years	144	216	

The ULN for lower socioeconomic status is 270 IU/ml and for higher socio economic status it is 201 IU/ml. Even though there seems to

be a difference between these groups, it is not statistically significant. The GMT is 153 IU/ml and 141 IU/ml respectively.

Table(4):

The comparison of GMT and ULN among socio-economic groups:

Socioeconomic status	**GMT**	**ULN**	**p-value**
Low	153	270	0.56
High	141	201	

Table (5) shows the comparison between the lower and higher socioeconomic classes with in the age group of 5 to 10 years. The GMT is 157 IU/ml among low socioeconomic group and 142 IU/ml among high socioeconomic group. The ULN is found to be 290 IU/ml among low socioeconomic group and it is 201 IU/ml among high socioeconomic group. The difference observed is not statistically significant (p=0.67).

Table (5):

GMT and ULN for the age group of 5-10 years among different socio-economic groups:

Socioeconomic class	**GMT**	**ULN**	**p-value**
Low	157	290	0.67
High	142	201	

Table (6) shows difference between lower and higher socioeconomic class in age group of 11 to 15 years. The GMT is 149 IU/ml among low socio economic group and 140 IU/ml among high socioeconomic group. The ULN is found to be 256 IU/ml among low socio economic group and it is 204 IU/ml among high socioeconomic group. Though there is a difference between two groups, it is not statistically significant (p=0.75).

Table (6)

For the age group of 11-15 years the socio-economic difference:

Socioeconomic class	**GMT**	**ULN**	**p-value**
Low	149	256	0.75
High	140	204	

Comparing the titer values among age groups studied with in the lower socio economic class shows age 5 to 10 years having higher values than 11 to 15 years, is also not significant.[Table (7); p=0.7]

Table (7)

GMT and ULN for lower socio-economic class among different age groups:

Age group	GMT	ULN	p-value
5-10 years	157	290	0.7
11-15 years	149	256	

But among higher socioeconomic class shows that age group 11 to 15 years have little higher value compared 5 to 10 years group, which is also not statistically significant. [Table (8) p=0.82]

Table (8)

GMT and ULN for higher socio-economic class among different age groups:

Age group	GMT	ULN	p-value
5-10 years	142	201	0.82
11-15 years	140	204	

DISCUSSION

One of the most important parameter to identify the recent streptococcal infection in a child who is suspected to have rheumatic fever is ASO titer. The evidence of recent streptococcal infection should be there to diagnose rheumatic fever which forms a part of Jones Criteria (1992)[10] .The ASO titer seems to be influenced by various demographic factors like age, environmental factors, etc leading on to wide range of titer values, observed from different studies globally. It has also been reported that the upper limit of normal titer varies over a period, even within the same geographical area. All these facts necessitate to define the upper limit of normal for individual population, as well as to be confirmed periodically to observe any change in the value to higher or lower side.

This goes without saying that the ULN of ASO titer to be redefined accordingly and made use for the diagnosis of acute rheumatic fever, otherwise leading to over or under diagnosis of acute rheumatic fever.

As we have scarce literature on this subject in Indian Sub Continent, that too from South India this study was done with the objective of finding out GMT and ULN of ASO titer for the 5-15 Years age group from the Urban Population of Chennai City.

The upper limit of normal of ASO in the present study is 227 IU/ml and the Geometric Mean titer is 147 IU/ml. The table shows the comparison among the present study and various other studies from literature.

Table (9):

Comparison of GMT and ULN of ASO titer by various studies:

S.No	Study	Sample size	*GMT*	ULN
1	Present	200	147	227
2	Sunil Sethi et al [15]	200	111.63	238.59
3	Karmarkar et al [16]	40	---	244, 305
4	Kaplan E L et al [18]	1131	89	240
5	Zaman M M et al [19]	361	241	390
6	Berriox et al [20]	135	110	---

The present study correlates well with most of the above said studies. But the studies by Zaman M Met al and Karmarkar et al showed a higher ULN than the present study. This may be due to seasonal or geographic variations. The socio economic variation was also not mentioned in their studies.

The ULN in 5 to 10 years is 233 IU/ml and in 11 to 15 years it is 216 IU/ml. Though there is a difference it is not statistically significant. The study from Australia by Dandin, et al also observed that 6 to 9 years having more titer than 10 to 14 years [17]. Renneberg [21] et al also observed age group of 9 to 12 years having high values than other age groups.

But study by Sunil Sethi, et al [15] showed higher ULN in 11 to 15 years than 5 to 10 years. But this difference was not statistically significant. Another study by Berriox et al, [20] also showed 10 to 14 years having more GMT compared to 5 to 9

years.

Children of 5 to 10 years are having more chance of getting recurrent streptococcal infection than 10 to 15 years, which may due to poor hygiene, vulnerability and over crowded classrooms in 5 to 10 years. But the exact cause is not known.

The children from Corporation schools that is from lower socio economic class children show a higher ULN than for children from higher socio economic class. Though the difference observed in our study is not statistically significant, there is well known correlation between overcrowding, lower standards of living and acute rheumatic fever. The children from Corporation schools are mostly from lower socioeconomic class and their standard of living also low. They are over crowed in schools as well as in houses. These may be the cause for the higher value among lower socio economic group. There is no study on socio economic variation in literature.

SUMMARY

- The upper limit of normal value of antistreptolysin O in the age group of 5 to 15 years is 227 IU/ml with in the urban limit of Chennai City during the period from July 2005 to June 2006. The geometric mean titer is 147 IU/ml.

- The ULN of ASO in 5 to 10 years is 233 IU/ml and in 11 to 15 years it is 216 IU/ml, and the GMT is 149 IU/ml and 144 IU/ml respectively.

- The ULN of ASO in lower socio economic group is 270 IU/ ml, and in higher socioeconomic group is 207 IU/ml, and the GMT is 153 IU/ml and 141 IU/ml respectively.

- Most of the children (49.5%) are having ASO titer of less than 150 IU/ml. Around 9.5% of children found to have titer above 300 IU/ml.

CONCLUSION

The antistreptolysin O level in healthy children of age group of 5 to 15 years in Chennai city is 227 IU/ml.

This level can be taken as upper limit of normal, while considering the diagnosis of post group A Streptococcal infections in our population.

ANNEXURE

DATA ENTRY FORM

Name: Address:

Age:

Sex: Socio economic class:

Complaints:

H/O sore throat : yes / no

H/O fever : yes / no

H/O antibiotic intake : yes / no

H/O arthritis / Arthralgia : yes / no

Examination:

General examination:

Throat:

CVS:

Titer:

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