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ANTI -STREPTOLYSIN O TITRE IN COMPARISM TO POSITIVE BLOOD CULTURE IN DETERMINING THE PREVALENCE OF GROUP A STREPTOCOCCUS INFECTION IN SELECTED PATIENTS IN ZARIA, NIGERIA

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ABSTRACTS: *The diagnosis of RF through recovery of streptococci from positive throat cultures and the use of immunological assays such as Anti-Streptolysin O (ASO) would provide useful in the diagnosis of streptococcal infections. The study therefore is aimed at determining the ASO titre in comparism to positive blood culture in determining the prevalence of GAS in the study area. A total of hundred swab and blood samples were collected for Streptococcus pyogenes isolation and characterization using cultural and confirmed using the Streptococcus identification kit. Qualitative and semi quantitative ASO determination was done using the ASO latex agglutination kit. The overall prevalence for Streptococcus pyogenes was 16% as determined by blood culture. Of the 16 patients that tested positive to cultural tests, 11 (68.75%) tested positive for ASO while the remaining 5(31.25%) were negative. The level of ASO in patients that tested positive for the presence of ASO ranged from 400IU/ml to 3200IU/ml. C10 and C13 showed the highest concentration of 3200IU/ml respectively with a mean of 1018.19IU/ml. Positive culture methods remain the effective method for diagnosis of Streptococcal infection. However, ASO remains a cheaper and easier method for diagnosis and could provide baseline information for use in diagnosis in developing countries though it has not been deployed in many clinical settings.*
KEYWORDS: Anti-Streptolysin O (ASO), latex agglutination, streptococcal infections, GAS

INTRODUCTION

Group A Streptococcus (GAS) are by far the most common bacterial cause of acute pharyngitis, accounting for approximately 15-30 % of cases in children and 5-10 % of cases in adults (Bisno, 2001). Certain M protein serotypes, such as M types 1, 3, 5, 6, 14, 18, 19, and 24 of GAS, are found associated with throat infections and rheumatic fever (Stollerman, 1997, Mandor *et al.,* 2013). The GAS strain producing scarlet fever does so because it carries the genes for one or more of the streptococcal pyrogenic exotoxins (Cunningham, 2000). Although they are not considered normal flora, pharyngeal carriage of GAS can occur without clinical symptoms of disease. GAS, which invade the skin and cause impetigo are different M protein serotypes from those that cause pharyngitis. In addition, some of the skin strains are associated with production of acute post streptococcal glomerulonephritis (Bisno and Stevens, 1996). In addition to suppurative infections, GAS is also able to cause acute rheumatic fever (ARF) and subsequent rheumatic heart disease (RHD) after infection in humans (Bisno, 1991). In this classic form, the disorder is acute, febrile, and largely self-limited. However, damage to heart valves may occur, which is referred to as rheumatic heart disease, total disability and, not infrequently, death may occur many years after the acute attack (Bisno and Stevens, 2000). Epidemiological data show that some GAS serotypes have been clearly associated with epidemics of rheumatic fever. M5 is the most common serotype, and M1, 3, 5, 6, 14, 18, 19, 24, and a few others are well represented. Such prevalent pharyngeal

types as M2, 4, and 12 are not associated with ARF (Stollerman, 1997). However, there is still much confusion about the notion of the rheumatogenic potential of specific M serotypes (Stollerman, 2001). The pathogenic mechanism involved in the development of ARF/RHD by group C and G Streptococci remains unclear; however it is evident that an abnormal humeral and cellular immune response maybe involved (Haidan *et al*., 2000, Hashikawa, *et al*., 2004).

The diagnosis of RF through recovery of streptococci from positive throat cultures are obtained in only about 11% individuals (Sethi, *et al*., 2003) and 2.5-35.4% of individuals with acute infection (Pichichero, *et al*., 1999). The use of immunological assays such as Anti-Streptolysin O (ASO) would provide useful in the diagnosis of streptococcal infections and their complications, and during follow-up, as well as in evaluating the effectiveness of treatments (Mahendrappa, 2010) as well as in situations when the throat culture technique is ineffective or when the patient has commenced antibiotics therapy. Significant findings have shown that an ASO-positive measurement might be used in conjunction with throat culture to identify GAS) (Manandhar *et al*., 2013). The presence of an immune response to either GAS somatic or extracellular antigens remains the most reliable means for documentation of bonafide infection (Johnson, *et al*., 2010). The study therefore is aimed at determining the ASO titre in comparism to positive blood culture in determining the prevalence of GAS in the study area.

MATERIALS AND METHOD

The Study Area and Population

The study area was Samaru Environmental Health Centre, Samaru, a densely populated, semi-urban area in the Zaria Local Government Area of Kaduna State. The Primary Health Centre is under the Kaduna State Government. Permit for the study was obtained from the Authorities of the Health Centre and verbal consent was obtained from all patients that participated in the study. The study population comprises of the patients aged 12 – 20 years reporting with suspected streptococcal infections to the Centre irrespective of gender.

Sample size, collection and handling

A total of hundred samples were collected; Blood and throat swab were collected from the same patient. Pharyngeal swabs and blood samples were collected from patients reporting with suspected streptococcal infections to the Centre. The pharyngeal swabs were collected using a sterile swab stick and a tongue depressor is used to put down the tongue so that the pharynx is visible and then the swab sticks was used to swab the pharynx gently. It is then returned into the swab holder and then transported to the Microbiology Laboratory for subsequent tests. A total of 3ml of venous blood was collected into sterile plain bottles using sterile 5ml syringes and transported to the Laboratory for further analysis.

Isolation and characterization of Streptococcus sp

The swabs were streaked on plates of blood agar labeled against the source of each of the specimens. The plates were incubated at 37°C for 24 hours after which, the plates were observed and the colonial morphology of colonies recorded. The phenotypically distinct colonies were sub-cultured on fresh blood agar to obtain pure bacteria culture. The freshly inoculated plates were

incubated at 37°C for 24 hours. A colony from each of the pure cultures was Gram stained. Based on the Gram reactions, further characterization for the identification of each of the isolates was done by subculture on other media or by further biochemical tests. Isolates that were Gram positive cocci arranged in chains were subjected to catalase test. And those found to be catalase negative was subjected to further tests such as voges proskauer test, bacitracin test and Pyrrolidonylarylamidase test (PYR). A presumptive isolates were confirmed using the Microgen Streptococcus identification kit

Determination of Anti-streptolysin-O in the study Population

The ASO test is a stabilized buffered suspension of polystyrene latex particles that have been coated with Streptolysin O. When the latex reagent is mixed with serum containing ASO, agglutination occurs. The sensitivity of the latex reagent has been adjusted to yield agglutination when the level of the ASO is greater than 200IU/ml. A level determined to be indicative of disease by epidemiological and clinical studies.

Qualitative test

The qualitative test is a screening test to determine the presence of the ASO antibody in the serum. The test was carried out by bringing all the tests reagents and serum too room temperature. The ASO latex vial was shaken gently to disperse and suspend latex particles. Positive and negative controls were tested with each series of test to be done.The disposable pipette provided, was used to place a drop of test serum onto a circle on the slide and one drop of the ASO latex was also delivered to the slide that contains the specimen, the resulting mixture was spread using the paddle end of the slide. The slide was gently tilted and rotated by hand for 2 minutes and was observed for macroscopic clumping under high intensity light. The reaction of the test serum to the ASO positive and negative sera was compared. Sera that tested positive in the screening / qualitative test were retested in the titration test to provide verification for borderline interpretations.

Semi-quantitative test

For each test to be titrated, six test-tubes were set-up and labeled 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and to each tube 0.2ml of physiological saline. To tube number one 0.2ml of undiluted test serum was added and two fold dilutions were serially made by mixing contents of tube number one with pipette and 0.2ml was transferred to tube number two. Each serial transfers was repeated for each tube. Agglutination shows positive result and a negative result is shown by a smooth milky suspension. The greatest dilution of the test sample showing agglutination is considered the endpoint

RESULTS

The overall prevalence for *Streptococcus pyogenes* in the work was 16 (16%) as determined by blood culture (Fig. 1). When the 16 patients that tested positive to cultural methods were subjected to ASO assay showed that, 11 (68.75%) of the patients tested positive while the remaining 5(31.25%) were negative (Fig. 2 and 3)

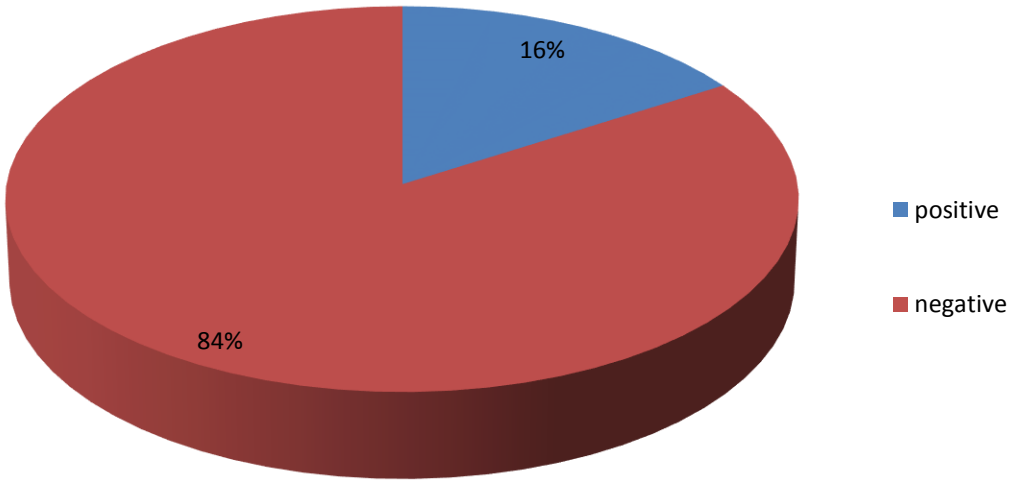

Fig. 1: Prevalence of *Streptococcus pyogenes* in the Study Population determined by throat culture

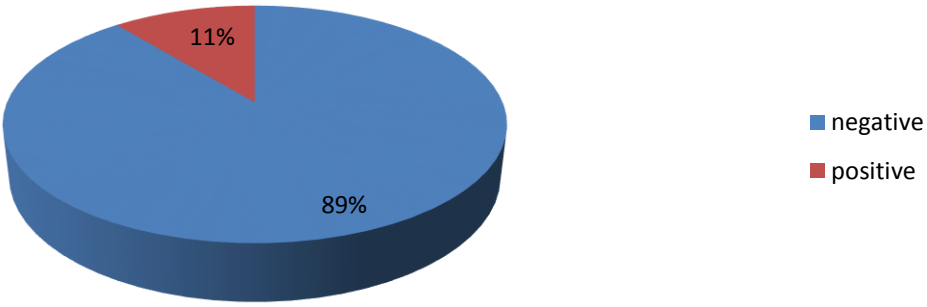

Fig.2: Determination of the presence of ASO in the Study Population

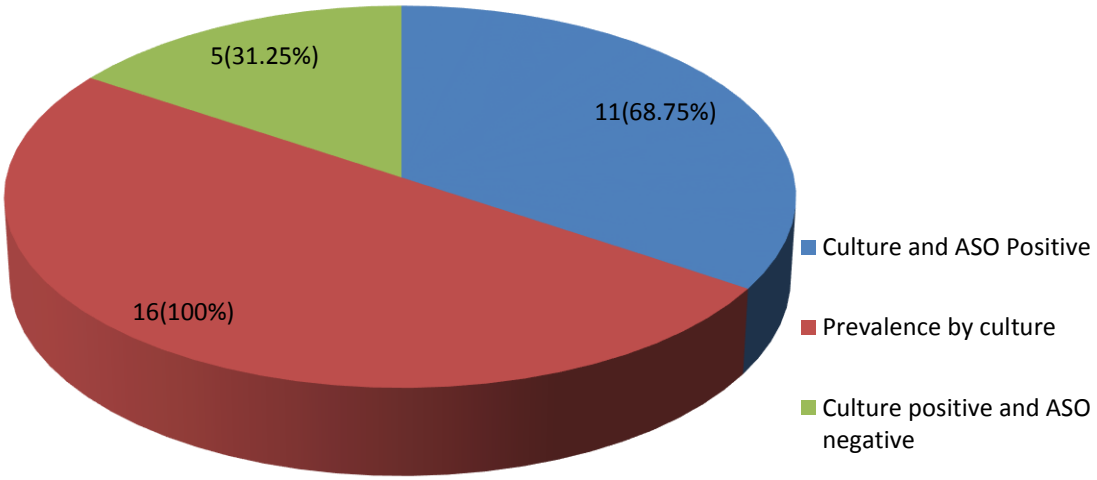

Fig. 3: The comparative study of both the ASO and Culture prevalence studied

The level of ASO in patients that tested positive for the presence of ASO varied from 400IU/ml to 3200IU/ml. C10 and C13 showed the highest concentration of 3200IU/ml respectively with a mean of 1018.19IU/ml (Table 1).

Table 1: The level of ASO in patients that tested positive for the presence of ASO Antibody

Specimen	Dilution (end point)	Concentration (IU/ml)
C3	1:2	400
C10	1:16	3200
C13	1:16	3200
C26	1:8	800
C29	1:8	800
C30	1:4	400
C33	1:8	800
C39	1:2	400
C42	1:2	400
C43	1:2	400
C45	1:2	400

DISCUSSION

The deployment of effective tool is of paramount importance in the diagnosis and effective control of infectious agent of diseases. There is therefore a continuous search for efficient protocol although this must be guided by cost as well as efficiency of time. In this study, the prevalence by culture was higher than that noted for the ASO assay, agreeing with the age long practice and that positive culture remains the gold standard in diagnosis. If done correctly, culture of a single swab on a blood agar plate has a sensitivity of 90-95% for the detection of the presence of GAS in the pharynx (Bisno, 2001). Other findings indicate 2.5-35.4% of individuals with acute infection (Pichichero, *et al.,* 1999). The culture positivity in this study was slightly lower than the 20.6% prevalence reported in children in Calabar (Mandor, *et. al.*, 2013); though lower than a previous study (13.3%) in Lagos, Nigeria (Lawal, *et al*., 1987). Charmaine, *et al*., (2006) reported 21.4% in Chennai, India while 15.4% was reported in the Tri-Island State of Grenada (Noel, *et al*., 2005). However, in some instances, positive culture may not provide a true diagnosis to the disease. Some reports have indicated the limitation of culture especially in cases of carrier state (Sethi, *et al*., 2003). As with many infections, pathogen recovery may also be hampered by culture due to empirically initiated antibiotics prior to hospital admission (Chang, *et al*., 2005; Berbari, *et al*., 2007).

Anti-streptolysin O (ASO) is the antibody response most often examined in serological tests to confirm antecedent streptococcal infection, and helps in the diagnosis of rheumatic fever (Cunningham, 2000). ASO remains a useful in the diagnosis of streptococcal infections and their complications, follow-up, as well as in evaluating the effectiveness of treatments (Periwal, *et al*., 2006; Danchin and Kelpie, 2007). ASO is helpful when the throat culture technique is ineffective or when the patient has already taken antibiotics. Since impoverished societies cannot afford other tests, such as throat culture, ASO is the only available test for diagnosing streptococcal infection. Significant findings have shown that an ASO-positive measurement might be used in conjunction with throat culture to identify Group A Streptococcus (GAS) carriers (Manandhar *et al*., 2013) necessitating the employment of the test in this work. Our finding indicates that 11% of the participants had significant ASO levels above the 200IU; a level higher than that reported in India

(Sethi, *et al*., 2003). Uc¸kay *et, al*. (2009) reported ASO levels of up to 800 IU in their work among orthopedic infections in Geneva.

ASO titers have been deployed exclusively for epidemiological studies and the clinical diagnosis of *S. pyogenes* infections and its sequelae, such as rheumatic fever, glomerulonephritis, and reactive arthritis after throat infections (Uc¸kay, *et al*., 2009). Some individuals are reported to harbor the same strain of GAS in the upper respiratory tract for long periods of time without symptoms and without an increase in ASO titers. This "carrier state" phenomenon remains poorly understood posing some limitations to clinicians and laboratory scientists studying the pathogenesis of streptococcal infections and their sequelae. (Johnson, *et al*., 2010)
However, ASO assay have not been deployed routinely in many laboratory in the study area and most work employ cultural techniques for diagnosis of streptococcal infections. There is need therefore to explore its diagnostic application in low income settings like Nigeria.

CONCLUSION

Positive culture methods remain the effective method for diagnosis of Streptococcal infection. The prevalence of *S. pyogenes* by positive culture method in the study population was 16% out of which, 11(68.75%) had significant levels of ASO while 5(31.25%) were negative. It was also found that 2 patients had ASO levels of 3200IU/ml and the mean ASO was 1018.19IU/ml. However, ASO remains a cheaper and easier method for diagnosis and could provide baseline information for use in diagnosis in developing countries though it has not been deployed in many clinical settings.

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