STREPTOCOCCAL THROAT INFECTION AMONG YEMENI CHILDREN

Madha Mohammed Sheet Saleh
Department of Medical Laboratory Science Technology, College of Health and Medical Technology, Baghdad – Iraq

Abstract

This package was conducted in Taiz/ Yemen as cross sectional study and mainly aimed to determine the prevalence rate of Group Aβ- herrolyhc streptococcus among school age children cases (1-15years) with pharyngitis and/ or tonsillitis. GAβHS was found in 28.6% out of the total 475 throat swab specimens, which were taken from cases with clinical sore throat. A peak of GAβHS bacterial isolates was noticed in the 6-10 years age group. The gender was found of no value as a risk factor in increasing or decreasing the prevalence rate of infection. Other risk factors were found variable in their influence as the family size, which was of high significant effect on the prevalence rate of GAβHS infection, and the seasonal variation as it was found that the highest prevalence rates at winter season (November and December) as well as rainy season (July and August). No association was pointed out between the GAβHS isolates and the results of anti-streptolysin. Antimicrobial sensitivity test revealed that amoxicillin was the antibiotic of choice for the presumptive treatment of GAβHS sore throat as 94.3% of the isolates were sensitive for this antibiotic.

التهاب البلعوم ببكتريا المسبحات القيحية بين الأطفال اليمنيين

مضاء محمد شيت صالح
قسم التحليلات المرضية، كلية التقنيات الصحية والطبية. بغداد– العراق.

الخلاصة

أجريت هذه الدراسة المقطعية في مدينة تعز–اليمن لتحديد نسبة انتشار بكتريا المسبحات القيحية المجموعة أ الحالة للدم نوع ب بين أطفال المدارس المرضى بالتهاب البلعوم واللوزتين. النتيجة كانت ايجابية في 28.6% من مجموع الحالات السريرية الكلية (475). مع وجود قمة في نسبة الإصابات في الفئة العمرية 6–10 سنوات. لم يكن لجنس الأطفال كعامل مؤثر أي اثر معنوي في نسبة الانتشار للمرض بينما كان لبعض العوامل الأخرى تأثيرا معنويا واضحا في ذلك مثل حجم الأسرة حيث ازدادت نسبة الانتشار في الأطفال الذين ينتمون إلى اسر ذات حجم عددي كبير وكذلك كان للفصل المناخي تأثيرا مشابها حيث كانت نسبة الانتشار المرضي عالية في موسم الشتاء وموسم الأمطار. لم تجد هذه الدراسة أي علاقة ما بين النتائج الموجبة لتلك البكتريا والنتائج المصلية لفحص مضاد الستربتولايسين او. بالنسبة لنتائج فحص حساسية تلك البكتريا لمجموعة من المضادات الحياتية فقد وجد ان أفضل مضاد حياتي افتراضي لعلاج هذا المرض هو الاموكسيسلين وبنسبة تحسس وصلت إلى 94.3% من مجموع العزلات المدروسة في هذا الفحص.

Introduction

Streptococcus pyogenes, or group A *Streptococcus* (GAS) causes numerous infections in humans, including pharyngitis, tonsillitis, scarlet fever, cellulitis, erysipelas, rheumatic fever, post streptococcal glomerulonephritis, necrotizing fasciitis, and lymphangitis. In streptococcal pharyngitis and tonsillitis, the pharyngeal acquisition is by person-to-person transmission of *Streptococcus pyogenes* primarily occurs through respiratory droplets, although it may also spread through body secretions from an infected patient [1]. Epidemics of pharyngitis have also occurred following ingestion of contaminated non-pasteurized milk or food, where pharyngitis in food-borne outbreaks is usually marked by short-than-usual incubation times (less than 2 days) and more severe acute symptoms [2]. The changing epidemiology of GAS and rheumatic fever is said to be related to changes in the distribution of serotypes [3], where certain virulent M types have been associated with invasive disease [4]. The prevalence, as well as the incidence rates of GAS sore throat among children below 15 years of age is almost well established in many localities in the world, but it is not in Yemen. Under the age of 3 years, pharyngitis is usually non-streptococcal, being caused by viral infections [5]. Age is not the only factor that contributes to the prevalence of infection but there are many other factors. Gender-associated variation was noticed in some studies [6], whereas season-associated variation was noticed in other studies [7].

Culture methods and the follow up bacteriological procedures are the gold standard for the diagnosis of GAS throat infection, whereas serological methods are mostly helpful in the diagnosis of rheumatic fever. Serological diagnosis of group A streptococcal infections is based on immune responses against the extracellular products streptolysin O, DNase B, hyaluronidase, NADase, and streptokinase [8]. The most popular and standardized serological test is still anti-streptolysin O (ASO) rather than ADNase B. ASO is not only useful in the diagnosis of streptococcal infections or their complications, but also in the follow-up process, and in evaluating the effectiveness of treatments. ASO is especially helpful when throat culture technique is improper or the patient has already taken antibiotics [9].

Penicillin remains the treatment of choice for group A streptococcal pharyngitis because of its proven efficacy, narrow spectrum, safety and low cost [10]. *In vitro*, no change was reported in penicillin sensitivity in collection of Group A Beta Haemolytic Streptococcus (GAβHS) from 1917 to 2004 [11 , 12]. On the contrary, other workers published a meticulously designed study in which injectable oral penicillin failed in 35% of children [13] or had no beneficial effect in children with sore throat on the average duration of symptoms [14]. Other reports observed that up to 25% of acute pharyngitis cases treated with penicillin having continued asymptomatic bacterial carriage within the nasopharynx [15]. Some studies suggested that using amoxicillin is as effective as penicillin V [16]. These aspects of treatment should be considered when selecting an antibacterial agent to treat GAβHS pharyngitis [17]. The cephalosporins appear to be satisfactory alternative to penicillin, particularly for patients who are allergic to penicillin [18]. This might be due to the superior efficacy of these drugs in eradicating carriage state [19]. Erythromycin considered as the drug of first choice in patients with an allergy to penicillin, however, azithromycin is better tolerated than erythromycin. [20].

Materials and methods

Media, solutions and reagents

Different culture media were used in this study including; blood agar base, Nutrient broth, MacConkey Agar (DIFCO), Crystal Violet (1 in 50,000) Blood Agar, brain heart infusion agar and Mueller- Hinton Agar (OXOID),

Different solutions and reagents were used in this study including; hydrogen peroxide 6 % for catalase enzyme test [21], McFarland solution (Tube 0.5) which was prepared as previously described [22], , sodium chloride solution (0.9%), *Streptococcus* grouping kit (OXOID), ASO latex (DIAMO), standard control strains which were obtained from American Type Culture Collection (ATCC) including; *Escherichia coli* V517 and S*taphylococcus aureus* 25923. The following table shows the types of antibiotics disks that were used in the antimicrobial susceptibility test.

Table 1: Types of antibiotic disks which were used in the anti

Antibiotic	Conc.	Antibioti
Penicillin G	10 Units	Ceftazidim
Ampicillin	10 µg	Azithromy
Amoxicillin	10 µg	Erythromy

Study samples

The study is a cross sectional in which the sample size was calculated by the statistical program of epi-info (version 6.04). The sample size was 475 children of age of 1 - 15 years who were attending the Al-Yemeni-Swedish hospital (out- and in-patients) and were suffering from throat infection as clinically inspected and primarily diagnosed by the hospital pediatricians. Throat swabs were taken from all cases in this group. Due to certain obstacles relating to parents permission, from only 191 children (4-15 years) out of these 475 cases blood sample beside the throat swab were taken. Some information were recorded concerning the gender, age, symptoms, family size, area of residence, antibiotics administration, history of the child's health including any previous sore throat, rheumatic fever or other infections. Throat swab specimens were processed immediately, whereas sera were separated from blood samples and stored in small aliquots at 20˚C or below in Appendrof tube until the time of testing. Types and numbers of the specimens that had been collected are shown in Table (2).

Table 2: Types and numbers of specimens.

Type of specimens	Number of specimens		
	Male	Female	Total
Throat wabs only	160	124	284
Throat and blood samples	94	97	191
Total	254	221	475

Methods

According to the diagnostic procedures recommended by [23, 24, 25 , 26], the isolation and identification of *S. pyogenes* from throat swabs of children were performed. The colonial morphology was studied after 24 hours and Gram staining was done for the β-haemolytic colonies. MacFaddin method was performed for catalase [21], Collee *et al*. method was used for the growth on MacConkey to differentiate between *Streptococcus pyogenes and Staphylococcus aureus* [27], whereas Maxted method was used for bacitracin susceptibility [28] to differentiate between GAS and other streptococci. Lancefield grouping was performed according to the procedure of the manufacturer (Oxoid). ASO antibodies in patient's sera were tested. A quantitative method was followed for all positive ASO sera to titrate the ASO antibodies [25]. Modified Kirby-Bauer Method was used for testing the antimicrobial sensitivity against a selected group of antibiotics [29]. For statistical analysis, Chi- square test was used. Differences between results were considered significant at P ≤ 0.05 as determined by the appropriative tests.

Results

Figure 1 shows the number and percentage of throat swab bacterial isolates among 475 symptomatic children.

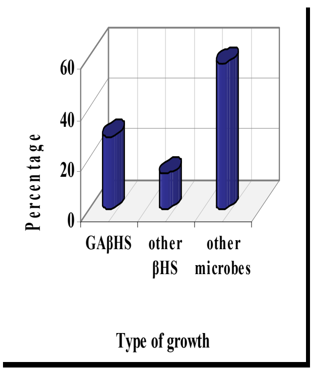

Figure 1: Culture results of bacterial isolates among 475 symptomatic children.

The distribution of positive cases by gender is shows in table 3.

Table 3: Frequency of GAβHS positive isolates by gender.

Gender	№ of studied cases	GAβHS positive isolates			
		№	%	X^2	P value
Male	254	74	29.1	0.81	0.66
Female	221	62	28.1	1.84	0.37

With respect to the distribution of GAβHS isolates by age, the greatest level of occurrence was in the second age group 6-10 years (40.4 %), with a statistical significant difference at $P < 0.05$, (Table 4). Figure. 2 shows this distribution more precisely, whereas Fig. (3) shows this distribution according to age and gender.

Table 4: Frequency of GAβHS positive isolates by age.

Age group	No. of studied cases	GAβHS positive isolates			
		№	%	x^2	P value
1-5 years	239	57	23.8	0.33	0.56
6-10 years	166	67	40.4	5.91	0.01
11-15 years	70	12	17.1	0.77	0.37

Figure 2: Frequency in details of GAβHS positive isolates by age among children with tonsillitis and/ or pharyngitis.

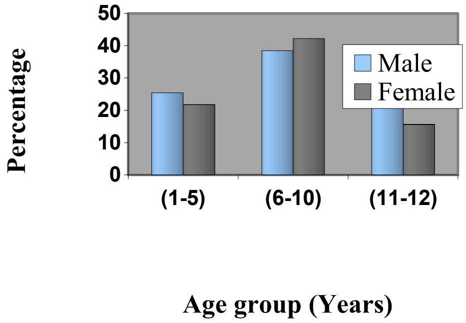

Figure 3: The frequency of GAβHS positive isolates according to the age and gender among symptomatic children with tonsillitis and/or pharyngitis.

Table 5 shows a positive correlation with a statistical significance ($p<0.05$) between the rate of prevalence of GAβHS isolates and the family size.

Table 5: Frequency of GAβHS positive isolates according to the family size.

Family size	Number of studied cases	GAβHS positive isolates			
		№	%	x^2	P
<4	47	11	23.4	0.24	0.62
4-6	189	47	24.9	0.31	0.57
>6	239	78	32.6	5.86	0.05

The distribution of GAβHS isolates according to geographical areas in this study is summarized in Table (6).

Table 6: Distribution of GAβHS isolates by the geographical areas.

Region	No. of studied cases	GAβHS positive isolates			
		№	№	x^2	P value
Urban	232	63	27.2	0.61	0.73
Suburban	131	37	28.2	12.2	0.01
Rural	112	36	32.1	13.6	0.001

Figure 4 shows that the prevalence of GAβHS sore throat exhibited two peaks, which were higher in the wet summer (July and August) months and the early winter (October, November and December). The results of serum-ASO among the total 191 blood samples is shown in table (7), whereas the titers of ASO in cases with positive ASO and GAβHS isolates is shown in Table (8).

Table 7: Correlation of the positive ASO results (≥ 200 IU/ml) with the culture results

Isolates	No. of studied	No. of Positive ASO titer	% of Positive ASO titer
GAβHS	64	37	57.8
Non-	33	23	69.7
Others	94	47	50
Total	191	107	56

Table 8: ASO levels (IU/ml) among symptomatic children with positive ASO and GAβHS isolates.

ASO	Positive isolates of GAβHS		N=64	
	№	%	x^2	P
Less	27	42.2	2.32	0.25
200	14	21.9	1.80	0.40
400	17	26.6	0.004	0.94
800	5	7.8	0.83	0.36
1600	1	1.6	-	-

The susceptibility of 70 isolates of GAβHS to 9 common antibiotics was investigated and the results are shown in table (9).

Table 9: Susceptibility of 70 isolates of GAβHS to antimicrobial agents

Antimicrobial agents	**Inhibition zone**					
	Sensitive		**Intermedi**		**Resistant**	
	No	%	No.	%	No.	%
Penicillin G	42	60.0	23	32.	5	7.1
Ampicillin	53	75.7	12	17.	5	7.1
Amoxicillin	66	94.3	2	2.8	2	2.8
Cephalexine	55	78.6	10	14.	5	7.1
Ceftazidime	34	48.6	13	18.	23	32.
Azithromyci	26	37.1	14	20.	30	42.
Erythromyci	23	32.9	16	22.	31	44.
Lincomycin	18	25.7	5	7.1	47	67.
Ceftriaxone	42	60.0	8	11.	20	28.

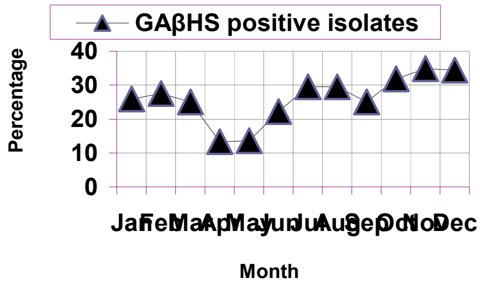

Figure 4: Monthly prevalence rate of GAβHS positive isolates over the one year study period.

Discussion

The first goal in this study was to estimate the prevalence rate of group A β-haemolytic streptococcal (GAβHS) infection among children at variable ages (from 1-15 years) who were suffering from sore throat at the time of their sampling in the pediatric hospitals and clinic in the city of Taiz. The criteria used for the diagnosis of GABHS were in fulfillment with the WHO requirements [30]. These criteria based, in part, on the clinical picture thus, specimens where only taken from children with tonsillitis and/or pharyngitis at the time of sampling. The other part of these criteria which was followed is the laboratory findings including the β-haemolytic activity of the anaerobic growth on blood agar (as a gold standard for the diagnosis of GAβHS) with a sensitivity of 90-95%, the growth inhibition zone around the bacitracin disc, and the undisputable serological results of Lancefield grouping method. As it is shown in Figure1, 136 cases representing 28.6% of the total 475 throat swab specimens were positive for GABHS. This is in a full agreement with what had been stated in many reference books of infectious diseases [31, 32, 33] that GAβHS accounts for nearly one third of all pharyngitis cases each year during spring and winter. Similar results were recorded in Egypt (27.7%) and Brazil (24.6%) [34], Taiwan (27%) [35], Iraq (31.2%) [36], Bangladesh (22%) [37] and Canada (29%) [38]. A lower prevalence rate was reported in some other studies in Tunisia (17.7%) [39], India (13.5%) [40] and Hong Kong (3%) [41], whereas a higher prevalence rate was pointed in other studies like that conducted in Croatia (42.0%) [34]. Increasing or decreasing in the prevalence rate of GAβHS in different regions in the world is a matter of extreme complexity as there are unlimited number of factors that can effect this rate including the socioeconomic states, sanitation level, endimicity of certain strains, climatic variation, crowdness condition, age, gender, technical and human errors and many others.

The age has been reported to be an important factor in the microbial etiology of GAβHS pharyngitis as well as in its carriage state. In this study the prevalence rate of GAβHS sore throat among children was higher in the age group of 6-10 (40.4%) compared to 23.8% and 17.1% in the age groups 1-5 years and 11-15 years respectively (Table 4). This result was statistically significant ($P < 0.05$). This result was within the ranges that had been reported in many other previous studies [42, 43, 44, and 45]. The reason of the occurrence of the peak incidence at the age group 6-10 years is not well established yet, but it could be relating to the increased activity of children at this age giving a higher chance for the exposure to infection than other ages. Besides, this is the school age in which children are mixing and contacting with each others in classrooms without ignoring the role of intra-familial transmission. Figure 2 explains more precisely this peak which occurs at 7 and 8 years. A similar age peak was found by some studies [13] whereas it was lower (5 years) or higher (11 years) in other studies [44, and 40 respectively].

Table 3 and Figure 3 show the frequency of positive isolates of GAβHS by gender. No statistical significant values ($P > 0.05$) were found between males and females. This result was in agreement with some other studies [40 and 46]. However, other studies reported a higher prevalence rate of GAβHS pharyngitis among females than males [47] or vice versa [48].

Increasing the number of family members lead in most occasions to increasing the rate of prevalence of many infectious diseases including GAβHS sore throat as a natural result of increasing the contact frequency. This fact was evident in this study and it seems that increasing family size plays a role in the prevalence of GAβHS infection (Tables 5). Similar results were found in other studies [49 and 50].

As shown in Figure 4, the prevalence of GAβHS sore throat exhibited two peaks; in the wet summer months and in the early winter months. Similar results were found by other workers [40 and 45]. In 2004, Mzoughi et al. observed that the isolation rates of GAβHS had peaked twice during the year from October to December and in June [39]. This result can be explained partially by the fact that during the rainy season and in winter, children mostly live indoors in crowded conditions, which may increase the rate of transmission of infection.

Table 6 shows that a higher prevalence rate of GAβHS sore throat was pointed in rural areas (32.1%) with high significant difference ($P < 0.05$) which could be due to poor medical care, poverty and unsanitary conditions.

One of the goals of this study was to find out any significant association between the results of throat culture and the serum anti-streptolysin-O antibody level among the clinically presented children with sore throat. As shown in Tables 7 and 8, no correlation was found between the culture results and the ASO results. These results were almost in agreement with the finding reported by others [51]. Explaining of such results is based on many facts. First, a detectable level of serum antibodies for any antigenic components of Streptococcus pyogenes (including streptolysin- O), non group A βHS or non βHS group (and even other bacteria) requires at least 10 to 14 days to appear in patients serum. Second, the ASO titer is elevated in response not only to GAβHS but also non group A βHS including group C and G streptococci, as well as to other non β-haemolytic streptococci groups [52]. Third, it is very likely that the ASO titer in both groups (GAβHS positive and negative isolates) in this study could be of accumulative nature as a result of a previous exposure to any streptococci that elicit the formation of ASO antibodies, i.e. the current ASO titer in both groups is not strongly associated with the current infection. A fourth factor that can play a role in this pattern of association between the culture results and ASO results is the age. The ASO titer is highly affected by age as was demonstrated by other workers [53] who reported that the ASO level as well as the ADNase B level is highly affected by the age. They could be as low as 85 IU/ml for ASO and 60 IU/ml for ADNase in preschool age children and rising to much more than that in the school age children. Kim and Lee stated in their study that the children with non-group A βHS also may exhibit higher levels of ASO than children with Streptococcus pyogenes [51].

Table 9 shows the results of susceptibility of 70 isolates of GAβHS to different antimicrobial agents. The sensitivity to Amoxicillin in this study is compatible to other studies [36, 42 and 50]. Amoxicillin is sometimes used as alternative for Penicillin V because of its improved palatability [20 and 54]. Although Penicillin is effective in the treatment of acute GAβHS pharyngitis, it is less effective in eradicating GAβHS than Amoxicillin. An explanation for penicillin failures involves the capacity of penicillin to eliminate normal oropharyngeal flora (mainly α-haemolytic streptococci) that provide an ecological barrier to GAβHS infection by production of bacteriocins [55]. Elimination of protective α-haemolytic streptococci has been implicated recurrent infection in GAβHS tonsillitis [56]. Cephalexine also seems to be effective more than Penicillin G. Other investigators [36 and 57] had reported similar results, however, cephalosporins are more expensive than oral penicillin or amoxicillin, which may precludes their routine use [20]. One of the interesting observations in this study was the number of GAβHS isolates resistance to this antibiotic which was more than the number of GAβHS isolates sensitive to erythromycin. However, many other countries, including Japan [58], Iraq [36], Italy [59], Germany [60], France [61], and Iran [50] do have a high prevalence of isolates that are resistant to erythromycin and other macrolide antibiotics.

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