CARRIAGE STATE OF GAβHS AMONG YEMENI SCHOOLCHILDREN AND THE UPPER LIMIT OF NORMAL FOR ASO IN DIFFERENT POPULATION GROUPS

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Abstract

The carriage state of Group A beta haemolytic Streptococcus is considered as an important spreading factor for the infection with Streptococcus pyogenes. To investigate that among Yemeni children, 172 apparently healthy school age children were tested by taking throat swab and blood samples. It was found that 15.1% of the total examined children were carrier for GAβHS, compared to 11.05% who were carriers for other groups of β-haemolytic Streptococci. No statistical significant difference was found concerning the age, gender or the area of residency of those children, but, a statistical variation been found when a high family index group was compared to a low family index group among which a higher carriage rate was found in those children from families of more than 6 members. Most GAβHS positive samples showed a positive results with ASO test (92.3%), however, other samples with positive results for non-βHS or non-streptococcal bacteria showed also different degrees for positivity with ASO test (57.9 and 70.8% respectively). The upper limit of normal for ASO titer among different population groups was higher in schoolchildren group (25%) compared to other groups (young adults and adults), and in females compared to males. No significant variation was noticed concerning people living in different areas of residency.

حالة حمل بكتريا المكورات السبحية مجموعة أ الحالة للدم نوع بيتا بين أطفال المدارس اليمنيين والحد الطبيعي الأعلى لمستوى أضداد سموم الستربتولايسين واو في مجاميع سكانية مختلفة

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الخلاصة

تعد حالة حمل بكتريا المكورات السبحية مجموعة أ الحالة للدم نوع بيتا مصدرا مهما لنقل الاخماج المتسببة عن هذه البكتريا. لتقصي هذه الحالة بين الأطفال اليمنيين، تم فحص ١٧٢ من أطفال المدارس ممن يبدون أصحاء بأخذ عينة المسحة الحنجرية وعينة الدم. وجد أن ١٥.١% من الحالات المفحوصة كانوا حاملين للجرثومة مقارنة مع ١١.٠٥% ممن كانوا حاملين للمجاميع الأخرى من المكورات السبحية الحالة للدم نوع بيتا. لم يكن للعمر او الجنس أو مناطق السكنى أي تأثير معنوي للتباين بين المجاميع، ولكن كان للمقياس العددي للعائلة تأثيرا معنويا للتباين حيث إن نسبة حاملي الجراثيم كانت أعلى ما بين الأطفال المنتمين لعوائل يزيد عدد أفرادها عن ٦. معظم الحالات الموجبة للمكورات السبحية مجموعة أ الحالة للدم نوع بيتا كانت موجبة أيضا لفحص أضداد سموم الستربتولايسين واو المصلي (٩٢.٣%) ومع ذلك فقد أبدى أفراد المجاميع

الأخرى ممن كانوا موجبين لحمل المجاميع الأخرى للمكورات السبحية الحالة للدم نوع بيتا او حاملين لبكتريا لا تعود أساسا للمكورات السبحية نسبا متفاوتة من الايجابية لفحص أضداد سموم الستربتولايسين واو المصلي (٥٧.٩ و٧٠.٨% على التوالي). الحدود العليا لمعيارية أضداد سموم الستربتولايسين واو كانت مرتفعة في مجموعة الأطفال (٢٥%) مقارنة بمجاميع البالغين اليافعين أو البالغين الكبار وكذلك في الإناث مقارنة بالذكور. لم يكن هناك فروقا معنوية في هذا المجال للساكنين في مناطق جغرافية مختلفة.

Introduction

Streptococcus pyogenes carriage state, has been defined as a recovery of Group A Beta Haemolytic Streptococcus (GAβHS) from the naso-pharynx or oro-pharynx in the absence of any evidence of acute infection [1]. Isolated GAβHS from carriers, however, may cause active throat infections.

Asymptomatic pharyngeal colonization by GAβHS occurs frequently and appears to follow the age and seasonal pattern of pharyngitis. The condition is most prevalent in the age group of 5 to 15 years and the highest prevalence occurring in 7-year-old children and rarely occurring in those under 3 years of age [2]. The asymptomatic prevalence is also higher (15% to 20%) in children compared to adults (<5%) [3, 4].

A report that compared between children living in temperate regions, with children living in the tropics showed that the latter were more likely to be asymptomatic carriers of GAβHS than the former [5]. Also children living in crowded conditions in temperate climates during the winter months are highly affected [4].

The upper limit of normal (ULN) for anti-streptolysin O (ASO) test is usually determined by assaying sera from a normal population and determining the highest titer present in 80-85 % of that population [6]. The ULNs of ASO varies according to age, area, study population, climatic conditions and detection method [7 , 8]. Fifty-five percent of schoolchildren in India had either ASO levels above 200 IU/ml [9], while 65% of children in a study preformed in Korean had measured above 200 IU/ml [10]. The value of ULN was highly variable in different studies at different places in the world which is usually highly influenced by individual's age. Klein *et al.* [11] reported the ULN thresholds of ASO by age groups, 85 IU/ml in preschool age children; 170 IU/ml in school age children; and 85 IU/ml in adults. Other studies reported that the ULN of ASO was 320 IU/ml in the school-age group [11], 240 IU/ml among children aged 2-12 years [7], 239 IU/ml among children ranged from 5-15 years [8] and 320 IU/ml in the school-age group (11 and 10).

This study aimed to determine the frequency of GAβHS carriers among Yemeni children of different age groups as well as to estimate the normal value of ASO in apparently healthy individuals according to their age groups.

Materials and methods

Culture media: Blood Agar Base (DIFCO), Crystal Violet (1 in 50 000) Blood Agar (prepared according to [12], Amies Transport Medium (DIFCO), Brain Heart Infusion Agar (OXOID), MacConkey Agar (DIFCO), Mueller-Hinton Agar (OXOID) for the Bacitracin sensitivity test.

Solutions and Reagents: Hydrogen Peroxide 6%, McFarland Solution (Tube 0.5), Sodium Chloride (0.9%), Streptococci Grouping Kit (OXOID), and ASO latex (DIAMO).

Study Area and Sample Size: The sample size was calculated by the statistical program of Epi-info (version 6.04). The experimental design of the study and the targeted population was as follows:

Group I: consists of 172 school children of ages 6-15 years who were apparently healthy and not clinically suffering at the time of sample collection from any signs or symptoms of throat infection and/or acute rheumatic fever. They were subdivided into 6-10 and 11-15 years children subgroups. The included schools in this study were all within the Taiz governorate of Yemen. Both throat swab and blood sample were collected from this group.

Group II: consists of 300 individuals of young and middle aged adults (16-36 years). They were subdivided into 16-25 years (young adults) and 26-36 years (adults) subgroups. All those were students or employees from the University of Taiz who were apparently healthy of throat infection as well as a current or previous signs and symptoms of rheumatic fever at the time of sample collection. Only blood samples were collected from this group.

All samples were collected along the period from June 2005 to December 2006. All samples

which were collected from children had been taken after filling an official consent by the child's parents. In a special form, all essential information including; gender, age, family size, geographical area of residency, antibiotics administration, the history of the child's health and any previous; sore throat, rheumatic fever or other infections were recorded.

Collection, Transport and Processing of Samples: Throat culture specimens were collected and transported directly to the research laboratory within 2 hours. When there was any delay over 2 hours, the samples were transported by Amies transport media, and processed the same day with the standard techniques. Venous blood samples were collected and sera were separated and placed in Appendorf tubes as small aliquots at -20˚C or below until the time of testing.

According to the diagnostic procedures recommended by [13, 14, 15, and 16] the isolation and identification of *S. pyogenes* from throat swabs of children were performed as follow:

Culture and biochemical tests: A standard method for inoculation of blood agar and crystal violet blood agar with the throat swab samples were used. The blood agar media were stabbed deeply to provide a reduced oxygen tension which is the most suitable atmosphere for the streptolysin O activity [17]. Growth on MacConkey agar was performed to differentiate between *Streptococcus pyogenes* and *Staphylococcus aureus* [15]. Bacitracin Susceptibility Test was performed according to the procedure previously recommended [18], to differentiate between GAβHS and other streptococci. This test was performed using Mueller Hinton agar with blood in a similar method to that of antibiotics sensitivity test [16].

The colonial morphology was studied after 24 hours and Gram staining technique was done for the β-haemolytic colonies [15].

Tube catalase test was performed to detect catalase production [19].

Serological Grouping (Lancefield Grouping): This test was performed according to the procedure of the manufacturer company (Oxoid).

Serological diagnosis: Detection and titration of ASO antibodies in patient's serum was performed first by a qualitative method to decide about the positive ASO sera. A quantitative method was followed for all positive ASO sera to titrate the ASO antibodies. This method was performed according to the procedure of the manufacturer company (Diamo).

Statistical Analysis: Chi- square test was used for statistical analysis. Differences between results were considered significant at $P \leq 0.05$ as determined by the appropriative tests.

Results

Among the 172 cases asymptomatic apparently healthy children studied cases, GAβHS positive colonies were recovered from 26 cases (15.1%), non-GAβHS positive colonies were recovered from 19 cases (11.05%), whereas other bacteria (non- *Streptococcus*) where recovered from 127 cases (73.8%) as it is shown in Table 1.

Table 2 shows that 13 out of 79 males (16.5%) and 13 out of 93 females (13.9%) were positive GAβHS carriers with statistically no significant difference ($P > 0.05$).

Table 3 shows that 9 out of 52 (17.3 %) in the age group 6-10 years and 17 out of 120 (14.2 %) in the age group 11-15 years were positive GAβHS carriers. Statistically, there was no significant difference between the two age groups ($P > 0.05$).

Table (4) shows that 3/32 (9.4%) of children living in families with 4-6 members compared to 23/124 (18.5%) of children living in families with more than 6 members, were positive GAβHS carriers with a statistically significant value ($P < 0.05$).

The distribution of GAβHS in children carriers according to the area of residency is summarized in Table (5). There were no significant differences ($P > 0.05$) in the occurrence of GAβHS in children who live in suburban compared to these who live in urban and rural areas.

Table 1: Types and numbers of isolated bacterial growth from throat swabs among asymptomatic apparently healthy children.

Organism	Asymptomatic apparently healthy children	
	№	%
GAβHS	٢٦	١٥.١
Other βHS	١٩	١١.٠٥
Other bacteria	١٢٧	٧٣.٨٤
Total	١٧٢	١٠٠.٠

Table 2: Frequency of GAβHS positive isolates by gender among children carriers.

Gender	№. of studied	GAβHS positive isolates			
		№	%	x^2	P value
M	79	١٣	١٦.٥	1.27	0.51
F	٩٣	١٣	١٣.٩	2.25	0.35

Table 3: Frequency of GAβHS isolates by age groups among children carriers.

Age group	№ of studied cases	GAβHS positive isolates			
		№	%	x^2	P value
6-10 Years	52	9	17.3	2.05	0.15
11-15 Years	120	17	14.2	0.21	0.89

Table 4: GAβHS positive isolates according to family size in children carriers.

Family size	№ of studied	GAβHS positive isolates			
		№	%	x^2	P value
<4	١٦	٠	0	-	-
4-6	٣٢	٣	٩.٤	٢.٠	٠.٣٢
>6	١٢٤	٢٣	١٨.٥	٢٠.٦	٠.٠١

Table 5: GAβHS positive isolates according to residency area in children carriers.

Area of residency	№ of studied cases	GAβHS positive isolates			
		№	%	x^2	P value
Urban	٧٩	١١	١٣.٩	٠.٧٨	٠.٣٧
Suburban	٦٩	١٣	١٨.٨	٤.٦٣	0.07
Rural	٢٤	٢	8.3	٢.٠٠	0.06

Table (6) shows the results of ASO titer in association with the culture results. A 24 out of 26 of positive GAβHS (92.3%) were ASO positive compared to the ASO positive results in the other-βHS carrier children (57.9%) and other bacteria (70.8%).

Table 6: Association between culture results and asymptomatic apparently healt

Isolates	№ of studied cases	№ of Positi titer
GAβHS	26	24
Other βHS	19	11

Table (7) shows the titer of ASO and their relationship with culture results in the asymptomatic apparently healthy children. The titers in the positive ASO cases were ranging from 200 IU/ml (61.5%) to 800 IU/ml (3.8%). This table shows no variations (P > 0.05) between the ASO levels and the positive GAβHS isolates cases.

Table 7: Correlation between the ASO levels (IU/ml) and the GAβHS positive isolates among children carriers

ASO titer	Positive isolates of GAβHS N=26			
	№	%	x^2	P value
Less than ٢٠٠	٢	٧.٧	٢.٠٠	٠.١٥
٢٠٠	١٦	٦١.٥	١.٥٨	٠.٢٢
٤٠٠	٧	٢٦.٩	١.٩٥	٠.٢٩
٨٠٠	١	٣.٨	-	-
1600	٠	٠	-	-

Table (8) describes the different factors including the age, gender and geographical distribution affecting the normal value of ASO. An ASO titer of > 200 IU/ml was more evident among schoolchildren which were 25% of the total examined cases compared to only 13.9% among the total examined cases of young adults or 12.2% among the total examined cases of adults. The statistical significance was noticed among the school age children group (P < 0.05) whereas there was no statistically significant difference (P > 0.05) in the other two groups. According to the gender, it was found a high significant difference between this factor and ASO titer (P < 0.05) on behave of females. No statistical difference was found in the normal ASO value among people living in different geographical areas.

Table 8: The effect of age, gender and geographical factors on normal ASO value.

Factors	№ of studied cases	ASO titer > 200 IU/ml				
		№	%	Mean	x^2	P value
AGE GROUP						
Schoolchildren (6-15 years)	١٧٢	٤٣	٢٥.٠	١.٩٨	٥.٤٧	٠.٠١
Young adults (16-25 years)	٢٥٩	٣٦	١٣.٩	١.٦	٠.٠٥	٠.٨٠
Adults (26-36 years)	٤١	5	١٢.٢	١.٦	٢.٢٢	٠.٣٢
GENDER						
Male	٢٢٢	٢٧	١٢.٢	١.٦١	١.١٧	٠.١٢
Female	٢٥٠	٥٧	٢٢.٨	١.٨٦	٢١.٦٦	٠.٠٠
REGION						
Urban	٢٤٩	٣٢	١٢.٩	١.٥٩	٠.٧٤	٠.٦٠
Suburban	١٨٠	٤٥	٢٥.٠	١.٩١	١.٨٤	٠.٠٩
Rural	٤٣	٧	١٦.٣	١.٩٣	١.٢٧	٠.٩٥

Discussion

Group A β-haemolytic streptococcal (GAβHS) pharyngitis is one of the most common bacterial diseases in human being. Healthy carriers of GAβHS are sources for bacterial dissemination and are able to communicate the disease and even lead to severe epidemics [10].

The first goal in this study was to estimate the prevalence rate of GAβHS isolates among children carriers with ages ranging from 6-15 years. As it shown in table (1) the overall isolates of GAβHS among this group were 26 out of 172 (15.1%). This result was compatible to some results of other studies in other parts of the world, as it was 13.1% in Saudi Arabia [20], 14 % in Tunisia [21], and 16.9% in Korea [22]. Lower rates of carriage state were reported in other places of the world; 5.9% in Babylon-Iraq [23], 8.4% in India [24], and 2.5% in England [25], whereas higher rates were reported in Turkey (25.9%) and Iran (28.5%) [26, and 27 respectively]. There was an exception of these results which was found in Kathmandu, Nepal as no GAβHS was found in carriers [28].

The prevalence rate of GAβHS carriage state depends widely on epidemiological and environmental factors. Significant geographical variation in the streptococcal groups isolated from the throat of healthy schoolchildren have been reported; group A are the most common in temperate countries, and groups C and G are the most common in tropical countries [29]. City of Taiz is of a moderate altitude of approximately 1300 m above the sea level with a summer temperature of 30-35 Ċ and winter temperature of 20-25 Ċ, hence, it can be categorized as moderately temperate area which can explain the partially high prevalence rate of GAβHS isolates among the asymptomatic schoolchildren carriers. The age has been reported to be an important factor in the carriage state of GAβHS. In this study, the effect of age on the rate of prevalence of GAβHS among the children carriers showed no statistically significant difference ($P > 0.05$) as shown in Table 3.

Tables (2), shows the frequency of GAβHS by gender among asymptomatic carriers. No statistical significant values ($P > 0.05$) were found between males and females. This result was in agreement with some other studies [30]. However, other works reported a higher prevalence rate of GAβHS carriage state among females than males [31] or vice versa [32 and 33].

Increasing the number of family members lead in most occasions to increase the rate of prevalence of many infectious diseases including GAβHS sore throat as a natural result of increasing the contact frequency. This was evident concerning the carrier group of GAβHS as it was significantly higher ($P < 0.05$) among children living within families of more than 6 members (18.5%) compared to 9.4% prevalence rate among children living within families of 6 or less members. This crowdness index was also pointed out in other studies [27].

No statistical difference in association between the area of residency and the carriage state rate was found in this study (Table 5). This might be due to that in Yemen the variation in the living standards (including the socioeconomic levels) is very narrow between urban and rural areas.

An association between the positive GAβHS culture results and the positive ASO results was found in this study (Tables 6 and 7). These findings were similar to those reported by other study [34]. They demonstrated elevated ASO responses in school children who carried A, C and G streptococci. However, in other study, it was found that ASO titers were positive in only 37% of GAβHS-carriers and 10.3% in GAβHS non-carrier children [26]. One important finding in our study concerning the results of ASO positive titers among other groups of βHS (non group A) and other bacteria as well which were 57.9 and 70.8% respectively, is that the high titer of ASO in patients as well as carriers is not a strong indication of GAβHS infection. It is very difficult to define as 'healthy normal' children whose antibody levels are above the upper limit of normal (ULN) threshold, as that is also one of the criteria for 'infection' [35]. The normal ASO level, according to most commercial antibody test is < 200 IU/ml in adult patients. Because such 'normal' level may only reflect appropriates titer for adults, correct interpretation of titer in children can be problematic. Elevated or rising titer of ASO is seen in 80% or more of the cases with acute rheumatic fever. Acute and convalescent sera should be obtained and tested simultaneously to decide a rising ASO titer, but this is not always feasible. Hence a single specimen when available requires to be compared with a pre-determined base line value or an ″upper limit of normal″ [8]. In this study we attempt to establish an ULN on different Yemeni population groups taking in consideration the above variables. Table (8) shows the results of such attempt.

Among the normal school age children, 25% had showed a titer of ASO above 200 IU/ml. The percent was lower than that (13.9% and 12.2%) among the young adults and adults groups respectively. These results were significant ($P < 0.05$) for only the school age children indicating a higher level of ASO titer among this group of normal compared to the other groups of normal (young adults and adults). This means that the ULN for ASO titer is > 200 IU/ml among school age children which is in agreement with other previous studies [8 and 7]. Having establishing that the ULN in school age children in our population was > 200 IU/ml would prove helpful in the interpretation of elevated ASO titers in cases of suspected ARF and should be of clinical value to physicians, epidemiologists and clinical laboratory personnel who may misinterpret streptococcal antibody titers because of the unawareness that children will, on an average, have higher titers than the adult values listed as ´normal` in the manufacturers inserts.

In the same table (8), there was a higher percent of ASO titer > 200 IU/ml among females (22.8%) than males (12.2%) with a statistical significant value ($P < 0.05$). On the contrary, no statistical significant was noticed ($P > 0.05$) in the percent of ASO titer > 200 IU/ml among different geographical regions. This can be due to the similarities in climatic conditions, socioeconomics, altitude and other factors between these regions. In conclusion, GAβHS carriage state rate among school age Yemeni children was moderately high in comparison with the rates in other climatically similar areas in the world. No statistical variation in the carriage state rates was noticed between different age groups, gender and areas of residency. However, such statistical variation was significant when the family index was high (family size was above 6 members). A weak association (not significant) was found between the carriage state of GAβHS and the positive ASO results. Finally, the ULN of a value higher than 200 IU/ml of ASO was significant among healthy school age children but it was not among healthy young adults and adults.

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