

Isolation ratio and T- serotyping of group A streptococci from pediatric upper respiratory tract infections in Turkey

Türkiye’de, üst solunum yolu enfeksiyonu olan çocuklarda A grubu streptokok izole edilme oranı ve T-serotiplenmesi

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ABSTRACT

Objective: Acute rheumatic fever can follow throat infections with group A streptococci. Certain serotypes of group A streptococci such as M1, M3, M5, M6, M14, M18, M19, M24 are associated with this disorder. Immunity to streptococci and to rheumatic fever depends on antibodies to the M proteins. Due to current scarcity of M-typing sera, many laboratories use T typing and opacity factor production for serotype identification of group A streptococci. In order to, investigate the most common serotypes of group A streptococci in our country in recent years we studied T-agglutination typing and opacity factor of 120 group A streptococci strains isolated from throat cultures of 930 children.

Methods: Diffuse, stable suspensions of group A streptococci were tested with polyvalent antisera (T,U,W,X,Y) by slide agglutination. Microplate method was used for opacity factor detection.

Results: T-protein -agglutination patterns U (2,4,6,28) were the most common among typeable strains. The rate of T-protein -agglutination patterns T (1,3,13, B3264) and X (8,14,25,Imp.19) were 20 % and 18 % respectively. Opacity factor production rate of isolated group A streptococci strains was 65 %.

Conclusion: To profit global assessment of rheumatic fever and rheumatic heart disease, more epidemiologic and serotyping research is required in our country. (*Anadolu Kardiyol Derg 2005; 5: 302-4*)

Key words: Group A streptococcus, isolation rate, serotyping

ÖZET

Amaç: Akut romatizmal ateş, A grubu streptokokların neden olduğu tonsillofarenjitlerden sonra gelişen non-süpüratif bir sekeldir. A grubu streptokokların M1, M3, M5, M6, M14, M18, M19, M24 gibi belirli M serotipleri bu tür sekellere yol açmaktadır. M proteinlere karşı oluşan antikolar streptokoklara karşı bağışıklığı sağlarken, romatizmal ateşe de neden olurlar. Anti-M serumları ticari olarak elde edilemediğinden, birçok laboratuvarında A grupları, T agglütinasyon paterni ve opasite faktörü ile serotiplenmektedir. Ülkemizde, son zamanlarda yaygın olan serotipleri belirlemek amacıyla, 930 çocuktan alınan boğaz sürüntülerinden izole edilen 120 streptokok kökeninin, T protein tipi ve opasite faktörü araştırılmıştır.

Yöntemler: T protein tipini belirlemek üzere, A grubu streptokok kökenlerinin homojen süspanسیونları, polivalan antiserumlarla (T, U, W, X, Y) lam agglütinasyon yöntemi ile karşılaştırılmıştır. Opasite faktörünü belirlemek için mikropak yöntemi kullanılmıştır.

Bulgular: Serotiplenebilen kökenler arasında en sık saptanan, T-protein agglütinasyon paterni U (2, 4, 6, 28) olmuştur (%30). Bunu T-protein agglütinasyon paterni olarak %20 ile T (1, 3, 13, B3264) ve % 18 ile X (8, 14, 25, Imp.19) izlemiştir.

Sonuç: Romatizmal ateş ve romatizmal kalp hastalıklarını genel olarak değerlendirebilmek için, ülkemizde daha fazla epidemiyolojik ve serotip çalışmalarına ihtiyaç vardır. (*Anadolu Kardiyol Derg 2005; 5: 302-4*)

Anahtar kelimeler: A grubu streptokok, izolasyon oranı, serotipleme

Introduction

Acute rheumatic fever (ARF) and rheumatic heart disease (RHD) are common in both developed and developing countries. Seasonal and climatic factors can influence the spread of group A streptococcal (GAS) infections and thereby affect the incidence of ARF. However, differences of distribution in the world in different communities of the same climate still can not be exp-

lained. In developing countries, the incidence of ARF is difficult to establish. However, RHD is a common clinical problem and ARF presumably occurs with corresponding frequency. The prevalence of ARF is 0.0367-0.107 % and the frequency of mitral valve replacement depending on rheumatic heart disease is 5500-6000 cases annually in our country (1,2). In a study, which has been planned in Ankara, Turkey, three cases out of 4086 schoolchildren were found to have RHD findings and fifteen children

had one episode of ARF (3). All cases of ARF occurred following a group A streptococcal upper respiratory tract infections. Certain M serotypes of GAS are strongly and repetitively associated with ARF (4). Serotypes M1,3,5,6,14,18,19,24 which are known as rheumatogenic streptococci do not produce opacity factor (OF) (5-8). The OF is a type specific enzyme and is associated with certain serotypes of M protein antigenically. Another useful epidemiological marker of GAS is T protein. The classical techniques for M protein serotyping, OF typing and T agglutination typing remain the gold standards in identifying group A streptococci. Because of the current scarcity of M typing sera determination of the T protein agglutination pattern and OF reaction are usually the main steps in the classification of GAS. T antigen patterns are useful characterization of GAS, especially when the streptococci are not typeable with existing M antisera. In order to detect the most serotype in our country in recent years we studied T-agglutination pattern and OF of group A streptococci isolated from throat cultures of children.

Methods

All GAS strains isolated from the throat cultures of 930 children (age range 4-14 years) with pharyngitis at the Hospital of Maltepe University School of Medicine in Istanbul, Turkey during the period of June 2002-January 2003 were identified. Each of GAS strain was obtained from different cases and repeated cultures were not included the study. Group A streptococcal strains were characterized by standard techniques including colonial morphology on sheep blood agar, bacitracin sensitivity testing, and serological grouping (Oxoid, Hemakim). T-protein agglutination patterns of strains were determined by the slide agglutination test with antisera obtained commercially (Denka Seiken Co.,Ltd. Tokyo, Japan). Diffuse, stable suspensions of GAS strains were tested with polyvalent T,U,W,X and Y antisera. The microtitre plate method for detection of OF involved the use of standard 96- well tissue culture plates. Hydrochloride extracts of strains were obtained as described before (9). Hydrochloride extracts of the streptococcus strains (10µL) were added into 100 µL of inactivated horse sera and incubated overnight at 350C in a moist atmosphere. Before the test results were examined 100 µL of normal saline was added to each well. Then it was visually examined for opacity by using a mirror (10,11).

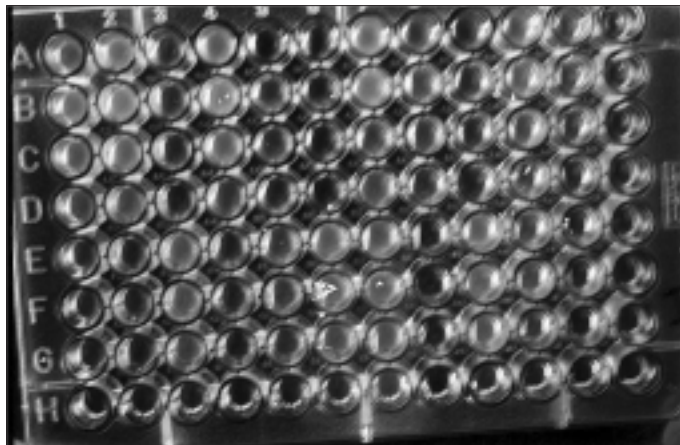


Figure 1. Microtitre plate of opacity factor detection.

Results

One hundred and twenty GAS strains were isolated from throat cultures of 930 children. Group A streptococcus's isolation rate from patients with pharyngitis was 13%. From 120 GAS strains 70% were typeable with polyvalent T- antisera. Microtitre plate of OF detection is shown in Figure 1. Table 1 summarizes the T-typing and OF production rates of GAS strains and Table 2 shows T-protein patterns of the examined 84 GAS strains, among then 65% were OF- positive and 35% OF- negative. Among typeable strains T - protein agglutination patterns U (2,4,6,28) was the most common (30%). Detection rate of T (1, 3,13, B3264) and X (8,14, 25, Imp.19) were 20 % and 18 % respectively.

Discussion

Group A Streptococcus's isolation rate was 13% in our study. Different isolation rates have been reported from various countries. Our isolation rate seems to be less compared to the rates demonstrated by two previous studies (12,13). Throat infection with GAS can lead to complications such as ARF and irreversible damage in the heart valves. In spite of effective antibiotic treatment of GAS infections, ARF and RHD are still unsolved health problems for most of developing countries. With different symptoms and atypical clinical course ARF is a mystery for most clinicians in our country too (14). It has been suggested that patients with ARF or RHD often have antibodies to OF's of M-types 4,9,22 and 29 (15). It is important to establish the epidemiological pattern of GAS in different countries and regions, and especially to serotype the isolated strains. This knowledge is important for the development and use of vaccines (16,17).

Specific correlations between OF result T-antigen pattern and M serotype of GAS have been reviewed by Johnson and Kaplan (18) . Obtaining M-antisera is very difficult, particularly for the OF producing strains. However, the strains can easily be serotyped by using human sera, which contains anti-OF antibody by inhibition method (9,19,20).

While most Asian isolates of GAS are OF negative and T-untypeable (21,22) , we have found that about 65% of strains are

Table 1. T-typeabilities and OF results of GAS strains

	OF- negative	OF- positive	TOTAL
Not T-typeable	9	27	36
T-typeable	33	51	84
TOTAL	42	78	120

GAS: Group A streptococci; OF: Opacity factor

Table 2. T-typing patterns and OF production of 84 GAS strains

T- patterns	OF- negative	OF- positive	TOTAL
U (2,4,6,28)	10	15	25
T (1,3,13,B3264)	7	10	17
X (8,14,25,Imp.19)	4	11	15
W (5,11,12,27,44)	5	8	13
Y (15,17,22,23,47)	5	7	12
More than one(T/X)	2	-	2
TOTAL	33	51	84

GAS: Group A streptococci; OF: Opacity factor

producing OF and 70% T-typeable with commercially available antisera. In our previous study, 51% of GAS isolates were OF positive and had low T-typeabilities (23). This difference with our new results may reflect the known variation in serotypes within time and we can estimate that ARF incidence will decrease.

Although, isolation rate of GAS have decreased and OF positive serotypes have increased since 1998, new cases of ARF are being continuously diagnosed (3,14). For global assessment of rheumatic fever and rheumatic heart disease, serotyping studies should be supported with long-time clinical observations. In our country, invasive disease and post- streptococcal complications such as ARF and RHD should continuously be monitored.

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