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Asymptomatic Infection by *Streptococcus pyogenes* in Schoolchildren and Diagnostic Usefulness of Antideoxyribonuclease B

This study is designed to evaluate the immune status of schoolchildren with respect to *Streptococcus pyogenes*, and to ascertain the usefulness of antideoxyribonuclease B (ADNase B). Antistreptolysin O (ASO) and ADNase B concentrations were measured quantitatively in 266 serum samples from healthy elementary school children in Seoul. Simultaneously, throat cultures were taken in order to isolate *S. pyogenes* and other beta-hemolytic streptococci (BHS). The upper limits of the normal (ULN) concentration of ASO and ADNase B were 326 IU/mL, and 362 IU/mL, respectively. The correlation between ADNase B (y) and ASO (x) was y=0.4x+173 (r=0.46). Mean ADNase B level (392 IU/mL) was significantly higher in children with *S. pyogenes* than in those with non-group A BHS (236 IU/mL) or no BHS (234 IU/mL). Some schoolchildren were proven, via ASO and ADNase B tests, to be harboring asymptomatic *S. pyogenes* infections. The high ULN of ASO and ADNase B in schoolchildren should be carefully considered, in order to interpret the data collected from the patients. We could add the ADNase B test to our set of diagnostic tools, which would allow us to more accurately detect and diagnose streptococcal infections, as ADNase B was more specifically related to the results of throat cultures, and there was little correlation between ASO and ADNase B.

Key Words : *Streptococcus pyogenes; Antideoxyribonuclease B; Antistreptolysin; Throat Culture; Asymptomatic Infection*

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INTRODUCTION

Streptococcus pyogenes (group A streptococci) infection is diagnosed by either bacterial culture or serological testing. For the serodiagnosis, antistreptolysin O (ASO) and antideoxyribonuclease B (ADNase B) are the most widely accepted tests (1-3). The most popular and standardized serological test is still ASO rather than ADNase B. ASO is not only useful in the diagnosis of streptococcal infections or complications, but also in the follow-up process, and in evaluating the effectiveness of treatments (4). ASO is especially helpful when throat culture technique is improper or the patient has already taken antibiotics (4). But, as ASO is not always elevated after streptococcal infection or sequelae, it is necessary to add the alternative serological test. Since ADNase B has a longer half-life than ASO, it can be a valuable tool in the diagnosis of remote past infections (5). In cases of poststreptococcal glomerulonephritis (PSGN) following skin infections or impetigo, ADNase B is elevated, but ASO levels do not tend to be elevated (4, 5). Previously, the ADNase B test was complicated, lacked reproducibility, and the reagents were difficult to obtain (6). With the development of immunochemical techniques such as turbidimetry or nephelometry, quantitative ADNase B analysis has become available (6). The upper limit of normal (ULN) in normal children should be elucidated first in order to interpret data accurately obtained from the patients (7).

The aim of this study was to measure ASO and ADNase B levels quantitatively to calculate the ULN and to compare to the results of throat cultures from schoolchildren.

MATERIALS AND METHODS

Throat cultures and venous blood samples were taken from 266 children aged from 7 to 12 in Seoul, Korea. The numbers of boys and girls were 140 and 128 respectively. These children were healthy, without any symptoms or signs of streptococcal pharyngitis. Bacterial cultures and identification were performed by bacitracin disk (0.04 U) and the latex agglutination method (A Strep AD, Denka Seiken, Tokyo, Japan). ASO was measured with a chemistry autoanalyzer (Hitachi 747, Tokyo, Japan) and ADNase B was measured with a nephelometer (Dade Behring Nephelometer 100, La Jolla, CA, U.S.A.). The reagents of ASO and ADNase B were purchased from Daichi (Tokyo, Japan) and Dade Behring, respectively. Mean ASO and ADNase B were calculated by school grade, and compared with the results of the throat cultures. The ULN of ASO or ADNase B was defined as the level of the 80th per-

centile of cumulative frequencies (8-10). The children were classified into three groups according to the results of throat culture, such as children with *S. pyogenes*, children with non-group A beta-hemolytic streptococci (BHS), and children with no BHS in their throats. The statistical significance of differences in ASO and ADNase B between each group was evaluated by Student's t-test, and the correlation between ASO and ADNase B was determined by regression analysis.

RESULTS

Mean ASO and ADNase B levels were 203 IU/mL (SD 217 IU/mL) and 251 IU/mL (SD 190 IU/mL), respectively. The ULNs for ASO and ADNase B were determined to be 326 IU/mL and 362 IU/mL, respectively (Table 1). The ranges of ASO and ADNase B were 1 to 1,712 IU/mL, and 77 (detection limit) to 1,616 IU/mL, respectively. Percentages of children with ADNase B concentrations higher than 400 IU/mL and 500 IU/mL were 17.8 and 6.8, respectively. *S. pyogenes* was most frequently detected in the 3rd grade (26.3%) and the 6th grade (18.0%), but ASO and ADNase B levels were highest in the 5th grade.

While ASO levels were highest in the children with non-group A BHS, ADNase B levels were significantly higher in children with *S. pyogenes* (Table 2). The correlation equation between ADNase B (y) and ASO (x) was y=0.4x+173 (r=0.46).

DISCUSSION

S. pyogenes produces several extracellular proteins, including streptolysin O, hyaluronidase, streptokinase, DNase, diphosphopyridine nucleotidase, and these antigens can also cause immune reactions (5). ASO tests have, lately, been most widely applied in clinical laboratory situations, using semiquantitative latex agglutination, the hemolysis inhibition method, quantitative turbidimetry, nephelometry or even the rapid chemistry autoanalyzer (9). The classical ADNase B method was somewhat complicated and less standardized (6). Recently developed turbidimetric and nephelometric methods are both rapid and reproducible (6). Quantitative analyses of ASO or ADNase B are useful for the interpretation of a single sample if we know the ULN of the population. But it is preferable to compare acute and convalescent levels, or levels both before and after treatment of streptococcal infections, rather than measuring a single level against an arbitrary standard (7). Although quantitative analysis is rapid and convenient for testing large numbers of samples, it still requires an accurate standardization, and an adequate quality of reagents.

ADNase B is valuable, especially when there is little or no elevation of ASO, even though the patient is infected with *S. pyogenes.* This is the case in which the victim develops impetigo, erysipelas, and PSGN secondary to skin infection (5). Fujikawa and Okuni (11) observed that ASO elevation occurs only in 60% of rheumatic fever (RF). But after they added either ADNase B or streptokinase test, they were able to diagnose RF with 95% accuracy. Ayoub and Wannamaker (4) recommended that it is also advisable to check a few other antibodies, because ASO can be normal, or throat culture can be negative, in some cases of RF or PSGN. They found that they could diagnose 80% of sequelae with one antibody, but 95% with two kinds of antibodies. As there was very little correlation between ASO and ADNase B in this study, it appears that it would, indeed, be helpful to screen ADNase B in addition to ASO to diagnose *S. pyogenes* infections.

ASO and ADNase B levels were not closely related to the isolation rate of *S. pyogenes* in each school grade. As group C or group G streptococci can induce ASO (5), the children with non-group A BHS also may exhibit higher levels of ASO than children with *S. pyogenes* (Table 2). Otherwise, these children might have a mixed infection, with non-group A BHS and *S. pyogenes*, or just a remote GAS infection. In contrast, ADNase B levels were specifically elevated in children with *S. pyogenes.*

It is very difficult to define as 'healthy normal' children whose antibody levels are above the ULN threshold, as that is also one of the criteria for 'infection' (3). Fifty-five percent of schoolchildren in India had either ASO levels or ADNase B levels above 200 IU/mL (10), while 65% of children in our study measured above 200 IU/mL. It should be emphasized that children have different criteria to interpret ASO or ADN ase B compared to the adults. The ULN of ASO or ADNase

Table 1. ASO and ADNase B levels (IU/mL) by school grade

School grade	ASO			ADNase B		
	No. of children	Mean	SD	No. of children*	Mean	SD
1	42	165	291	38	205	265
2	39	153	124	36	181	132
3	57	216	229	48	239	162
4	46	162	169	43	225	164
5	43	277	228	38	368	204
6	39	244	191	33	297	129
Total	266	203	217	236	251	190

*Some children were not available to test ADNase B test due to small amount of serum sample.

Table 2. ASO and ADNase B levels (IU/mL) according to the results of throat culture

Throat culture	ASO			ADNase B		
	No. of children	Mean	SD	No. of children	Mean	SD
S. pyogenes	33	352†	233	28	392†	179
Non-group A BHS*	13	418†	236	9	236	81
No BHS	220	168	199	199	234	192

* β-hemolytic streptococci. †$p<0.05$ with *S. pyogenes* and non-group A BHS and no BHS group.

B varies according to age or area (1, 12, 13). The ULN of ASO and ADNase B of children in Seoul were found to be 326 IU/ mL and 362 IU/mL, respectively. Although the measurement methods were different, Klein et al. (13) reported the ULN thresholds of ASO and ADNase B by age groups, as 85 IU/mL for ASO and 60 IU/mL for ADNase in preschool-age children; 170 IU/mL for both ASO and ADNase in school-age children; and 85 IU/mL for both ASO and ADNase in adults. Kaplan et al. (14) reported that the ULN of ADNase B (400 IU/mL) was higher than that of ASO (320 IU/mL) in the school-age group, a similar finding to ours. Ayoub and Wannamaker (2) reported the ULNs of ASO and ADNase B to be 166 IU/mL and 250 IU/mL, respectively. Taken together, it appears that the ULNs of ASO or ADNase B varies according to age, study population, and detection method (9). Generally the ULN of ADNase B appeared to be higher than that of ASO by our study and above reports.

Some elementary school children without history of recent upper respiratory tract infections or skin infections manifested very high ASO or ADNase B levels, indicating frequent asymptomatic infections in this age group. In the recent recurrence of RF in the U.S.A., almost half of the cases did not recall having previous pharyngitis symptoms (15), suggesting that asymptomatic infections could precede RF. Whether we should treat these asymptomatic infections or not has not been determined (1, 14, 16).

In conclusion, some school children were proved, by ASO and ADNase B tests, to have asymptomatic *S. pyogenes* infections. Because the ULNs of ASO and ADNase B were so high in schoolchildren, clinicians should be careful in interpreting the results of tests. ADNase B test is an efficient method for the accurate diagnosis of streptococcal infections, because ADNase B was more specifically related to throat culture results, and there was little correlation between ASO and ADN ase B.

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