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EVALUATION OF SOME DIFFERENT METHODS FOR THE DIAGNOSIS OF STREPTOCOCCAL INFECTION

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ABSTRACT

The classical haemolytic and latex agglutination tests were used for determination of antistreptolysin O titer (ASO) in 63 patient serum samples presenting in ENT and Pediatric Departments in Assiut University Hospitals. At the same time, throat swab was collected from each patient and cultured on blood agar. There was an agreement between the two serological tests in 73. 33% of the cases; the remaining 26.67% of cases were only positive with the haemolytic test. Therefore, the latex test may be suggested for preliminary screening of the sera beforeembarking on determination of ASO. From the obtained data an agreement was apparent between the haemolytic test and isolation method in 95.56% of the examined cases.

INTRODUCTION

The most common bacterial infections of the upper respiratory tract are caused by various streptococci. The group A, B-haemolytic streptococci are responsible for a great variety of clinical diseases. In some cases the initial infections is followed by scarlet fever, pyelonephritis and possible rheumatic heart diseases (Buffaloe and Ferguson, 1981). Diagnosis of group A, B-haemolytic streptococcal infection may be facilitated by detecting antibodies specific for the haemolysins. Streptolysin O is antigenic and persons with Strept..Pyogenes infections develop peak antibody titers against the haemolysin about 2 weeks after initial infection.

These antistreptolysin O (ASO) titers are useful in diagnosing post streptococcal sequelae, rheumatic fever and glomerulonephritis, (Mc'ane and Kandel, 1985). The risk of potential complications of streptococcal infections emphasizes the need for rapid and accurate identification of Streptococcus Pyogenes from infected throat. The different methods of serotyping B-haemolytic streptococci were mentioned by many investigators (Baenkler et al., 1969 and Ganezarski et al., 1969). The classical haemolytic test used for detection of ASO is a time consuming test, this lead to the development of antistreptolysin O latex test (ASLO). The ASLO is a rapid test based on a visible agglutination of the patient serum on a slide with polystyrene latex particles sensitized with streptolysin O (Ramello et al., 1970; Dixon and Grocholski, 1970; Flumara, 1972). Several investigators obtained . valuable results with the ASLO test, when used for distinguishing between relapses and reinfection antistreptolysin O titer (Edward, 1964, Kaplan, 1981, Feigin, 1983 and Gerber et al., 1985). The aim of this work is to compare between the classical haemolytic test and the latex agglutination test for determination of ASO titer to select one for routine use.

EXPERIMENTAL

Material and Methods:

Children between 2 and 16 years of age presenting in ENT and pediatric departments in Assiut University Hospitals with clinical finding suggesting tonsilitis and pharyngitis, were involved in this study. Throat swabs were obtained from 63 patients by rubbing a sterile cotton-tipped swab over the posterior portion of the pharynx and both tonsils or tonsilar fossae. The swab was then immediately streaked onto a blood agar plate and a bacitracin disk was placed on the primary inoculum. After overnight incubation, at 37°C the plate was examined for the presence of B-haemolytic streptococci. Films were prepared from B-haemolytic minute colonies and stained with Gram stain. B-haemolytic streptococci that were sensitive to bacitracin, were presumptively identified as group A.

Evalution of some Different Methods for the Diagnosis of Streptococcal Infection

If the sensitivity to bacitracin could not be determined after overnight incubation due to the number of colonies developed or the location of the later was far from the disk, representative colonies were subcultured and sensitivity to bacitracin was determined on a separate plate (Buchanan and Gibbon, 1974; Roe et al., 1984; Gerber et al., 1985). Blood specimens were collected from those 63 patients to determine the ASO using both the latex and the haemolytic tests. All sera were stored at -70°C and later analyzed simultaneously according to the methods described by Edward (1946) and Ramello et al. (1970).

Glassical haemolytic test:

Several dilutions of the serum sample 1/50 - 1/1200 (one ml each) were prepared. Add 0.5 ml of titrated streptolysin O, incubate for 15 min. in a 37°C water bath. Add 0.5 ml of 5% rabbit red cells to each tube, incubate for 45 minutes at 37°C. The test include two control, red cell control that give no haemalysis and streptolysin control that give total haemolysis at the end of the reaction. Serum titer of antistreptolysin O is the inverse of the highest

Latex antistreptolysin O test (ASLO):

Reagents:

Latex-ASL reagent: Consists of an aqueous suspension of polystyrene latex particles sensitized with streptolysin O, a purified protein preparation from cultures of B-haemolytic C-streptococci.

ASL control serum, positive: Is a stabilized human serum containing at least 50 IU antistreptolysin O/ml.

ASL control serum, negative: Is a stabilized human serum containing less than 10 IU antistreptolysin O/ml.

Method: Bring serum samples and reagents to room temperature. Dilute patient serum 1+5 with 0.9% saline solution. Apply one drop (about 40 ul) of the diluted serum or positive and negative control serum to slides. Shake the latex-ASL reagent well, add one drop (about 40 ul) to the drops of sera, mix well with stirring rods, and rotate the slides. After 2 min. Check for agglutination, and at the same time compare with the reactions of the control sera. Marked agglutination indicates an antistreptolysin O content about 200 IU/ml ± 15% Marked agglutination of the serum dilutions: 1+10 = 400 IU/ml, 1+15 = 600 IU/ml, 1+20 = 800 IU/ml, 1+30 = 1200 IU/ml, 1+40 = 1600 IU/ml.

RESULTS AND DISCUSSION

The number of bacterial isolates from the examined patients was given in Table(1):B-haemolytic streptococci was isolated from the majority of the cases either in pure form (69.84%) or mixed with other organisms (4.76%), organisms other than B-haemolytic streptococci were isolated from 25.4% of the cases. The number of cases showed positive reaction with the classical haemolytic and latex agglutination tests were 45 (71.43%) and 33(52.38%) respectively. The correlation between the classical haemolytic test and the isolation of the causative organism was reported in Table (3). It was shown that B-haemolytic streptococci could be isolated from 43 out of the 63 examined patients and the sera of those patients showed antibodies to the classical test. Seven cases were positive for streptococcal isolation but they are negative for antibodies.

The approach to the detection of group A streptococci in throat specimens with the use of conventional culture technology was mentioned by Schwartz and Guinell, 1980 and Todd, 1982. Although some authors have questioned the validity of this approach (Mondzac, 1967), there are now several well controlled studies that

demonstrate an acceptable accuracy of laboratory cultures using sheep blood agar and primary plate bacitracin grouping (Sprunt et al., 1974 and lyerly et al ., 1980). In our study the isolation rate of streptococci was 74.6%; similar results were obtained by Schwartz and Guinell (1980), Todd (1982) and Roe et al. (1984). Concerning the ASO titers of the examined cases, in this study, about 55.5% showed values> 200 unites. A siminlar observation were reported by Gunatillake and Perera (1970) where they determined the ASO titers in sera of 257 children in a rural population of ceylon; they found that the greatest number of children showed ASO values between 100-166 units. The percentage of children showing values over 166 units increased with age until a maximum of 54% was reached between 9-10 years. Also, Gerber et al. (1985) reported that ASO titers are increased with the age as determined by both tests. There is a wide agreement between the results of latex agglutination and classical haemolytic test within the ranges of error for the two methods. In the present study such agreement reached 73% of the examined cases which is nearly similar to the findings of Bach at al. (1972), Guerrero (1970). Klein et al. (1970) and Ramello et al (1970). Patients with streptococcal sore throat develop dangerous complications; such complications cane be avoided by early diagnosis of streptococcal infection. Prevention of rheumatic heart disease is the major consideration in proper management of streptococcal sore throat. Effective antibiotics, usually penicillin, administered within the first 7 days of symptoms, prevent such dangerous sequelae. From our investigation as seen in Table 3), the serological methods can be used for rapid diagnosis of streptococcal infection. There is a great agreement between isolation of streptococci and detection of ASO. A possible explanation for large number of the observed culture could be an intra familial exposure and transmission. The patient who had negative culture with positive titer was treated successfully with antibiotics.

The risk of intrafamilial exposure and reacquistion of the homologous serotype of group A, B-haemolytic streptococci was great among the families with treatment failures than among those with patients who were treated successfully (Kaplan et al., 1981) The who recommends utilization of ASL for the diagnosis of streptococcal infections (El Kholy, A., (1978).

Evaluation of some Different Methods for the Diagnosis of Srteptococcal I Infection.

Table 1 . Frequency of different bacterial isolates from 63 patients.

Organism	N. of cases	%
B-haemolytic streptococci	44	69.84
B-haemolytic streptococci mixed with other organisms	3	4.76
Organisms other than B-haemolytic streptococci;	16	25.40
Staph. aureus	9	14.28
Strept. pneumoniae	3	4.77
H.influenza	3	4.77
Staph. albus	1	1.58
Total	63	100

Table (2): Relation between the two serological tests.

		No. of positive cases	No. of negative cases	Titer											
				50 µ	100 µ	200 µ	300 µ	400 µ	500 µ	600 µ	700 µ	800 µ	900 µ	1000 µ	1200 µ
- Classical haemolytic	No.	45	18	5	5	13	--	9	--	1	--	9	--	--	3
- Technique (ASO)	%	71.43	28.57	7.94	7.94	20.63	--	14.29	--	1.59	--	14.29	--	--	4.76
- Antistreptolysin	No.	33	30	--	--	11	1	8	--	1	--	9	---	--	3
Technique (Latex) (ASLO)	%	52.38	47.62	--	--	17.46	1.57	12.70	--	1.59	--	14.29	--	--	4.76

Table (3): Relation between cultural and antibody detection with the classical haemolytic test.

Findings	No. of cases	%
1- Positive streptococcal growth with detection of antibodies	43	68.25
2- Positive streptococcal growth without detection of antibodies	7	11.11
3- Negative streptococcal growth with detection of antibodies	2	3.17
4- Negative streptococcal growth without detection of antibodies	11	17.46
Total	63	100

REFERENCES

1) G.L. Bach, R.Cadotte, R.A.Wiatr, M.Bhorade, and T.O.Anderson, Amer. J. Clin. Path., 57, 209. (1972).

2) H.W.Baenkler, F.Scheiffarth, and F.Legler. Zrheumaforsch, 28, 5, 6 (208-211). (1969).

3) R.E.Buchanan and A.Gibbons. Berge's manual of Determinative Bacteriology. The williams and wilkins Company, Bultimore. (1974).

4) N.D.Buffaloe, and D.V.Ferguson. "Microbiology" 2 Ed., by Houghton Mifflin Company, Boston (1981).

5) J.M.S.Dixon and J.J.Grocholski. Appl. Microbiol., 19, 621-623. (1970).

6) E.A.Edward. J. Bacteriol., 87 1254-1255. (1964).

7) A.El-Kholy. Bull. WHO, 56, 887. (1978).

8) R.D.Feigin. Neelsen Textbook of Pediatrics, Ed. 12. Philadelphia, W.B.Saunders Co. pp. 632-637. (1983).

9) A.S. Flumara. Am. J. Clin. Path., 57, 538-540.(1972)

10) A.Ganezarski, Z.Jkvzeminski. and K.Kulesza. Zable. Bakteriol. Akad. Med Lodz-przeg lepidem 32,2, 273-277 (1969).

11) A.M.Gaber, L.J.Spadaccini, L.L.Wright, L.Deatsch, L.E.Kaplan. A.J.D.C. 139, 1145-1148. (1985).

12) S.R.Guervero. Laborato (Granada) 50, 298 (307-317). (1970).

13) P.P.Gunatillake and T.O.Perea. J. Hyg. (London), 68,1 , 13-17 (1970).

14) E.H.Kaplan, A.S.Gastanaday, B.B.Huwe. J. Lab. Clin. Med., 98, 326-335 (1981).

15) L.E.Kaplan. A.J.D.C., 138, 1145-1198 (1985).

16) G.Klein, C.N.Baker and M.D.Moody. J. Appl. Microbiol., 19,1 ,60-61 (1970).

17) W.H.Lyerly, J.W.Bass and L.B.Harden J. Pediatric, 96, 431-433. (1980).

18) L.Mckane and J.Kandel. "Microbiology, Essentials and Applications" By McGraw-Hill Book Company, New York. (1985).

19) A.M.Mondzac J.A.M.A., 200, 208. (1967).

20) A.Ramello, S.Motone, and P.G.Pich. Laboratorio (Granada) 50, 296 , 125-130 , (1970).

21) M.H.Roe, P.R.Talliver, P.L.Lewis and J.K.Todd. A.J.D.C., 138: 589-591(1984).

22) R.H.Schwartz and R.K.Guinell, Am. Fam. Physician, 21, 71-78 (1980).

23) J.K.Todd. Pediatr. infect. Dis., 1; 265-270 (1982).

24) K.Sprunt, D.Vial and R.S. Asnes. Pediatr., 54, 718-728. (1974).

تقييم بعض الطرق المتبعة فى تشخيص عدوى ميكروب المكور السبحى

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لقد تضمنت هذة الدراسة استعمال اختبارى التلزن وتكسير الدم فى الكشف عن الاجسام المضادة لسموم الاستبرتوليسن (٥) فى أمصال ثلاثة وستون مريضا من قسم الانف والاذن والحنجره بالمستشفى الجامعى بأسيوط . وفى نفس الوقت قد تم أخذ مسحات الزور من هؤلاء المرضى وكان هناك تطابق فى النتائج التى حصل عليها باستعمال هذين الاختبارين فى ٣٣ر٧٣ / من هذة الحالات وباقى الحالات وهى ٦٧ر٢٦ / قد تم تشخيصها باستخدام اختبار تكسير الدم فقط.

ولهذا يتضح باستخدام اختبار التلزن فى المسح السيرولوجى لعدوى ميكروب المكور السبحى . ومن النتائج التى تم الحصول عليها وضح أن هناك تطابق تام بين اختبار تكسير الدم وعزل الميكروب من المرضى .

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