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Molecular study of Beta-Haemolytic Streptococci in Patients with Tonsillitis In Ramadi City

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ASTRACT

Objectives: To evaluate the sensitivity and specificity of polymerase chain reaction PCR technique in early detection of infection with group A beta-haemplytic streptococci in comparing with conventional diagnostic methods among the patients with tonsillitis.

Methods: One hundred ninety one swabs and blood specimens were obtained from patients with tonsillitis in addition to samples of healthy control group during a period between 2008-2009 in general Ramadi hospital, Iraq and the tests (PCR) and other bacteriological and serological test at the same time on these samples.

Results: Our data show that in recurrent tonsillitis group out of 72 GBHS isolates 43 (59.7%) were detected by PCR, however 15(20.8%) were positive by serogrouping. Out of 75 bacterial isolates, 22 (29.4%) isolates were GABHS and 15 (83.5%) of these were GABHS detected by PCR and only 4 (5.3%) of GABHS detected by serogrouping methods in acute recurrent group, and our results revealed that the inoculation of penicillin and L-therionine to the brain heart infusion broth improved the DNA extraction from gram positive bacteria (GBHS).

Conclusion: Our study confirmed that the PCR technique more sensitive and highly specific in early detection of GBHS among the patients with tonsillitis in comparing with conventional; diagnostic methods.

Keywords: type A Streptococcus , PCR, Tonsillitis.

INTRODUCTION

Tonsillitis is disease frequently occurs and worldwide distribution and one of the most prevalent infections in children and adolescents Asher, B. *et al*. (2001). The signs are similar in bacterial and viral pharyngitis the etiological agents might be viral or bacterial, Gerber, M. A. (1998). The most important bacterial cause is group A streptococcus (GAS) which is responsible for about 15% of all cases 80-90% of cases are caused by viruses particularly adenovirus, Durand, M. *et al*.(1998). The viral infection usually self-limiting with symptoms lasting less than five days, Rotbart, H. (1998)., while bacterial infection is considered a serious infection when complication occurs, World Health Organization. (1998). *Steptrococcus pyogenes* (group A) is still the most frequent cause of tonsillitis and can lead to sever squeal including rheumatic fever and glomerulonephritis, Bisno, A. L. (1991). During recent years, various new techniques have been adopted for the diagnosis of *Streptococcus pyogenes*, no ably in the field of molecular biology and standard polymerase chain reaction (PCR) which is currently the method of choice for putative transcriptional regulatory gene, Liu, D. *et al*. (2005). The main aim of the present study was to show the sensitivity and specificity technique in detection of infection with infection with beta-haemolytic streptococci in the patients with tonsillitis.

METHODS

One hundred ninety one swabs and blood specimens were obtained from patients with tonsillitis in addition to samples of healthy control group during a period between 2008-2009 in general Ramadi hospital, Iraq. Group one consist of 109 patients with recurrent tonsillitis, group two consists of 45 patients with tonsillectomy (tonsillitis) and group three consist of 37 patients with recurrent acute tonsillitis . DNA extracted from beta-hemolytic streptococci according to the method described by Jenemy Carson (1999), with simple modification by inoculated of throat swabs and blood samples on 5% sheep blood agar plate, and also inoculated in 25ml of brain heart infusion broth (BHI) supplemented with 2 g/liter of L-therionine, culture were incubated at 37 c overnight with shaking after that 0.1 gm/L of penicillin G was added and incubated for further 1.5 hr. on the hand several colonies were used to inoculated 25ml brain heart infusion broth without supplement with L-therionine plus penicillin G, culture was incubated at 37 c overnight. DNA was extracted by alkaline lyses method (1% SDS, 0.2, 0.2 N NaOH) and 1.5 ml of culture was poured into centrifuge tube and was centrifuged at 12000 x g for 30 second at 4 c in a centrifuge. The remainder of the culture was store at 4 C. the medium was removed by aspiration, and the bacterial pellet left as dry as possible and bacterial lyses by alkaline methods for extraction. The DNA was a modification of methods of Birnoboim, H. C. and Doly J. (1979). And then the DNA was stored at-20 C. All samples of bacterial culture from throat swab and blood were examined for DNA load were examined for DNA which were assayed by PCR amplification process using specific primer which were chosen from alpha DNA (Alpha DNA, Co., Canada) according to Lui *et al*. (2005).

The oligonucleotide sequence putative transcriptional regulatory if Spy1258 and PCR program which used in amplification of the target DNA was shown in Tables 1,2 and PCR products of the sample were detected and analyzed after amplification by agarose gel electrophoresis followed by detection of the specific bands in ultraviolet light (Figs. 1 and 2).

Table 1: The PCR program which used in the amplification of the targets DNA.

PCR Program			
Initial Denaturation	**2 min**	94 ℃	
Denaturation	**20 sec**	94 ℃	**30 cycles**
Annealing	**20 sec**	55 ℃	
Extension	**45 sec**	72°C	
Final extension	**2 min**	72°C	

Table 2: The original PCR reagents and final concentrations which were used in procedure.

Component	Volume	Final concentration
Go Taq Green Master Mix 2 x	12.5 µl	1 x
Forward primer	2.5 µl	10 µM
Reverse primer	2.5 µl	10 µM
DNA template	5 µl	250 ng

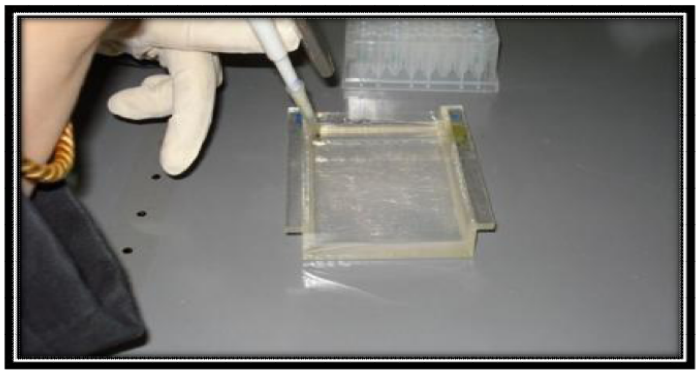

Fig. 1: Loading of DNA samples.

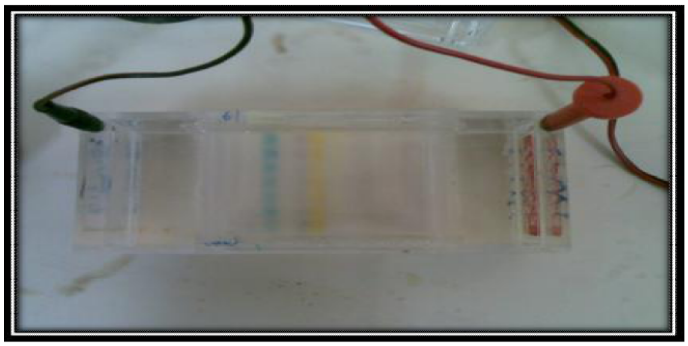

Fig. 2: Agarose gel electrophoresis.

RESULTS

The results showed that in recurrent tonsillitis group, out of 72 isolates, GBHS were detected in 15 (20.8%) by serogrouping while 43 (59.7%) were detected by PCR, however with regard to other serogrouping isolates 36 (50%) GFBHS were positive by serogrouping and 100% were positive by PCR. And for GABHS 20 (55.6%) were positive by PCR, 17(23.6%) GCBHS were positive by serogrouping and 7 (41.2%) were positive by PCR for GABHS (Table 3).

Table 3: The population group (suspected recurrent tonsillitis and control groups) in relation to diagnostic tools (blood culture, throat culture and PCR) for GBHS.

Specimens	Patient n= (109)				Control n=(20)			
	culture (n=(233) isolates)		PCR (n=(72) isolates)		culture		PCR	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Blood Culture	0 (0.0%)	109 (100%)	-- --	-- --	0 (0.0%)	20 (100%)	-- --	-- --
Throat Culture BHS isolates	72 (30.9%)	37 (34%)	43 (59.7%)	29 (40.3%)	0 (0.0%)	20 (100%)	0 (0.0%)	20 (100%)

In acute recurrent tonsillitis group throat swabs and blood samples were taken from 37 individuals, out of 75 bacterial isolates, 22 (29.4%) isolates

were GBHS. Out of 22 GBHS isolates; GABHS were detected in 4 (5.3%) by serogrouping method, while 4 (100%) were positive by PCR. on the other hand, 15(83.3%) were positive for GABHS by PCR from GBHS, with regard to blood samples 15 (40.6%) were found to be positive for GABHS for the same number of individuals (Table 4).

Table 4: The population groups (suspected acute & control group) in relation to diagnostic tools.

Population Groups	Exam. NO.	Throat Swab NO=37						Blood Sample N=37	
		Culture N = (75) isolates		PCR(GBHS) N = 18 isolates		PCR (GABHS) isolates N = 4		PCR N= 37	
		GBHS	GABHS	GABHS		GABHS		GABHS	
				positive	negative	positive	negative	positive	negative
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Suspected Recurrent Acute Group	37	18 (24.0%)	4 (5.3%)	15 (83.3%)	0 (0.0%)	4 (100%)	0 (0.0%)	15 (40.6%)	22 (59.4%)
Control Group	20	0 (0.0%)	0 (0.0%)	- -	- -	- -	- -	0 (0.0%)	20 (100%)

Result revealed that small DNA diagnostic band were detected at 407 bp of all these GBHS isolates and for GABHS, there were differences between DNA extracted from bacteria inoculated in brain heart infusion broth with or without L-therionine and Penicillin G 100%, 0% respectively (Figs. 3 and 4). No results were found either by PCR or serogrouping method in healthy control group. Serological groups A,B,C,D, and F were represented in recurrent tonsillitis individuals with high percentage of GFBHS 50% whereas in recurrent acute individuals serogroups A,B,C were represented with high percentage of GCBHS 72.7% (Table 5 & Fig. 5).

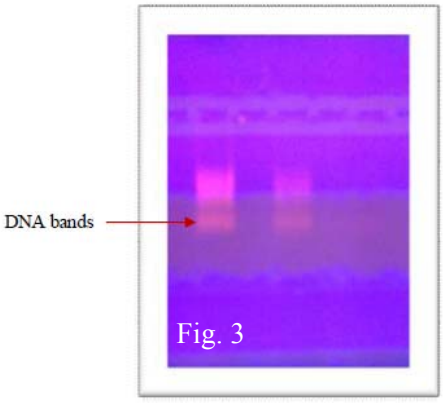

Fig. 3: Agarose gel electrophoresis of DNAs extracted from bacterial growth culture (*Streptococcus pyogenes*) cultivated in Brain heart broth & supplemented with L-therionine & Penicillin G, from seven isolates (*Streptococcus pyogenes*) under an experimental condition (DNA concentration measured prior PCR). The phptocomposition of the figure was obtained from the Digital Camera film. The time and voltage of electrophoresis was 1.5 hr, 70 volt respectively.

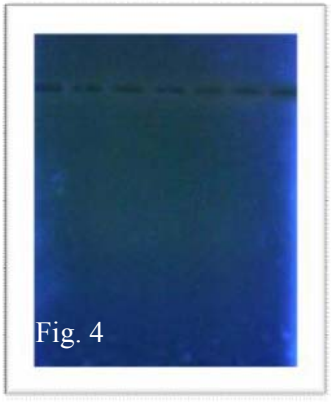

Fig. 4: Agarose gel electrophoresis. DNAs extracted from bacterial growth culture (*Streptococcus pyogenes*) cultivated in Brain heart infusion broth & no supplemented with L-therionine & Penicillin G, from seven isolates (*Streptococcus pyogenes*) under an experimental condition (DNA concentration measured prior PCR). The phptocomposition of the figure was obtained from the Digital Camera film. The time and voltage of electrophoresis was 1.5 hr, 70 volt respectively.

Table 5: The population group (suspected recurrent and control group) in relation to diagnostic tools for GBHS.

Serogroups	Patient Exam. n = (109)				Control n =20	
	Throat culture n=(233) isolates		BHS –PCR for GABHS n = (72)		Culture & PCR	
	Positive NO. (%)	Negative NO. (%)	Positive NO. (%)	Negative NO. (%)	Positive NO. (%)	Negative NO. (%)
GABHS	15 (20.8%)	57 (79. 2 %)	15 (100%)	0 (0.0%)	0 (0.0%)	20 (100%)
GFBHS	36 (50%)	36 (50%)	20 (55.6%)	16 (44.4%)	0 (0.0%)	20 (100%)
GCBHS	17 (23.6%)	55 (76.4%)	7 (41.2%)	10 (58.8%)	0 (0.0%)	20 (100%)
GBBHS	2 (2.8%)	70 (97.2%)	1 (50%)	1 (50%)	0 (0.0%)	20 (100%)
GDBHS	2 (2.8%)	70 (97.2%)	0 (0.0%)	2 (100%)	0 (0.0%)	20 (100%)

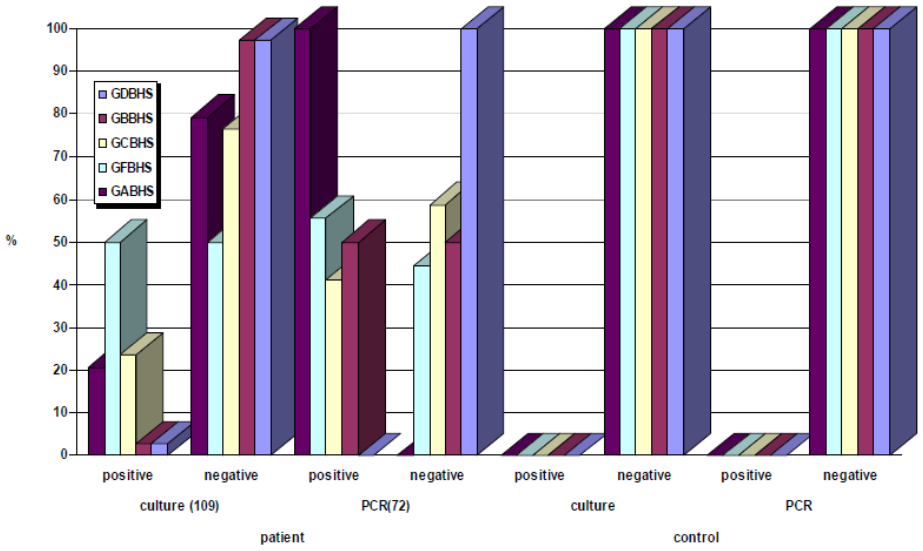

Fig. 5: The population group (suspected recurrent & Control group) in relation to diagnostic tools for GBHS.

DISCUSSION

This study revealed that GABHS (*Streptococcus pyogenes*) was detected by serogrouping and identified by PCR primer from a putative transcriptional regulatory (PTR) gene, Liu, D. *et al*. (2005). The results of present study showed that the GABHS was a major cause of recurrent tonsillitis as confirmed by positive culture of these microorganisms in 30.9% of samples. These results were in agreement with the findings from several studies were done in many countries, Al-Gebori, A. R. Q. (2007) and Martin, D.R. (2004). Our study finding that no isolates of any bacteria from blood culture in patients with recurrent tonsillitis and these results was in contrast with the other reports which indicated that positive blood culture (invasive group) due to bactermia, Kao, F. L. H. and Chia–Hui, K. (2005); Weiss K. *et al*. (2001). Our study suggested that this negative result of blood culture might be due to administration of antibiotic before collecting of blood from patients who

were no in febrile period in which the bacteria were shedding from blood. The results of this study that revealed 59.7% GABHS diagnosed by PCR and 20.8% of these microorganisms detected by serogrouping method and this was agreed with findings of other study, Kasenomm, P. *et al*. (2004), who found that 42% GABHS positive by PCR and 30% positive by culture. The present study indicated highly specificity and sensitivity of PCR than culture technique and PCR is the best choice for diagnosis of infection with beta-hemolytic streptococci and these finding was in contrast with findings from several studies in many countries which indicated the specificity and sensitivity of culture technique in comparing with PCR (20-21).

The result of the present study showed that small diagnostic bands of GABHS were detected at 407 bp such result was found to be similar to that reported by Liu, D. *et al*. (2005). The comparison between DNA isolation from BHI with or without supplement with L-therionine and penicillin G in isolation and extraction of DNA from gram positive bacteria due to L-therionine cause weakness of cell wall while the penicillin G cause distraction of the cell wall of these bacteria. This finding was in agreement with that reported by Jermy carson (1999)**.**

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ARABIC SUMMARY

دراسة جزيئية للمكورات المسبحية المحللة للدم نوع بيتا للمرضى المصابين بالتهاب اللوزتين في مدينة الرمادي

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الأهداف : تقييم حساسية وخصوصية تقنية PCR في التشخيص المبكر لإصابة بالمسبحيات نوع بيتا للمرضى المصابين بالتهاب اللوزتين مقارنة مع الطرق التشخيصية الاعتيادية الأخرى.
الطرائق: أجريت هذه الدراسة على ١٩١ عينه مسحة بلعوم ونموذج دم مأخوذة من مرضى مصابين بالتهاب اللوزتين، إضافة إلى عينات من أشخاص أصحاء في مستشفى الرمادي العام خلال الفترة المحصورة بين عامي ٢٠٠٨ إلى ٢٠٠٩ حيث طبقت اختبارات PCR والاختبارات البكتيريولوجية والسيريولوجية الأخرى على هذه العينات في آن واحد.
النتائج: أظهرت النتائج الدراسة من مجموع ٧٢ عزلة من المكورات المسبحية نوع بيتا تم عزلها من أشخاص مصابين بالتهاب اللوزتين المتكرر، ٤٣ (٥٩،٧%) شخصت بطريقة PCR مقارنة مع ١٥ (٢٠،٨%) شخصت بالطرق البكتيريولوجية والسيريولوجية الأخرى. ومن مجموع ٧٥ عزلة بكتيرية تم عزلها من أشخاص مصابين بالتهاب اللوزتين الحاد المتكرر كان منها ٢٢(٢٩،٤%) مسبحيات محللة للدم نوع بيتا ، ١٥ (٨٣،٥%) منها كانت GABHS باستخدام تقنية PCR مقارنة مع ٤ (٥،٤%) شخصت بطرق المصلية الأخرى. كما بينت النتائج أن إضافة L- penicillin , therionine إلى مرق نقيع القلب والمخ المستخدم في عزل يحسن من استخلاص ألدنا بصورة نقية من المسبحيات المحللة للدم نوع بيتا.
الاستنتاج: يمكن اعتبار تقنية تفاعل البلمرة المتسلسل PCR ذو حساسية وخصوصية عالية في تشخيص المبكر للمكورات المسبحيات نو ع بيتا للمرضى المصابين بالتهاب اللوزتين مقارنة مع الطرق التشخيصية الاعتيادية الأخرى.