Plant Archives Volume 20 No. 2, 2020 pp. 6135-6138 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR IDENTIFICATION OF SCPA AND SMEZ VIRULENCE GENES IN *STREPTOCOCCUS PYOGENES* ISOLATED FROM THALASSEMIC PATIENTS

Worood AM AL-Jobouri[1], Jawad KT AL-Khafaji[1] and Ahmed Shemran[2]

[1]Department of microbiology, College of Medicine, University of Babylon, Iraq.
[2]Babylon-Teaching Hospital for Maternity and Pediatrics, Babylon-Health Department, Iraq.

Abstract

The present study focus on isolation and identification of *Streptococcus pyogenes* in patient with thalassemia. In this case-study design include a total of 145 individuals were subjected for this study with age range (1-60 years). All samples were collected from thalassemic patients in thalassemia center of Babylon teaching Hospital for Maternity and Pediatrics from julay 2019 till December 2019. Demographic data including age, sex, Family history, history of splenectomy, Thalassemia with other diseases and Vaccination were studied for all patients. The results show30 (20.6%) bacterial isolates were identified as *Streptococcus pyogenes* , 101(69.6%) positive cultures for other bacteria and 44 represent no growth. molecular techniques for detection of some virulence factors of *S. pyogenes* by using conventional PCR technique for (scpA, smeZ genes). The result show 26 (86.6%) give positive reaction for scpA gene and 23 (76.6%) of isolates positive result with smeZ gene.

Introduction

Thalassemia is internationally the most common genetic disorder, characterized by reduced hemoglobin production. Two main types; alpha thalassemia (defect in production of the ± globin chain) and beta thalassemia (defect in production of the β globin chain) (Unissa *et al*., 2018). Thalassemia syndrome can be divided into three subgroups according to clinical severity: thalassemia major, thalassemia intermedia and thalassemia minor (Weatherall, 2012). The incidence of infection in patients with thalassemia ranges from 22.5% to 66% (Rahav *et al*., 2006; Sakran *et al*., 2012).

Most of the studies on bacterial infections in thalassemia patients have concentrated on those patients with thalassemia major. Infection with *Streptococcus pyogenes*, a beta-hemolytic bacterium belonging to sero group A of Lancefield, also known as group A streptococci (GAS), causes a wide range of diseases in humans (Shulman *et al*., 2012).

Streptococcus pyogenes is gram positive, aerotolerant bacterium, non-motile and non-sporingcocci, extracellular, pathogenic, part of the skin microbiota. The organism usually colonizes the throat, rectum, genital mucosa and skin. These bacteria can cause a wide range of diseases including streptococcal pharyngitis, rheumatic heart disease, rheumatic fever and scarlet fever. (Efstratiou & Lamagni, 2017).

The scpA gene produced by GAS specifically to cleave the human serum chemotaxin at binding site of leukocyte , Acts as a virulence factor delay the influx of inflammatory cells and Streptococci clearance during the First hours following infection (Hamim *et al*., 2017). C5a peptidase is a major surface virulence protein promoting local infection with group A streptococci. Necessary to reduce the influx of neutrophils early in infection by cleaving a potent neutrophil chemotaxin C5a produced by complement system as the bacteria are attempting to colonize the host's tissue. a highly specific protease and adhesin/invasion. As a result, SCPA inhibits chemotaxis, delaying phagocyte infiltration, thus delaying the clearance of bacteria from the mucosal and sub dermal surfaces and enabling the organism to establish an infection site (Shet *et al*., 2003~ Hidalgo-Grass, 2006).

Streptococcal mitogenic exotoxin Z (smeZ) was stimulate the immune system and its linked to different disease like acute rheumatic fever, scarlet fever and toxic shock syndrome (Degaim *et al*., 2019). smeZ was as virulence determinants implicated in the 'initiation of the

systemic toxicity which linked with fierceness of diseases and severe infections caused by *S. pyogenes,* a smeZ gene was the most exhibiting an effective super antigen, contributed imperative role, and coded to highly mitogenic proteins produced by numerous isolates of S. pyogenes (Norrby-Teglund *et al*., 2001).

Materials and Methods

Ethical approval: This research was approved by the Medicine College Ethics Committee, university of Babylon, Babylon Province, Iraq.

Samples collection

A 145 sample were collected (60 throat swabs, 25 ear swabs, 20 blood samples, 40 sputum samples) from patients attending to Babylon – Teaching Hospital for Maternity and Pediatrics in Babylon – Iraq during the period 16 June to 10 November 2019. The age groups of patients were different.

Isolation and identification of *S. pyogenes*

Identification of *Streptococcus pyogenes* depended on the following: morphological characteristics, Gram stain reaction, conventional biochemical tests and commercial biochemical (Vitek 2 system -Biomerieux-France)) and serological tests. ((Forbes *et al*., 2007). The most important method to identification the presence of GAS by culture on blood agar plate, this method distinguished *S. pyogenes* from other Streptococcus. *S. pyogenes* strains have ability to completely lyse red cells (beta hemolysis) while other streptococci exhibit partial or no lysis of red cells. Other method for distinguished *S. pyogenes* from other Streptococcus by use 0.04 units Bacitracin discs (Abraham & SiSTlA, 2016).

Extraction of bacterial DNA:

After incubation of *Streptococcus pyogenes* strains on brain-heart infusion broth (MacFaddin, 2000), at 37°C for 24 hr . The bacterial DNA extracted from a fresh culture in a Brain Heart Infusion broth according to thegenomic DNA purification kit supplemented by the manufacturing company (Geneaid, UK).

Thermal Cycling Procedure

This reaction performed in PCR using Thermal Cycling Procedure, to generate large gene copies. The total volume of reaction is 25µl, according to polymer chain reaction technique is complete on an automated cycle. The thermal cycling procedure includes heat and cools the tube that containing the reaction mixture in a short time, this procedure performed in 30 cycles. This method includes:

1. Denaturation achieved at 94°C.
2. annealing achieved in 59°C.
3. Extension achieved in 72°C.

Molecular detection of (scpA, SmeZ) virulence gene of *Streptococcus pyogenes*

Based on the manufacturer's instructions as stock suspension, the DNA primers were re-suspended by dissolving the lyophilized product after spinning briefly with nuclease-free water. Working primer tube was prepared by diluted with TE buffer. The final pica moles are based on each primer procedure.usingspecific primer pairs table 1.

Detection of Amplified Products

The successful PCR amplification was confirmed with agarose gel electrophoresis. Use UV trans illuminator for observation of DNA bands, then photographed the gel by using a digital camera.

Statistical analysis

Statistical Package of Social Sciences (SPSS) version 25 (Inc., Chicago, IL, USA) computer software was used for results analysis. chi-square test (X2) and correlation was used to calculate value significant. The level of statistical significance was set at alpha equal to 0.05 (a = 0.05). When P value <0.05 was considered statistically significant, and when P value <0.01 was considered high statistically significant (Al-Ukaelii and Al-Shaeb, 1998).

Results

A total number of samples 145 was taken, 30(29.7 %) bacterial isolates from positive culture (101) were identified as *Streptococcus pyogenes*, 101(69.6%) positive cultures for other bacteria and 44 represent no growth.

All positive isolates in present study were confirmed with vitek 2 system and API 20 Strep, which has high sensitivity and specificity.

Molecular detection of virulence factors of GAS by PCR

Molecular detection of scpA gene in *Streptococcus pyogenes*

Molecular detection of scpA gene by using two

Table 1: Primer sequences used in this study.

gene	Primer sequence	Size (bp)	Reference
scpA	F:GCTCGGTTACCTCACTTGTCCR:CAATAGCAGCAAACAAGTCACC	622	Borek *et al.,* 2012
SmeZ	F:TTTCTCGTCCTGTGTTTGGAR:TTCCAATCAAATGGGACGGAGAACA	246	Borek *et al.,* 2012

specific primer with molecular size (622 bp) table 1, the results show 26(86.6%) give positive reaction for this gene as shown in Fig. 1. ladder with 1500 pb. 1,3,4,5,6, 8,9,10 isolates of *Streptococcus pyogenes* show positive results.

Moleculer detection of smeZ gene in *Streptococcus pyogenes*

By using specific primer table 1 for detection of smeZ gene with molecular size (246 bp). The result in Fig. 2 show 23 (76.6%) of isolates positive result with smeZ gene. 1, 2, 5, 6, 9 isolates of *Streptococcus pyogenes* show positive results.

Discussion

The results of the present study showed 145 was taken, 30 (29.7%) bacterial isolates from positive culture (101) were identified as *Streptococcus pyogenes*, 101(69.6%) positive cultures for other bacteria and 44 represent no growth. All positive isolates in present study were confirmed with vitek 2 system and API 20 Strep, which has high sensitivity and specificity. The infections more likely in thalassemia patients due to Decreased innate immunity, Transfusion-related infections, Splenectomy, Central venous catheters, Iron overload and Stem cell transplantation. The results of study by Al-Khafajii *et al*., (2009). Show that *Streprococcus pyogenes* was the major gram-positive isolate with percentage 41.17%, also this results agreed with Ofek *et al*., (2002) which recorded *Streptococcus pyogenes* was

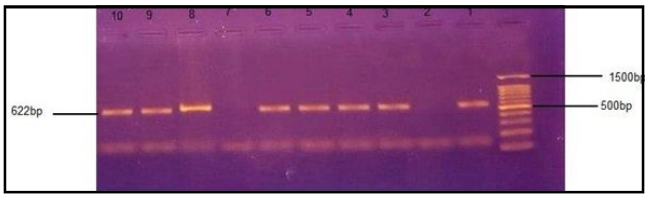

Fig. 1: Gel electrophoresis of the amplified products of *Streptococcus pyogenes* scpA gene on 1.5 % agarose gel at 70 volt for one hour visualized under UV after staining with ethedium bromid. L: ladder with 1500 pb.

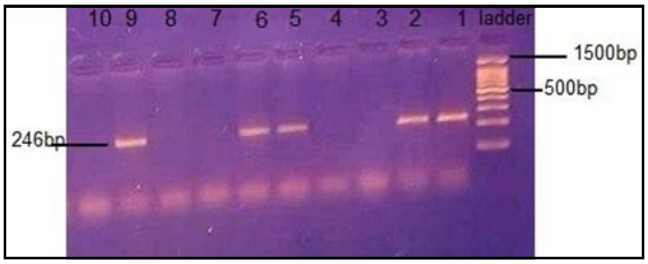

Fig. 2: Gel electrophoresis of the amplified products of *Streptococcus pyogenes* smeZ gene on 1.5 % agarose gel at 70 volt for one hour visualized under UV after staining with ethedium bromid. L: ladder with 1500 pb.

the most infectious agent in immuno-compromized pateints.

In contrast, the records of a total of 211 patients, severe bacterial infections were found in 11 patients (5.2%) in patients with NTDT. In this study the most common causative organisms were Klebsiella species (36.4%), followed by *Burkholderia pseudomallei* (27.2%), Group B Streptococcus (9.1) (Teawtrakul *et al*., 2015).

Molecular detection of scpA gene and smeZ gene by using two specific primer table 1 with molecular size (622 bp, 246 bp), the results in Fig. 1, 2 show 26 (86.6%) of isolates with scpA gene and 23 (76.6%) of isolates with smeZ. The scpA gene produced by GAS specifically to cleave the human serum chemotaxin at binding site of leukocyte, Acts as a virulence factor delay the influx of inflammatory cells and Streptococci clearance during the First hours following infection. (Hamim *et al*., 2017). In study of Ibrahim *et al*., (2016) detect all isolates show positive reaction with bacterial C5a peptidase (scpA). scpA was positive in all strains of *S. pyogenes* (Türk *et al*., 2018). A 53.9% of *S. pyogenes* showed positive reaction with scpA gene (Hamim *et al*., 2017).

Streptococcal mitogenic exotoxin Z (smeZ) was stimulate the immune system, and its linked to different disease like acute rheumatic fever, scarlet fever and toxic shock syndrome (Degaim *et al*., 2019). In study of Türk *et al*., (2018) smeZ act as the most frequent gene Among streptococcal pyrogenic exotoxin which detect in (90.0%) of isolates. The smeZ detect in all isolates (100.00%), isolated from scarlet fever patients (Liu *et al*., 2014). The PCR results showed that 50 percent of isolates contain smeZ genes, the SmeZ was a virulence-determinant of the systemic toxicity associated with the prevalence of diseases and serious infections caused by *S . Pyogenes* (Degaim *et al*., 2019).

Conclusion

Early screening and management of specific complications linked to diseases should Considered to improve quality of life in patients with thalassemia according to their clinical risk factors.

Commercial and automated techniques make streptococcal infection simple and fast to diagnose.

The species' high genomic identity of DNA adds to the identification process.

References

Abraham, T. and S. Sistia (2016). Identification of *Streptococcus pyogenes*–Phenotypic Tests vs Molecular Assay

(spy1258PCR): A Comparative Study. *Journal of clinical and diagnostic research, JCDR,* **10(7):** 12-14.

Al-Khafajii, H.A., M.M. Abboud and M.A.K. Al-saadi (2009). Immune Response in Thalassemic Patients Associated with Bacterial Infections . *Thesis of M.Sc. in college of Medical Microbiology-Babylon,* **45:** 107-120.

Al-Ukaelii, S.A. and S.M. Al- Shaeb (1998). Statically Analysis by used SPSS Program .Al-Shoroq house for Publishers and advertisement Amaan, Jordan.

Degaim, Z.D., E.D. Taher and M.S. Mohammed Jasim (2019). Molecular study of spy1258 and smeZ genes in Group A Streptococcal Tonsillitis. *J. Pure Appl Microbiol.,* **13(1):** 433-439.þ

Efstratiou, A. and T. Lamagni (2017). Epidemiology of *Streptococcus pyogenes*. In *Streptococcus pyogenes:* basic biology to clinical manifestations. University of Oklahoma Health Sciences Center.þ.

Forbes, B.A., F.S. Daniel and S.W. Alice (2007). Baily and Scoh's. Diagnostic microbiology. 12thed., Mosby Elsevier company-USA, 62-465.þ.

Hamim, S.S., K.R. Hussein and Y.Y.A. Kamel (2017). Molecular profile of scpA and sdaB virulence genes in *Streptococcus pyogenes* isolated from pharyngitis. *Al-Qadisiyah Medical Journal,* **13(24):** 67-71.þ

Hidalgo-Grass, C. (2006). Mishalian I. Dan-Goor M. Belotserkovsky I. Eran Y. Nizet V. A streptococcal protease that degrades CXC chemokines and impairs bacterial clearance from infected tissues. *EMBO. J.,* **25(19):** 4628-4637.þ

Ibrahim, J., J.A. Eisen, G. Jospin, D.A. Coil, G. Khazen and S. Tokajian (2016). Genome analysis of *Streptococcus pyogenes* associated with pharyngitis and skin infections. *PloS one,* **11(12):** e0168177.þ

Liu, Z., M. Fang, B. Hu, Z. Bi, Z. Kou, Y. Ren and B. Chen (2014). Molecular types of group A Streptococcus isolated from scarlet fever patients and asymptomatic carriers in Shandong province, 2013. *Zhonghualiuxingbingxuezazhi = Zhonghualiuxingbingxuezazhi,* **35(12):** 1375-1378.þ

MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria, Williams and Wilkins. Philadelphia, PA, 113-121.þ

Norrby-Teglund, A., P. Thulin, B.S. Gan, M. Kotb, A. McGeer, J. Andersson and D.E. Low (2001). Evidence for super antigen involvement in severe group a streptococcal tissue infections. *The Journal of infectious diseases,* **184(7):** 853-860.þ

Ofek, I., D.L. Hasty and R.J. Doly (2002). Bacterial Adhesion. Am. Soc. Microbiol. Washington, DC. pp. 63-69.

Rahav, G., V. Volach, M. Shapiro, D. Rund, E.A. Rachmilewitz and A. Goldfarb (2006). Severe infections in thalassaemic patients: prevalence and predisposing factors. *British journal of haematology,* **133(6):** 667-674.þ

Sakran, W., C. Levin, Y. Kenes, R. Colodner and A. Koren (2012). Clinical spectrum of serious bacterial infections among splenectomized patients with hemoglobinopathies in Israel: a 37-year follow-up study. *Infection,* **40(1):** 35-39.þ

Shet, A., E.L. Kaplan, D.R. Johnson and P.P. Cleary (2003). Immune response to group A streptococcal C5a peptidase in children: implications for vaccine development. *The Journal of infectious diseases,* **188(6):** 809-817.þ

Shulman, S.T., A.L. Bisno, H.W. Clegg, M.A. Gerber, E.L. Kaplan, G. Lee and C. Van Beneden (2012). Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. *Clinical infectious diseases,* **55(10):** e86-e102.þ

Teawtrakul, N., A. Jetsrisuparb, C. Sirijerachai, K. Chansung and C. Wanitpongpun (2015). Severe bacterial infections in patients with non-transfusion-dependent thalassemia: prevalence and clinical risk factors. *International Journal of Infectious Diseases,* **39:** 53-56.þ

Türk, H.D., S. Yüksekkaya, T. Seyhan, D. F1nd1k, I. Tuncer and U. Arslan (2018). Investigation of *Streptococcus pyogenes* virulence factors and typing by multiple locus variable number tandem repeat fingerprinting (MLVF) method. *Mikrobiyoloji bulteni,* **52(3):** 233-246.þ

Unissa, R., B. Monica, S. Konakanchi, R. Darak, S.L. Keerthana and S.A. Kumar (2018). Thalassemia: A Review. *Asian Journal of Pharmaceutical Research,* **8(3):** 195-202.þ

Weatherall, D.J. (2012). The definition and epidemiology of non-transfusion-dependent thalassemia. *Blood reviews,* **26:** S3-S6.þ