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

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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 july 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 show 30 (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 α 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-sporingcoci, 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 the genomic 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 using specific 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 (α = 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
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.

**Molecular 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 Streptococcus 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 the most infectious agent in immuno-compromized patients.

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**


