Study of Fibrinolytic Enzyme (Streptokinase) Produced from Clinical *Streptococcus pyogenes* Isolates

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Abstract

Thirty samples of bacterial isolates were collected from patients of Hilla Teaching Hospital during the period from 1st - 31 st / July – 2014 . These samples were isolated from upper respiratory tract (pharynx) of both sex patients .

The swabs were cultured immediately on tryptic soya agar and incubated at 37 °C for 24 hr . The isolates were identified according to cultural properties and biochemical testes . The results showed that these isolates were belonged to *Streptococcus pyogenes* . The ability of these isolates to produce streptokinase was studied . The results exhibited presence of two active SPA1 and SPA2 .

The activity of the produced enzyme was studied by fibrin plate method .

Furthermore the effect of pH at different values and different temperatures (-5,5,25,35 and 55C) were examined . The results revealed that the optimum pH was 6 , while the optimum temperature was 35°C .

Key words : Streptokinase , *Streptococcus pyogenes* , Fibrinolytic Enzyme

الخلاصة :-

تم في الدراسة الحالية جمع ثلاثون مسحة من بلعوم مرضى الجهاز التنفسي العلوي في مستشفى الحلة الجراحي للفترة من 1- 31 تموز / 2014 ومن كلا الجنسين وقدر زرعت المسحات مباشرة وعلى وسط الصويا الصلب . شخصت عزلات بكتريا المسبحات القيحية اعتمادا على الصفات الزرعية والفحوصات البايوكيمياوية . وقد أظهرت النتائج وجود عزلتين كفوأتين في أنتاج الأنزيم الحال للخثرة الدموية بطريقة طبق الفايبرين ، وقد رمز لهما (المسبحية القيحية 1و 2) . وقد درس تأثير كل من الأس الهيدروجيني ودرجات الحرارةالمختلفة (-5 و 5 و35 و55 م)على فعالية الأنزيم المنتج ، وقد تبين أن أفضل أس هيدروجيني 6 ، وأفضل درجة حرارة هي 35 °م .

الكلمات المفتاحية: ستربتوكاينيز، المسبحات القيحية، الانزيم الحال للخثرة الدموية.

Introduction

Streptococcus pyogenes is a major human pathogen responsible for numerous diseases ranging from uncomplicated skin and throat infections to severe, life threatening invasive disease such as necrotizing fasciitis and streptococcal toxic shock syndrome . These severe invasive infections progress rapidly and produce high rates of morbidity and mortality despite the implementation of aggressive treatment plans. The activation of plasminogen and the acquisition of plasmin activity at the bacterial cell surface are critical for the invasive pathogenesis of this organism. To facilitate this process, *S. pyogenes* secrete streptokinase, a potent plasminogen activating protein. Here, we describe the role of streptokinase in invasive pathogenesis and discuss some potentially useful strategies that disrupt streptokinase mediated plasminogen activation and could be employed to treat severe invasive *S. pyogenes* infections (Bisno and Stevens, 1996) *Streptococcus*

pyogenes (Group A streptococcus; GAS) is a Gram – positive , β hemolytic human pathogen associated with diverse infections ranging from asymptomatic mild respiratory and skin ailments such as pharyngitis and impetigo to life – threatening

froms of invasive disease such as necrotizing fasciitis and streptococcal toxic shock syndrome .

Superficial self – limiting infection of the upper – respiratory tract is the most common disease manifestation of GAS infection .

Systemic diseases arise from the capacity of GAS to degrade and cross epidermal and mucosal barriers and invade deep subcutaneous tissues . Although invasive GAS disease is much less common , its significance remains high due to the rapidity of spread and severity of symptoms (Bisno and Brito, 2003) .

The overall burden of GAS disease worldwide is of significant concern (Carapetis *et al.,* 2005)

The aim of the present study was to isolate GAS from respiratory tract patients and to study their fibrinolytic activity .

Materials and Methods

Collection of Samples :

Thirty throat swab samples from upper respiratory tract patient of both genders (Males and Females) in Al – Hillah Teaching Hospital . These swabs were cultured on tryptic soya agar at 37° C for 24 hr , medium and refrigerated

After that loopful of this culture was screened on the surface of brain heart infusion agar for the same period in order to obtain isolated colonies .

Identification of Isolates

These isolates were identified according to Microscopic features and Biochemical tests (Benson, 1998)

Production of crud SK enzyme :

Five Milliliters of bacterial culture on tryptic soya agar were centrifuged at 5000 rpm for 5 min . Then the supernatant was filtered throughout Millipore filter (0.45µl) .

Measurement of Enzymatic Activity

Enzymatic activity was measured by Fibrin plate method . This method include mixing of 2.5 ml of casein with 2.5 ml of sterile human serum with 15 ml of melted agar , and let it to solidify at room temperature .

Then 0.5 cm pores were done on the surface of agar by crock porer and 25µl of crude enzyme was added in pores .

These plates were incubated at 37° C for 24 hr . The enzymatic activity was measured by calculate the diameter of lysis zone around bacterial colonies .

Determination of optimum pH

Several buffer solutions at different pH values were prepared (4,5,6,7,9,11) . A volume of 0.1 ml of crude enzyme solution was added to each of these buffer , then the enzyme activity of them was measured in order to determine the optimum pH degree for bacterial isolates enzyme .

Determination of Optimum Temperature

A volume of 9 ml of buffer at pH 7 was prepared and 0.1 of crude enzyme solution was added , then it leaved for 30 min at different temperature (-5 , 5, 25 , 35 and 55) C and enzymatic activity was measured (Dubey *et al* , 2011)

Results and Discussion

Thirty throat swabs were collected from upper respiratory tract patient from Al- Hillah Teaching Hospital (Table – 1)

Table -1 – Number and percentages of bacterial isolates

Gender	Age (year)	Number	Percentage
Female	1- 10	**5**	16.6
	11- 20	3	10
	21 - 30	4	13.3
	31 - 40	2	6.6
Male	1- 10	6	20
	11- 20	3	10
	21 - 30	5	16.6
	31 - 40	2	6.6
Total		30	100 %

All of these isolates were subjected to identification methods .

The results showed that there were 12 bacterial isolates belonged to *Streptococcus pyogenes* according to cultural features and biochemical tests (Table – 2)

Table – 2- identification tests for *Streptococcus pyogenes* :

BIOCHEMICAL TEST	RESULTS
Gram stain	+
Cellular shape	Cocci
Colony feature	White , Large opaque
Arrangement	Strep
Indol	-
Methyl red	-
Vogas proskauer	+
Simmon citrate	-
Starch hydrolysis	-
Gelatin liquification	-
Motility	-
Licithinase	-
Oxidase	-
Catalase	+
Sugars fermentation	
Glucose	+
Sucrose	-
Lactose	-
Arabinose	+
Galactose	+
Maltose	-
Nitrate reduction	-

The streptokinase activity was estimated according to fibrin plate method .The enzymatic activity was measured by mixing of 2.5 ml of casein with 2.5 ml of sterile human serum with 15 ml of melted agar, and let it to solidify at room temperature. Then 0.5 cm pores were done on the surface of agar by crock porer and 25 µl of crude enzyme was added in pores . These plates were incubated at 37 C for 24 hr . The

enzymatic activity was measured by calculate the diameter of lysis zone around bacterial colonies .

From these samples there were two isolates which were active in the production of streptokinase (fibrinoplytic) enzyme . These isolates were coded SPA1 and SPA2 .

The effect of pH on activity of produced streptokinase , six different values of pH were used (Cunningham 2000, Efstration 1990, Stevens etl 1989, Stevens DL 2001 and Johnson DR 1992)). The results exhibited that 6 was the optimum pH for enzyme activity (Table – 3) .

Table -3- Effect of pH on activity of produced streptokinase

pH	Enzyme activity U/ ml	
	SPA1	SPA2
4	14.6	13.8
5	15.5	14.4
6	17.8	15.3
7	16.5	14.4
9	12.6	13.6
11	12.7	13.3

The second parameter studied in this research is the effect of temperature on the activity of production streptokinase by the two active isolates SAP1 and SAP2 , therefore five different degrees were used (-5, 5, 25, 35, 55)° C .

The results revealed the optimum temperature for the activity of enzyme was 35 °C

Table-4- Effect of pH on activity of produced streptokinase

Temp. °C	Enzyme activity U/ ml	
	SPA1	SPA2
-5	15.7	17.2
5	15.3	17.1
25	17.3	18.2
35	18.3	19.2
55	12.5	11.6

Invasive GAS disease such as necrotizing fasciitis requires bacterial entry into areas of the human body that are normally sterile through degradation of internal host tissues barriers .

A number of secreted GAS proteins potentially play a role in this process by degrading DNA , hyaluronic acid deposits , and host proteins .

GAS produces several streptodornases , proteins with DNase activity , that are thought to be involved in GAS pathogenesis by participating in the clearing of pus at the site of local infection and consequently aiding invasion [Cunningham ,2000 ; Norrby-Teglund *etal.,* 1996] and by playing a role in evasion of the host immune system through degradation of neutrophil extracellular DNA traps .

Most known streptococcal DNases (types A , C and D) are phage – encoded proteins [Johnson , 1992].

The broad – spectrum cysteine protease SpeB is an extracellular protein secreted as a 40 kDa zymogen that is proteolitically cleaved to its 28 kDa active from . It is found in all GAS strains but differentially secreted [Norrby-Teglund *etal.,* 1996].

SpeB is responsible for cleaving cytokine precursors, immunoglobulins, cell receptors and ECM proteins [36 – 38] .

In accordance with these properties , inactivation of SpeB in some murine models of infection resulted in decreased lethality and reduced dissemination to organs highlighting a key role of SpeB activity in GAS virulence [Kotb *et al.,* 2002] .

The exploitation of host components is to increase the chance of survival and spread within the human host. It is an essential invasive pathogenic mechanism of GAS . Human plasminogen is often used by invasive bacteria as a virulence factor and this process has been recognized as a critical step in GAS invasion .

In the healthy host , active plasmin dissolves intravascular fibrin clots and participates in the repair and remodeling of tissues by activating host extracellular metalloproteases and collagenases . These same mechanisms are exploited by GAS to promote systemic spread [Dubey , 2011] .

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