Detection of Phospholipase Enzyme in Bacterial Associated with Vaginitis

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Abstract

In this study, (90) vaginal swabs were taken from women suffering from vaginitis, who were admitted to Babylon hospital for maternal and pediatrics, and Teaching Hospital of Hilla, during the period from November 2012 to march 2013 in Babylon province . The patient's age ranged from (18 - 54 years). 76 vaginal swabs were taken from non pregnant women , and 14 from pregnant women, 88 samples gave positive results of bacterial growth and only two samples gave no growth bacterial. The most common species of opportunistic bacterial types were, *Streptococcus agalactiae (28.4%), S. aureus (21.5%) , Streptococcus mutans (15.9 %)* , Coagulase – negative staphylococcus (12.5%), *Escherichia coli(7.9%),* Acinetobacter *(5.9%), Enterococcus (5.9%), Neisseria.gonorrhoea(2.2%).* Production of exteracellular phospholipase D in the presence of arachidonic acid was also detected. It was found that all isolates have ability to produce this enzyme . Also , phospholipase extract was added to the culture media of *candida albicans* , and it was found that there was an increase in number of *candida*(which was isolated in this study) after the addition of enzyme extract.

الخلاصه

تم في هذه الدراسة اخذ ٩٠ مسحه مهبليه من النساء اللواتي يعانين من التهاب المهبل وراجعن مستشفى بابل للولادة والأطفال ومستشفى الحلة التعليمي ، للفترة مابين تشرين الأول ٢٠١٢ والى آذار ٢٠١٣ في محافظه بابل ، تتراوح أعمار المريضات بين(١٨ – ٤٥) سنه. تم اخذ ٧٦ مسحه من غير الحوامل و١٤ مسحه من الحوامل .٨٨ مسحه كانت موجبه للنمو البكتيري و٢ فقط كانت سالبه للنمو البكتيري.

كانت أكثر أنواع البكتيريا الانتهازية التي عزلت هي *streptococcus agalactia* ٢٨,٤ % ثم جائت بعدها *S. areus*(٢١,٥ %) و *St. mutans*(١٥,٩%) و *Coagulase –nagative staph*(١٢,٥%) و *E.coli* (٧,٩%) و *Enterococcus* و *Acinetobacter* (٥,٩ %) و *N.gonorrhea*(٢,٢%)

وكذالك تم الكشف من إنتاج إنزيم الفوسفولايبز نوع D الخارج الخلوي بوجود الارشدونك أسد ، وجد أن معظم العزلات لها ألقدره على إنتاج إنزيم الفوسفولابيز

كذالك عند أضافه مستخلص إنزيم الفوسفولايبز D للوسط ألزرعي *candida albicans* وجد إن هناك زيادة في عدد خلايا *candida* (التي عزلت في هذه الدراسة) بعده أضافه مستخلص الإنزيم.

Introduction

The genital tract is the portal of entry for numerous sexually and non-sexually transmitted diseases. A number of bacterial and non-bacterial infections exist that affect the female reproductive tract and cause vaginal discharge. Vaginal discharge is a common symptom in primary health care and is often the second most common gynecological problem after menstrual disorders [1]. Bacterial vaginitis is an infection of the lower female genital tract that

occurs predominantly in women after marriage . It is caused by an alteration in the normal vaginal flora in which the normally predominant *Lactobacilli* are replaced by pathogenic bacteria [2].

The most common organisms present in healthy vagina are *Lactobacilli,* and *candidia Albicans* in the most common, However many bacteria may associate with vaginitis such as *staphylococcus aureus*, *Escherichia coli*, Group B streptococcus, *Listeria monocytogenes*, coagulase_negative staphylococcis, *Acinetobacter* spp., *Neisseria. gonorrhoea, Enterococcus spp. Streptococcus mutans* [3].

The most important virulence factor are the phospholipases produced by bacteria . There are many ways by which bacterial phospholipases contribute to the development of disease ,direct effects of phospholipases resulting from the hydrolysis of phospholipids leading to loss of membrane integrity and cytotoxicity [4,5]. Certain microbial phospholipases are known to possess haemolytic activity. Phospholipase C from certain microbial source was shown to be devoid of the amino acid sequence associated with haemolytic activity [6].

This study mainly aims to detect phospholipase D activity in microorganisms associated with vaginitis.

Material and Methods

collection of samples : The samples were generally collected from women with vaginitis by using a vaginal speculum Specimens were immediately inoculated on blood agar plates, nutrients agar, Columbia- bloob agar plates and MacConkey's, plates. All plates were incubated at 37°C for 24 – 48 hrs.

Phospholipase D Production

Nutrient agar media was used for the detection of phospholipase D enzyme. After sterilization in autoclave and cooling to 50°C , 1% of arachidonic acid (sterilized by filtration) was added. After the inoculation of this media with bacterial isolates and incubation at 37°C for 24 hours; 3ml of glacial acetic acid was added to precipitate the lipid. The formation of transparent area around the colony of bacteria indicated a positive result for phospholipase enzyme (personal communication by Dr. Mohammad Sabri).

Growth of *candida albicans* in presence of bacterial phosphlipases extract (personal communication by Dr. Mohammad Sabri).

1- Bacteria supernatant which was confirmed to possess phospholipases is subdetacted for this experiments

2- Candida was previously cultivated on brain heart infusion (BHI) agar for 24 hr. at 37°C

3-The yeast was then transferred to BHI broth and then dilutions were done to calculate the colony forming unit (CFU).

4- Then,250 µl of yeast growth was mixed with 100 µl of bacterial laysate.

5- After incubation for 24 hr. at 37 °C on BHI agar with 1% arachidonic acid ,dilutions were also done and the CFU was calculated for each

Results

Isolation and Characterization:

Ninty (90) vaginal swabs were taken from women with abnormal vaginal discharge , who were admitted to Babylon hospital for maternal and pediatrics, Hilla teaching hospital, during the period from November 2012 to March 2013 in Babylon province . The patient's age ranged from (18 - 54 years).(76) vaginal swabs were taken from non pregnant women, and (14) taken from pregnant women, of these 90 swabs,28swabs were from

women who have post abortion.as shown in table 1

Table 1 number of isolates among pregnant and nonpregnant women

Type of bacterial growth	No. of isolates		%
Gram positive	Pregnant	7	7.9
	Non pregnant	67	76.1
Gram negative	Pregnant	6	6.9
	Non pregnant	8	9
No ,growth	Nonpregnant	1	1.1
	Pregnant	1	1.1
Total		90	100%

Opportunistic Bacterial Isolates in Women with Vaginitis

Some cases of vaginitis were caused by single bacterial type ,*candida albicans* was also isolated from cases. The followed types of bacteria were detected, group B streptococci GBS (25), *Staphylococcus aureus* (19) ,*strepcoccus mutans* (14), coagulase – negative staphyloccocis (11), *E coli* (7) , *acinetobacter(5) and enteroccoci* (5) and *N. gonorrhoeae*(2)

Table 2 Types of bacterial isolates from women with vaginitis.

Bacteria	No. (%)
St. agalactia	25 (28.4)
S. aureus	19(21.5)
Streptococcus mutans	14 (15.9)
Coagulase –negative staphylococcus	11(12.5)
Escherichia coli	7(7.9)
Acinetobacter	5(5.9)
Enterococci	5(5.9)
N.*gonorrhoeae*	2(2.2)

3.2. Phospholipase D production

It was found that most bacterial isolates have the ability to produce phospholipase D enzyme extracellular when arachidonic acid as a substrate is used. It was noticed that phospholipase D is mostly produced by Gram positive bacteria and in less degree by Gram negative bacteria. .shown in table 3.

Table 3 production of PLD by bacterial isolates

Bacteria	**Positive production**	**Percentage (%)**
S. agalactia (25)	15	60
S. aureus (19)	17	89.4
S. mutans (14)	7	50
C.N.S (11)	8	72.7
Enterococci (5)	0	0
E. coli (7)	5	71.4
Acinetobacter (5)	3	60
(2) N.gonorrhoeae	0	0
Total (88)	55	62.5

Growth of *candida albicans* in prsenc of bacterial phosphlipases extract

In this study *candida albicans* was grown in the presence of PLD extract after incubation for 48 hrs. it was found that the number of candida was increase.

Table 4 Shows NO. of candida albicans after and befor addition of phospholipase D extract

Source	Yeast NO before addition of PLD extract	Yeast NO after addition of PLD extract
Sample NO.1 *S. aureus*	2.5 X 10^7	3.5 X 10^8
Sample NO.2 *St. mutans*	1.5 X 10^7	3.6 X 10^9
Sample NO.3 *E.coli*	1.1X 10^7	2.7X 10^8

Discussion

The outcome of our study revealed that prevalence of isolated among nonpregnant women is high when compared with pregnant women, also it was observed that gram positive bacteria is more prevalence than gram negative bacteria where the rate of isolation of gram positive is 67 % among nonpregnant women. This result is identical to that results obtained from local study in Hilla province where gram positive bacteria was more predominant than gram negative (Taisser 2009 ; ZainabAdil 2011).

Also, This study revealed that *S. agalactia* constitutes one of the predominant organisms incriminated as the major causes of viginitis , because out of the 88 positive isolates, *S. agalagtia* constituted 25 (28.4%) out of the organisms isolated from the pregnant and non-pregnant women , this was followed by *Staphylococcus aureus* 19(21.5 %), *S. mutans 14*(15.9%), Coagulase – negative staphylococcis 11(12.5%), E. coli 7(7.9%) *Acinetobacter* 5(5.9%), *Enterococci* 5(5.9%) and *N. gonorrhoeae* . From these results , *S. agalagtiae* is predominant among

Gram positive whereas *E. coli* predominant among Gram negative.

Phospholipase D enzyme was screened to show the ability of bacterial isolates to produce this enzyme extracellularly .It was found that most bacterial isolates have the ability to produce phospholipase D enzyme extracellular when arachidonic acid as a substrate is used. It was noticed that phospholipase D is mostly produced by Gram positive bacteria and in less degree by Gram negative bacteria. The results of this study revealed that most isolates of *S. aureus* , C.N.S and *S.agalatiae* were able to produce this enzyme (89.4%, 72.7%, 60 % respectively). It was clear that *staphylococci* isolates are highly predominant of this enzyme followed by *streptococci* which are presented by *St. agalactiae* and *S. mutans* where their isolates produce this enzyme at rate above 50 %. In contrast to *enterococcus* which failed to produce this enzyme , this may be due to absence of genes involved for production of enzyme , or the time of enzyme induction is not enough or there is a problem in secretion of such enzymes. Also ,*Escherichia coli* isolates were able to produce this enzyme extracellarly as in Gram positive bacteria although vagina is not the natural habitat of this enzyme .Moreover *Acinetobacter* isolates have the ability to produce this enzyme at a rate 60%. This is a real pathogen in vaginitis and also it resembles *N. gonorrh*ea . It's diplococci but the later failed to produce this enzyme . The results of this study resemble the study done by walev *et al*. [7] who found that *S. aureus* have the ability to produce PLD but previous studies done by Colee and Proulx [8] have found that *S. aureus* have no ability to produce this enzyme. The results shown that 71.9% *E.coli* produced phospholipase D, Lee *et al* , [9] reported that *E. coli* phospholipase D. Songer *et al* [10] , also found that *E. coli* was expression of the phospholipase D gene . 60 % of *Acinetobacter* isolates also had been product phospholipase D Jacobs *et al* [11], reported that phspholipase D major virulence factor of *Acinetobacter*. Also 50% of *S. mutans* produced PLD. In this study, the results showed that all *Enterococcus* and N. gonorrhea isolates were negative for PLD production. Bacterial PLD are important to adhere to and to invade primary cervical cells [12] . In this study *candida albicans* were grown in the presence of PLD extract after incubation for 48 hrs. It was found that the number of candida was increase. When PLD extract of *S. arueus , S. mutans* and *E. coli* it was used. It was ,known that PLD increase the rate of *candida* cell division , this will explain why *candida albican* were overcome in some of vaginitis although it is not a real pathogen , this will return to the ability of bacterial pathogen to produce extracellular PLD which in turn will increase the division of *candida* and hence increase the number of it. Mclain and Dolan [12] showed that PLD required for dimorphic transition in *candida albican*. However, some studies indicated that PLD is very important in adhesion and also in stimulation of sporulation in some fungi.

References

1- Isibor, J. O.1, Samuel, S. O., Nwaham, C. I., Amanre I. N., Igbinovia, Oand Akhile, A. O. 2011. Prevalence of bacterial and *Candida albicans* infection amongst women attending Irrua Specialist TeachingHospital, Irrua, Nigeria African Journal of Microbiology Research Vol. 5(20) : 3126-3130

2- Tagg, J. R., Dajani, A.S. and Wannamaker ,L.W. 2007. Bacteriocins

of gram positive bacteria, *Bacteriol. Rev*., 40: 722-756.

3- Donders, G.G.G.; Vereecken, A.; Bosmans, E.; Dekeersmaecker, A.; Salembier, G.; and Spitz, B. (2005). Aerobic vaginitis: A bnormal vaginal flora entity that is distinct from bacterial vaginisis. Gynecol. Obstet. Rep. Med. In Daily Practice, 1279: 118-29.

4- Sitkiewicz, I., Stockbauer, K.E., and Musser, J.M., 2006. Secreted bacterial phospholipase A2 enzymes: better living through phospholipolysis. Trends in Microbiol. 15(2), 63-69.

5- Hurley, B.P., and McCormick, B.A., 2008. Multiple Roles of Phospholipase A2 during Lung Infection and Inflammation. Infect. Immun. 76(6), 2259-2272.

6- Zofia Olempska-B. 2008. Phospholipase C Expressed in *Pichia pastoris.* Chemical and Technical Assessment (CTA)1-7.

7- Iwan Walev , Ulrich Weller , Suanne Strauch, Timothy Foster, and Sucharit Bhakdi 1996 . Selective Killing of Human Monocytes and Cytokine ReleaseProvoked by Sphingomyelinase (Beta-Toxin) of *Staphylococcus aureus* Infection and Immunity,. 64(8) 2974–2979.

8- Coleeand R. and proulx. 1975. Phospholipase D Activity of Gram-Negative Bacteria. J. of Bacteriolgy.124(3): 1148-1152.

9- Lee JS, Bat-Ochir M, Demirev AV, Nam DH2009 .Molecular cloning of the phospholipase D gene from Streptomyces sp. YU100 and its expression in Escherichia coli.J Microbiol. 47(1):116-22.

10- Songer JG, Libby SJ, Iandolo JJ, Cuevas WA1990 . Cloning and expression of the phospholipase D gene from Corynebacteriumpseu-dotuberculosis in Escherichia coli.J .of Infect Immun. ;58(1):131-6.

11- Anna C. Jacobs, Indriati Hood, Kelli L. Boyd, Patrick D. Olson, John M. Morrison , Steven Carson, Khalid Sayood, Peter C. Iwen, Eric P. Skaar, and Paul M. Dunman 2010. Inactivation of phospholipase D diminishes Acinetobacter baumannii pathogenesis. Infect Immun. 78(5):1952-1962.

12- Edwards JL, Entz DD, Apicella MA: Gonococcal phospholipase D modulates the expression and function of complement receptor 3 in primary cervical epithelial cells. Infect Immun 2003, 71:6381-6391.

13- Nealoo McLain and Joseph W. Dolan 1997. Phospholipase D activity is required for dimorphic transition in *Candida albicas.* J. of Microbiology 143: 3521-3526.