Effect Of Use of IUD On Infection with Candida SPP in Woman With Vulvovaginitis

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Abstract

This study conducted to investigate the effect of using Intrauterine contraceptive devices (IUD) on ratio of candidal Vulvovaginitis infection and detection of Candida albicans virulence factors . In this pilot study, there are 80 women were submitted in the current study. culturing methods and multiplex PCR were used in order to achieve this aim

The results of current study showed that candida spp. detection in rate of 57.5 and in rate of 40.0%. Candida albicans Formed 73.9% and 31.2%% in women used IUD and in women not used IUD respectively. Result of multiplex PCR showed that Candida albicans HWPI, PLB1 and ALSI virulence detected in rate of 9.0%, 54.5% and 72.7%

The conclude from this study that IUD using consider enhance factors to infected with candidal Vulvovaginitis, and Candida albicans is dominant SPP was detected in our study.

Introduction

Vulvovaginitis is an inflammation of the vagina, Many infectious etiology of ulvovaginitis, example: *Candida* species, Tricophyton spp, *Nisseria gonorrheae, Trichomonus vaginalis* these infectious agents caused itching ,burning vaginal discharges. These agents transferred during sexual meeting or due to disturbance in vaginal environmental that to change in number and type of normal flora. (Qwun ,2000; Bhatla, 2001).

Contraception is techniques that prevent fertilization or to interrupt pregnancy at various stages, which include contraception that prevent fertilization or contragestion that preventing the implantation and abortion (Hadley, 2000)

Intrauterine contraceptive devices (IUD): are usually have (T) like shape and placed inside the uterus which may be either contain copper (Copper is toxic to sperms and stimulation of uterus and fallopian tubes to produce spermicidal fluid), or forrelease a progestin which effect on ovulation and increase in the mucosa thickness of alluterine cervix (Grimes, 2004).

Candida is a genus of yeasts, most of them conceder as normal flora on normal adult skin, mucous membranes of the respiratory, gastrointestinal, and female genital tracts while some *Candida* species cause disease. The infection caused by candidia called candidiasis , (Ryan and Ray, 2004).

When immune system comprised, *C. albicans* will shift from yeast formed mycelial fungal form to yeast formed invade the body. And may be caused superficial, mucocutaneous and systematic invasive (Dean, 2009; Ferreira *et al.*, 2010).

Candida albicans has many virulence factors , HWP1 (Hyphal wall protein 1) is

glycosyl phosphatidylinositol linked mannoprotein play role on adhesion. phospholipases protein 1 (PLB1 p) which help on candida to caused systemic infection. And agglunin-like sequence 1 gene (*ALS1)* that mediates aachment toendothelial cells (Abdul-Lateef *et al*., 2015; Samaranayake *et al*.,2005; Fu *et al.*,2002; Inci *et al*.,2013)

Material and methods

- Patients : 80 women patients suffering from Vulvovaginitis in age of 25-40years (40 of them used IUD) arrived to out patients clinic.

- Sample: vaginal swabs collected by sterilized cotton swabs, then direct culture on sabouraud dextrose agar and incubated at 30ºC for 24-48hrs , Single colony purified by subculturing . staining by then Lactophenol cotton blue and group of biochemical test were applied and according to (Ryan and Ray, 2004)

- DNA excretion: Genomic DNA Extraction kit (AccuPrep Bioneer Corporation) used for isolation and purification and according to menfecuted company

- Primers : table (1) describe primer used for detection of candida spp.

Table (1) primer used for detection of candida spp

Candida spp.	Primers sequencing		DNA amplification size	References
C. albicans	F	AGCTGCCGCCAGAGGTCTAA	466	Trost et al., 2004
	R	TTCTTTTCCTCCGCTTATTG		
C. tropicalis	F	GATTTGCTTAATTGCCCCAC	583	
	R	GTCAAACTTGGTCATTTA		
C. glabrata	F	TTGTCTGAGCTCGGAGAGAG	929	
	R	GTCAAACTTGGTCATTTA		

Reaction mixture : as in table (2).

Table (2): Reaction mixture used for detection of candida spp.

Compounds used in preparation of Reaction Mixture	Volume (microliter)
Taq PCR Master Mix KIT (Qiagen, Germany)	12
Primer (0.3 microliter from each primer)	1.8
DNA Template	3
DNA free water (Qiagen, Germany)	8.2
Total	25

- Thermocyclar programs : as in table (3)

Table (3): Thermocyclar programs used for detection of candida spp

Stage	Temperature (c°)	Time	Cycles (numbers)
First Denaturation step	94	10 mints	1
Denaturation step	94	15 seconds	40
Primer-annealing step	54	30 seconds	
DNA extension step	65	45 seconds	
Final DNA extension step	65	5 mints	1
End Temperature	4	————	————

- Determine of Candida albicans virulence factors: for determination of *Candida albicans* virulence factors ,following steps were conducted
- Primers: the primer used for detection of *Candida albicans* virulence factors as in table (4)

Table (4): Primers determine of *Candida albicans* virulence factors

Candida albicans virulence factors	Primers sequencing		DNA amplification size	References
HWP1	F	ATG ACT CCA GCT GGT TC	572	Shrief et al.,2019
	R	TAG ATC AAG AAT GCA GC		
PLB1	F	ATGATTTGCATCATTTG	751	
	R	AGTACTGGAGCTCTAC		
ALS1	F	GAC TAG TGA ACC AAC AAA TAC CAG A	318	Inci, et al.,2013
	R	CCA GAA GAA ACA GCA GGT GA		

- Reaction mixture used for detection of Candida albicans virulence factors: as in table (5).

Table (5): Reaction mixture used for detection of Candida albicans virulence factors

Compounds used in preparation of Reaction Mixture	Volume (microliter)
Taq PCR Master Mix KIT (Qiagen, Germany)	12
Primer (0.3 microliter from each primers)	1.8
DNA Template	3
DNA free water (Qiagen, Germany)	8.2
Total	25

- Thermocyclar program used in detection of *Candida albicans* virulence factors: as in table (6).

Table (6): Thermocyclar program used in detection of *Candida albicans* virulence factors

Stage	Temperature (c°)	Time	Cycles (numbers)
First Denaturation step	94	4mints	1
Denaturation step	94	30 seconds	35
Primer-annealing step	52	1mint	
DNA extension step	72	2mints	
Final DNA extension step	72	5 mints	1
End Temperature	4	————	————

Results and discussion

According to result of culture, candida were detection in rate of 57.5% in Patients used IUD and in rate of 40.0% in Patients not used IUD as in table (7) and figure (1).

Table (7): Distribution of candidia spp. Between contraceptive and non contraceptive users

Patients with Vulvovaginitis	Number of sample gave positive culture for candidia spp	Rate of sample gave positive culture for candidia spp
Patients used IUD (40 cases)	23	57.5%
Patients not used IUD (40 cases)	16	40.0%
Total	39	48.7%

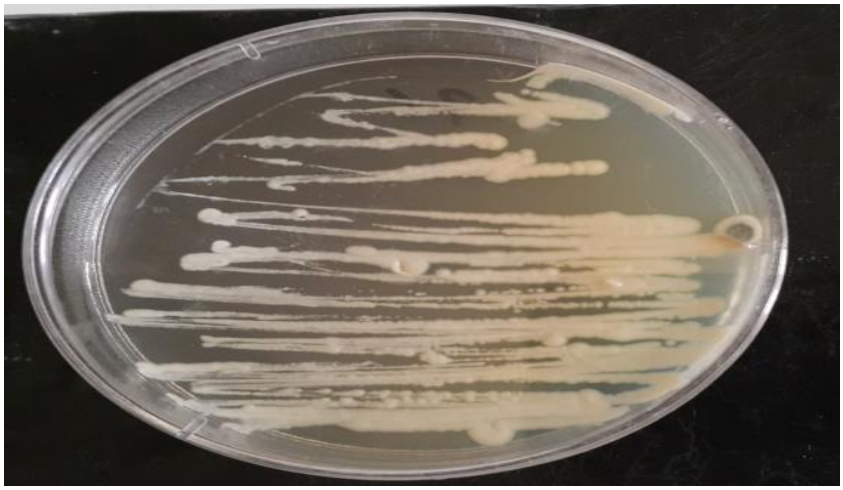

Figure (1): candida colony on SDA

- The results of current study showed that candida detected in rate of (57.5%) is more than results that reordered by (Abu-Elteen *et al.* ,2001) : 44.9%, (Bauters *et al.* ,2002) : 21.7%, and (Cetin *et al.*, 2007) : 44.2%. while the results of current study is less than result recorded by (Ribeiro *et al.*, 2001) which 79.3%
- The current results showed that candidia spp isolated from patients used IUD more than from patients not used IUD, this results agreed with results of (Cetin *et al.* ,2007) whose recorded isolation rate were 44.2% and 37.9% in contraceptive users, non-contraceptive users respectively.

Contraceptives caused increase glycogen levels that increase lactic acid bacteria which decomposing glycogen that cause increase mannoproteins which enhance infection and Vulvovaginal candidiasis (Boyd, 1988).

- From the results of multiplex PCR , *Candida albicans* detection in rate of 73.9% and 31.2%. *Candida tropicalis* detection in rate of 13.0% and 56.2% while *Candida glabrata* detection in rate of 13% and 12.5% from Patients used IUD and from Patients not used IUD respectively. Table (8) and figure (2).

Table (8) Species of candida that isolated in the current study

Candida spp.	Candida isolated from Patients used IUD (23)		Candida isolated from Patients used IUD (16)	
	No	Rate	No.	Rate
Candida albicans	17	73.9%	5	31.2%
Candida tropicalis	3	13.0%	9	56.2%
Candida glabrata	3	13.0%	2	12.5%
Total	23	100%	16	100%

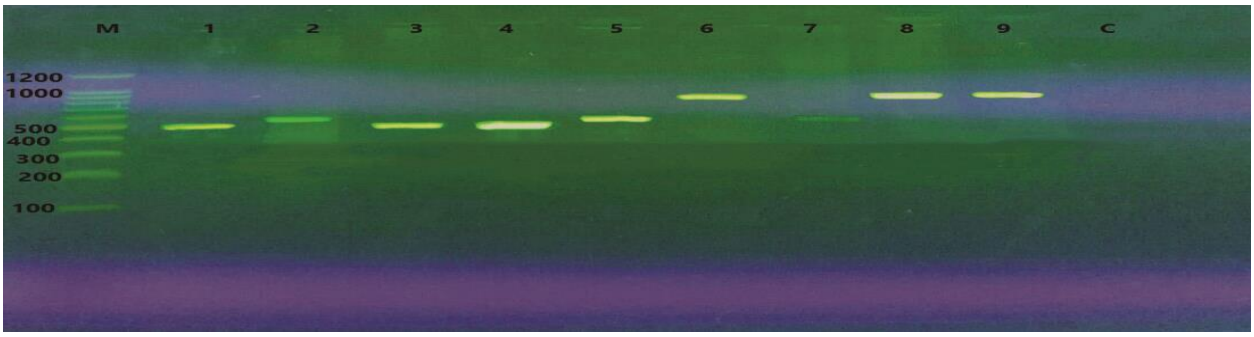

Figure (2): electrophoresis of multiplex PCR of Candida spp. , M: 100bp DNA marker, C: control negative, lane 1,3,4,7: *Candida albicans* with band in size 466bp, lane 2,5: *Candida tropicalis* with band in size 583bp, lane 6,8,9: *Candida glabrata* with band in size 929bp.

- *Candida albicans* is the highest, this results agreed with results of (Ribeiro *et al.*, 2001; Linhares *et al.*, 2001)
- Detection of *Candida albicans* virulence factors: from table (9) and figure (3), showed that detection of *HWPI, PLB1* and *ALSI* were detected in rate of 9.0%, 54.5% and 72.7% respectively

Table (9): *Candida albicans* virulence factors

Candida albicans virulence factors (22 isolates)	No	Rate
PLB1	12	54.5%
HWP1	2	9.0%
ALS1.	16	72.7%

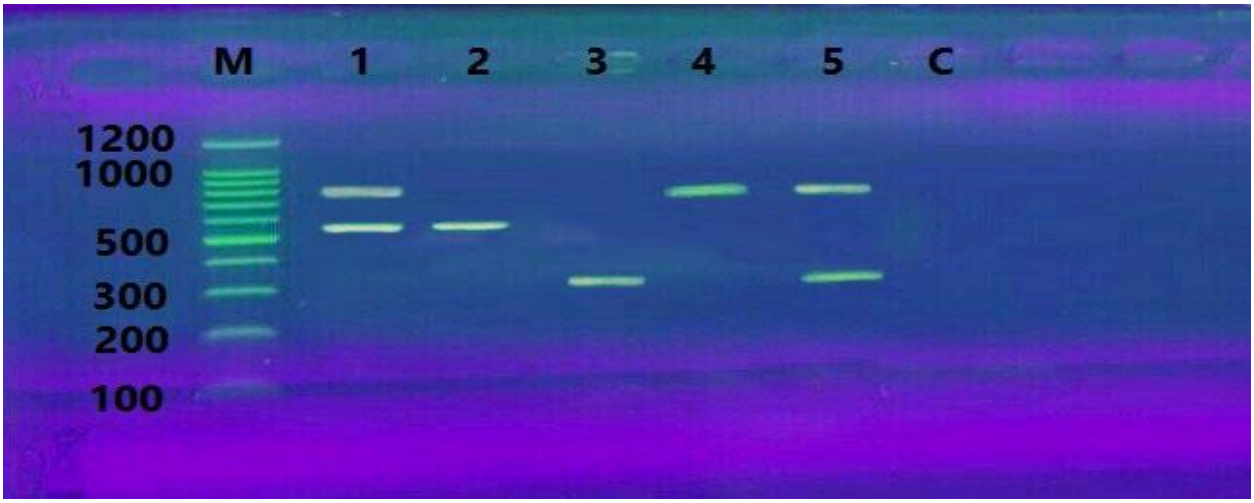

Figure (3): electrophoresis of *Candida albicans* virulence factors, M: 100bp DNA marker, C: control negative, lane 1: *Candida albicans* has *HWPI* (with band in size 572bp) and *PLB1*

(with band in size 751bp), lane 2: *Candida albicans* has HWPI with band in size 572bp, lane 3: *Candida albicans* has *ALSI* with band in size 318bp, Lane 4: lane 4: *Candida albicans* has *BLPI* with band in size 752bp, lane 5: *Candida albicans* has *HWPI* (with band in size 572bp) and *ALSI* (with band in size 318)

This result agreed with result recorded by (Abdul-Lateef et al., 2015; Samaranayake *et al*.,2005; Fu *et al*.,2002; Inci *et al*.,2013) with different in detected rate . this may be due to differ in type of infection and study location

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