Biofilm Formation on Intrauterine Device and Associated Infections

Sabah Abd Al-Kareem Al-Kattan*, Daniz Tarik Burhan* , Shafaq Tarik Burhan**

ABSTRACT:

BACKGROUND:

Intrauterine devices are highly effective methods of contraception; but considered to cause pelvic inflammatory diseases by its colonization by bacteria and /or fungi and form biofilms consisting of layers of host cells and bacteria or fungi embedded within a matrix material.

OBJECTIVE:

To screen the microbial biofilms on intrauterine devices, and its associated infection in vagina and cervix in intrauterine devices users.

METHODS:

A case series design was adopted, composed of 50 participants. High vaginal and cervical swabs were taken and intrauterine devices were removed and sent for microbiological examination.

RESULTS:

Eighty four percent of removed intrauterine devices were infected with biofilm containing different types of microorganisms and 92.8% of these infected IUDs were associated with high vaginal and cervical infection.

E.coli was found to be most common microorganism 61.5% of the infections, *staphylococcus aureus* 43.6%, *Pseudomonas* spp. 15.3%, *candida albicans* 10.3%, *Neisseria gonorrhoea* 5.1%.

CONCLUSION:

There is high frequency of microbial biofilms formation on intrauterine devices and also high percentage of associated cervico-vaginal infections in intrauterine devices users.

***KEY WORDS*:** intra uterine devices, biofilm, microbiological examination.

INTRODUCTION:

Intrauterine device (IUD) is an effective, long-term, safe and convenient option for many women and it is one of the most popular methods of contraception being used by about 100 million users worldwide .[1,2]All devices have one or two nylon filaments (tail) protrudes through the cervical canal into upper part of vagina allowing easy removal. [1,3]

Lago et al., in 2003 [4] found that the prevalence of cervico-vaginal infections was 29.1% and that bacterial vaginosis was frequently found 19.7% among IUDs users 6 months after insertion.

Bacteria in most environment are exist predominantly in multicellular surface, well-organized, cooperating communities of microorganisms called biofilms adherent to surface like plastic, glasses, metal, minerals and biotic surface [5-7]. This biofilm is enclosed in a matrix of polysaccharide material. This film is responsible for chronic bacterial infection and infection on medical devices as IUDs resulting in pelvic inflammatory disease(PID) [5, 8-12]. The tail portion of the IUD may be the primary source of contamination.[13]

The biofilm bacteria are usually resistant to attack by antimicrobial agents and host phagocytes.This is one reason why infections caused by these micro-organisms are hard to treat without removal of the devices(Pal et al., 2005) [14].

The aim of the present study is to screen the microbial biofilms on intrauterine devices, and its associated infection in vagina and cervix in intrauterine devices users.

PATIENTS AND METHODS:

A case series study of clinical and microbiological data of 50 participants was carried out over a 9 months period extended from 1st-October 2010 to 31st-June-2011 in the Family Planning Clinic in Al-Khansa' Maternity Teaching Hospital, Mosul, Iraq.

Full history was taken from participants about their age, duration of use of IUD, reason of removal, symptoms of infection, history of

*Department of Obestetric and Gynecology Al-Kansaa Maternity Teaching Hospital/ Mosul- Iraq.

**Department of microbiology, College of Science, University of Mosul/ Mosul-Iraq.

previous infection, taking treatment or not and any associated medical disease.

After informed consent was obtained. The examination was done in lithotomy position under aseptic technique. The external genitalia were inspected under a good light. After this a bivalve (Cusco's) speculum was inserted to visualize the cervix, careful examination of the cervix for any lesion such as polyps, or ectopy, any discharge from cervical canal, also thread of IUD inspected for presence or absence, high vaginal swab(HVS) and cervical swabs(CVS) were taken by using swabs with its transport media. After removal of first speculum, careful cleaning of the vagina and cervix with povidine iodine 10% and another sterile Cusco's speculum was used for removing IUD without touching the vaginal wall or the opener instrument to prevent contamination. The thread that is attached to the device was cut with sterile scissor as threads in previous study found to harbor microbes [15]. We put the IUD in brain heart infusion broth (BHI) (fig.1) which is prepared as mentioned in Cruckshank. [16]

Three samples were taken from each woman. First high vaginal swab, second cervical swab, and thirdly IUD . All samples were brought to the laboratory in the microbiological department in collage of science during one hour for investigation. The IUD samples were put in shacking incubator at 37 °C for two hours to increase bacterial releasing from biofilm. Then each one of the three samples was cultured on each following media:

1. Blood Agar: used for fastidious gram positive and gram negative bacteria and for *Neisseria gonorrhea* with 10% of CO_2 incubation.

2. MacConkey's Agar: used for determination gram negative lactose and non-lactose fermenter bacilli.

3. Manitol Salt Agar: used for determination of *Staphylococcus aureus*.

4. Saproid Dextrose Agar: which is used for *Candida albicans* determination.

5. Cetramid Agar: which is used for *Pseudomonas* spp. determination.

Then the cultures were incubated at 37 °C for 24-84 hours after that smears of each colony on these media were done and stained with gram stain which was prepared as described in Prescott.[17] The slides were examined by light microscope with oil immersion lens and the results were reported.

Data tabulation and statistical analysis performed by using Minitab version 16.2 statistical software program. Z-test for two proportions was applied in comparing different proportions and Fisher's exact test (non-parametric) was also performed for small proportions.

P-Value < 0.05 were consider statistically significant.

RESULTS:

The mean age of the women was 33.92 ± 6.99 years (range: 20 – 46 years) old. The results of this study showed that the most common reason for removing IUDs was infections of varying degrees, including PID 42%, while 32% of IUDs were removed because of heavy menstrual bleeding (HMB) and abnormal uterine bleeding, 10% due to finishing the duration of use, 10% of participants want to get pregnant and 6% of participants needing MRI for other medical problems (table 1).

After 48 hours of IUDs cultures, the results showed that 42 IUDs (84%) were infected with microorganisms and formed biofilm (fig 2).

Out of 42 infected IUDs, 39 IUDs (92.8 %) had associated infection with the same microorganisms present in high vaginal swab and cervical swab for each participant as it shown in figure (3).

This study found that the associated microorganisms were predominantly composed of E. coli 61.5%, Staphylococcus aureus 43.6%, Pseudomonas spp. 15.3%, Candida albicans 10.3%, Neisseria gonorrhoea 5.1%, finally lactobacillus spp., Enterobacter spp., Klebsiella spp., and Staphylococcus epidermidis with 2.5% for each microorganism and each sample of HVS, CVS, and IUD have more than one microorganisms and associated with each other and exist together as shown in table (2).

Sixty percent of participants with infected IUDs in this study had history of previous infections and treated with antimicrobial agents depending on culture and sensitivity of HVS, but we found there was no improvement by clinical examination, and 40% were not treated as it illustrated in figure (4).

DISCUSSION:

Pal et al.[14] found that the main cause of IUDs removal was inflammation of varying degrees including PID. This agreed with findings of the present study. Gristina [18] found that the 1st cause was infection followed by heavy menstrual bleeding and abnormal uterine bleeding so this agrees with our results. Kulsum et al [19] explained the reasons of discontinuation are unacceptable

vaginal bleeding, pain and infection was the last cause.

Previous survey performed by Pruthi et. al.[15] has revealed that 75% of the IUDS recovered from patients suffering from reproductive tract infections were covered with a consortium of microbes. This is agree with this study.

Sharief in Basra [20] found that genital infection was high among women using an IUDs. Pruthi et. al.[15]. found that the microbial flora obtained from the vaginal swabs and IUDs matched to a large extent, more over, Lago et al. in 2003 [4] found that the prevalence of cervicovaginal infections was high among IUDs users and this is in agreement with this study.

Tatum *et al.* [21] showed that the thread attached to the tail of the IUDs is perhaps one of the routes of microbial migration from the vagina to the uterus [8,13,15,22]. A previous study has indicated that there was less incidence of biofilm formation on IUDs that didn't have a tail protruding into the cervical region.[15,22,23]

The predominant microorganism in the present study is *E.coli* which concordant with the results of Pruthi *et al.,*[15] Similar to our result. Pal *et al.,* [14] found that *Enterobacter* was the predominant microorganism while *E.coli* the second most common microorganism which is inconsistent with our result where the second most common microorganism was *Staphylococcus aureus*. This could be due to different cultural setting.

Each sample of IUD, HVS and CVS have more than one organism associated with each other and exist together as it illustrated in table (2), and this due to microbial diversity in the biofilm ranging from normal flora like *Lactobacillus* to fastidious pathogenic bacteria like *Neisseria gonorrhea*, and this go with Donaln [7] who said that biofilms may be composed of single or multiple species depending on the device and its duration of use in the patients. Sharief in Basra [20] found that there was a strong association of *Klebseilla* and *candida* spp. in vaginal swab of women with IUD use 14.5% for each one and much less association with isolation of *E-coli* and *Staphylococcus aureus* 4.35% and 8.7% respectively, also there is a significant differences between our results and sharief's results regarding *E-coli, Staphylococcus aureus, Pseudomonas spp.* and *Klebseilla spp.*with P-value (0.001, 0.001, 0.009, 0.016) respectively, table (3), which could be due to different cultural setting or the difference in samples size.

John *et al.* [24], referred that the bacteria sequestered in biofilms exhibit increased tolerance to the normal antibiotics therapies. Pruthi et al.[15] also found that biofilm formation may be one of the major causes for persistent infection and antibiotic resistance in IUDs users. Although the mechanism of this resistance is not known, current hypotheses on the subject include the heterogeneity of biofilm-incased bacteria and the decreased penetration of antibiotics due to interaction with exopolysaccharide matrix, this may explain the persistence of infection in our participants in spite of treatment. After a period of treatment, biofilm will work as a microorganisms releasing machine when it reach a mature stage the biofilm will release planktonic bacteria which also can travel through IUD's thread to infect cervix and high vagina. Auler et al.[25] also found that biofilm formation on IUDs is the cause of recurrent vulvovaginal candidiasis therefore the study found a close cycle of cervix and high vagina and IUDs infections.

Figure 1: IUD in brain heart infusion broth (BHI).

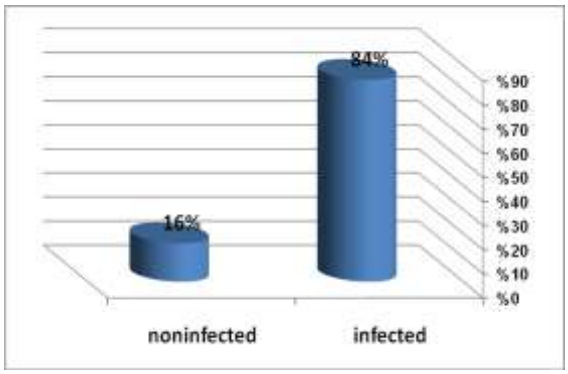

Figure 2: Percentage of infected IUDs in the study sample (n=50).

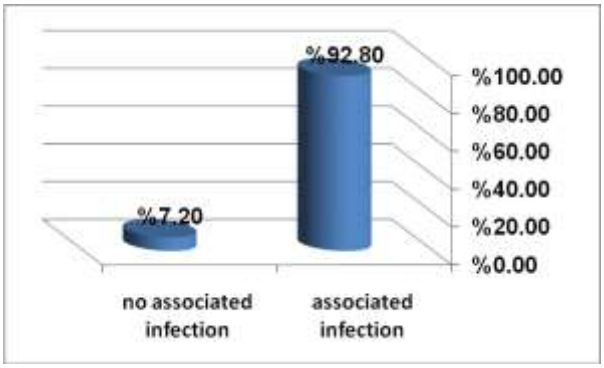

Fig 3: Frequency of associated high vaginal and cervical infection.

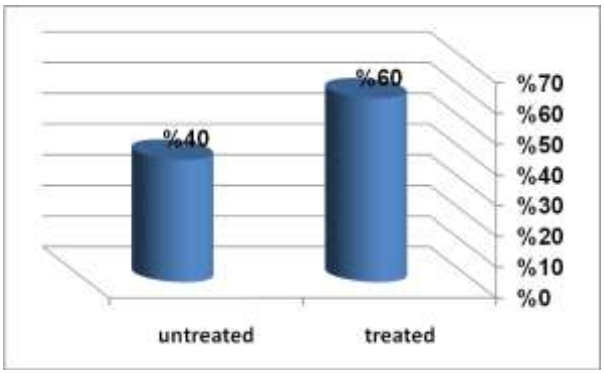

Figure 4: Frequency of history of antimicrobial agent's therapy.

Table 1: The frequency of the causes of IUDs removal.

The cause of IUDs removal	number	%
Inflammation and PID	21	42.0
Heavy menstrual bleeding and abnormal uterine bleeding	16	32.0
Finished duration	5	10.0
Want to get pregnant	5	10.0
Need MRI	3	6.0
Total	50	100%

Table 2: Frequency of detailed culture results of associated infections found in IUCDs, CVS and HVS (n = 39).

Types of microorganism	NO.	%
Escherichia coli	24	61.5
Staphylococcus aureus	17	43.6
Pseudomonas spp.	6	15.3
Candida albicans	4	10.3
Neisseria gonorrhoea	2	5.1
Lactobacillus spp.	1	2.5
Klebsiella spp.	1	2.5
Enterobacter spp.	1	2.5
Staphylococcus epidermidis	1	2.5

Table 3: Comparison between the present study and Sharief`s study in Basra regarding type of microorganism.

Types of microorganism	Present study [n = 39]		Sharief`s study[20] [n = 69]		P-value*
	No.	%	No.	%	
Escherichia coli	24	61.5	3	4.3	0.001
Staphylococcus aureus	17	43.6	6	8.7	0.001
Pseudomonas spp.	6	15.3	1	1.4	0.009**
Candida albicans	4	10.3	10	14.5	0.511
Neisseria gonorrhoea	2	5.1	8	11.6	0.216
Klebsiella spp.	1	2.5	10	14.5	0.016**

* Z-test for two proportions was used.
** Fisher`s exact test was used.

CONCLUSIONS AND RECOMMENDATIONS:

Although a large number of women prefer IUDs as a method of contraception, but inflammation and pelvic inflammatory disease is the main cause of IUDs removal.

There is high frequency of microbial biofilms formation on IUDs and also high percentage of associated cervico-vaginal infections in IUDs users. Further studies should be done using a scanning and transmission electron microscope which showed highly organized and often densely packed micro-colonies of bacteria.

REFERENCES:

1. Anna G, Ailsa E. Intrauterine Systems: Handbook of Family planning and Reproductive Healthcare. 5th ed .2008:129-140.
2. Ronald TB, Alan H.D., Lauren N.T, Murphy Goodwin, Neri-Laufer: Contraception and Family planning : Current Diagnosis and Treatment.10th ed. 2007:589-91.
3. Anna Glasier D, Keith Edmonds: Contraception: Dewhurstes's Textbook of Obstetric and Gynaecology. 7th ed. 2007:309-11.
4. Ferraz do Lago R, Simoes JA, Bahamondes L, Camargo RPS, Perrotti M, &Monterio I. Follow-up of users of intrauterine device with or without bacterial vaginosis and other cervicovaginal infections. *Contraception J* 2003;68:105-9.

5. Rodney M. Donlan. Biofilms and Device-Associated Infections. *Emerging Infectious Diseases J* 2001;7:277-81.

6. Davey, M.E. and O'Tool, G.A. Microbial biofilm from ecology to molecular genetics. *Microbiol Mol. Biol. Rev. J* 2000;64:847-67.

7. Donlan RM, biofilms. microbial life on surfaces . *Emerging Infectious Diseases J* 2002;8:881-90.

8. Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: Importance and applications. *Indian Journal of Biotechnology*. 2009;8:159-68.

9. Hall-Stoodley L, Costerton JW and stoodley P. : Bacterial biofilm from the natural environment to infectious disease .*Nat. Rev. Microbiol. J* 2004;2: 25-108.

10. KumarCG: Significance of microbial biofilm in food industry. *Int. J Food Microbiol.* 1998 ;42:9-27.

11. Deibel V. Biofilms. *J Food Safety* 2001:1-6.

12. Joesoef M.R. Karundeng A, Runtupalit C, Moran J S, Lewis J S and Ryan C A et al : High rate of bacterial Vaginosis among women with intrauterine device in Mando, Indonesia. *Contraception J* 2001;64:169-72.

13. Marrie T J, Costerton J W. :A scanning and transmission electron microscopic study of the surface of the intrauterine contraceptive devices .*Am. J Obstet. Gynaecol*. 1983;146:384-94.

14. Pal Z, Urban E, Dosa E, Pal A & Nagy E : Biofilm formation on intrauterine devices in relation to duration of use . *Med Microbiolo J* 2005;54:1199-203.

15. Pruthi V, Al-Janabi A, BM J Pereira : Characterization of biofilm formed on intrauterine devices. *Indian J of Medical Microbiology* 2003;21:161-65.

16. Cruckshank R, Duguid JP, Marmion B P and Swain RHA., *Microbiology* 12th ed.1975;95-100.

17. Prescott,L.M.,Harley, J.P. and Klein, D.A., *Microbiology*.3rd ed.1996;120-32.

18. Gristina G.A: Biofilms and chronic bacterial infections. *Clincal Microbiol NewsJ* 1994;16:171-76.

19. Kulsum J, David M, Luesley MA , Philip N, Baker DM :Contraception , Sterilization and termination of Pregnancy: Obstetric and Gynaecology An evidence -based text for MRCOG . 2nd ed. 2010 :546-49.

20. Sharief M. Genital infections among women using various contraceptive methods in Basra, Iraq. *Eastern Mediterranean Health Journal*.1998;4:487-92.

21. Tatum, H. J., F. H. Schmidt, D. Phillips, M. McCarty, and W. M. O'Leary**.** The Dalkon Shield controversy. *JAMA* 1975;231**:**711-17.

22. Coughin RW, Mullen D, Brancieri M, Rezman V, Vieth RF. *J Biomater Sci Polym Ed*.1999;10:827-44 10487317 Cit:1

23. Donlan RM, Costerton JW : Biofilm survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev J* 2002;15:167-93.

24. John C, Beverly L, Jared L , Rachel L ,Richard AG, Bruce S et al. Treatment of biofilm infections on implants with low frequency ultrasound and antibiotics. *Am J Infect control* 2005;33:78-82.

25. Auler M E, Morreira D, Rodrigues FF, Abrao M S, Margarido PF, Matsumoto FE et al. Biofilm formation on intrauterine device in patient with recurrent vulvovaginal candidiasis .*Med Mycol J* 2010;48:211-16.