

MICROBIOLOGICAL ASSESSMENT OF FEMALE UROGENITAL MICROBIOME IN PREGNANT & NON-PREGNANT WOMEN, AND THE MULTIPLE COMPLICATIONS DEVELOPED IN MOTHERS AND NEWBORNS

Farzad Shayeghi¹

1. Faculty of medicine, Islamic Azad University, Tehran Medical Sciences Branch, Iran

Esfandiar Matini²

2. Faculty of medicine, Islamic Azad University, Tehran Medical Sciences Branch, Iran

Vahideh Lazemi³

3. School of Nursing and Midwifery, Islamic Azad University, Tehran Medical Sciences branch, Iran

Seyed Ali Hosseini Zavareh⁴

4. Medical Student, Department of Medicine, Islamic Azad University, Tehran Medical Sciences branch, Iran

Mersedeh Moradi⁵

5. School of Nursing and Midwifery, Islamic Azad University, Tehran Medical Sciences branch Iran,

Samaneh Vafaei⁶

6. Department of Modern Science, Islamic Azad University, Tehran Medical Sciences branch, Iran

Farshad Moosavi⁷

7. School of Health and Medical engineering, Islamic Azad University, Tehran Medical Sciences branch, Iran

Mohammad Niatibozcheloei⁸

8. Medical Student, School of Medicine, Islamic Azad University, Tehran Medical Branch, Iran

Arad Hashemi⁹

9. Medical Student, Department of Medicine, Islamic Azad University, Najafabad Branch, Iran

Roqayeh Hakimi¹⁰

10. Paramedicine Department, Islamic Azad University, Tehran Medical Sciences branch

Faezeh Abdollahi¹¹

11. School of Nursing and Midwifery, Islamic Azad University, Chalous Medical Sciences branch

Ashkan Rezvani Joybari¹²

12. Medical Student, Department of Medicine, Islamic Azad University, Zahedan Medical Sciences branch

Faezeh Seyf¹³

13. School of Nursing and Midwifery, Islamic Azad University, Tehran Medical Sciences branch

Amirali Rahmani¹⁴

14. Paramedicine Department, Islamic Azad University, Tehran Medical Sciences branch

Roghayeh Ghodsian Nezhad¹⁵

15. School of Health and Medical engineering, Mazandaran University of Medical Sciences

Nima Mojri^{16*}

16. Paramedicine Department, Islamic Azad University, Tehran Medical Sciences branch, Iran

*Corresponding Author: Paramedicine Department, Islamic Azad University, Tehran Medical Sciences branch. Email: nimamojri7600@gmail.com Tel: +989389452251

Abstract

Introduction: Nowadays in regarding to peoples inadvertency to personal health issues and unfavorable changes of ethical terms between fellows, unfortunately most of the women are disposed to numerous healthy problems. In this study we have made our attempt to evaluate the contamination and disorders which would imperil the individual's wellbeing.

Materials & Methods: In this descriptive cross-sectional study, totally 1550 women including 529 pregnant and 1021 non-pregnant women were randomly selected. All of them were cared until delivery and newborns were also examined and monitored for health status. After sampling, the specimens were examined precisely by use of principal scientific sources and standard bacteriological methods which are mentioned farther.

Results: Infections recorded in this article are classified into 4 general categories of bacterial, fungal, viral and protozoa Infection. *Candida* accounted for the highest number of contamination in both pregnant and non-pregnant women. **Conclusion:** For the females, in fact, not paying attention to personal health may lead to problems such as fertility disorders, Vulvovaginitis, Salpingitis, Pelvic Inflammatory Disease (PID) and etc. It can also cause problems for the fetus in pregnant women including low birth weight, Chorioamnionitis, Systemic complications and even miscarriage.

Keywords: Female Urogenital Microbiota – Microbial problems – Pregnancy – Newborn Congenital Disorders – Sexual Transmitted Diseases.

INTRODUCTION

Paying attention to the health of the genital area has always been of special importance owing to the fact that the related infections and complications may have caused some serious problems in people's lives. One of the most important aspects of each individual's personal life is the issue of sex and reproduction in which the personal hygiene will take substantial regard. Clearly, not paying attention to health issues and related principles can be associated with the transmission of microbial contamination. Actually the females are more susceptible to infections and damages followed by sexual matters in comparison to males whom are mostly known as a carrier of disease. So this may have essential impression on women's menstrual, fertility, pregnancy and sexual life (1). In any case, each person's epidemiological knowledge is generally proportion to the rate of prevention and incidence of the disease. On the other hand, cultural and social issues such as low cultural level, premature delivery and low birth weight of the fetus as well as low level of healthcare information, embarrassment of being examined by a doctor and the

taboo of such issues, prescribing over-the-counter medications or the prevalence of home remedies as well as normalization of serious clinical symptoms in women have a significant effect on individual's wellbeing. These mentioned factors along with other issues such as social economic problems and the government's policies such as hiding and not disclosing the extent of pollution outbreak by some countries, can lead to a silent epidemic among the people and even threatening the health of future generations (2–5).

Nowadays, for corrupted effect of cyberspace and immoral sites, the ethical framework in people relationship has been undergone exorbitance changes. These include multiple sexual partners, unprotected and high-risk relationships and etc., which in turn can increase the transmission of infections between people. For example, in an article (6) examining genital infections among immigrant women in Vietnam between the ages of 18 and 49, it is found that sexually transmitted diseases (STIs), such as HIV, were on the rise and about 50% of the samples are HIV positive.

Also, according to the article (4) and the studies conducted, it was found that

among the total statistical society of the research, about 64% of women generally used cloth, 43% used sanitary napkins and 16% used sanitary handkerchiefs for menstrual bleeding. According to their results, these covers themselves significantly increased the amount of pollution on genital areas.

Overall, as reported by previous studies, the most common infection in reproductive tracts (RTI) is vulvovaginal candidiasis, and the most common sexually transmitted infection (STI) is *Trichomonas vaginalis* (7). They can also cause some serious problems such as infertility in women, premature termination of pregnancy, premature delivery and low birth weight of the fetus as well as other infections and fetal problems such as scalded skin syndrome (SSS), Conjunctivitis, meningitis and pneumonia.

Regarding contraception and their effectiveness in preventing infections, it should be noted that none of them prevent STIs. Occasionally they can even cause severe problems on its own, such as increased irregular bleeding in the first few periods after menstruation, increased risk of blood clots, and problems such as increased appetite, decreased bone density, hair loss, nausea, allergies to spermicides, and also increase the risk of various infections and diseases. In addition, in women with other diseases, the contraception materials such as Intrauterine devices (IUD), medicine and etc. can cause drug interactions, so they are not suitable for everyone. Moreover, some of contraceptive drugs must always be used at regular occasions of time, so that they may not be effective enough if left unchecked.

Finally, according to the above, the importance of personal health issues and the examination for preventing and controlling infections and possible complications in future is determined. Therefore, in this article, an attempt was made to conduct a more detailed research on the prevalence of various types of infections among women.

MATERIALS AND METHODS

This article is a descriptive cross-sectional study to investigate the type and extent of female genital tract infection and to increase awareness and attract the attention of physicians in this field and even ordinary people. In this study, 1550 women were randomly selected from different parts of Tehran City. Sample collection and review lasted from October 2020 to July 2021.

Initially, 1800 women were evaluated to participate in this study and for various reasons, only 1550 people were selected, including 529 pregnant women and 1021 non-pregnant women (8-10). In order to select individuals, factors such as living area, literacy level, family income, education, occupation and age of individuals were assessed in order to conduct comprehensive examinations and clinical trials, as well as external interfering factors.

First, a consent form and a questionnaire including information such as history of medical treatment as well as taking certain medications, specific diseases, number of giving birth, history of abortion, use of contraceptives and supplies, addictions, regular or irregular menstruation, number of children, type of previous delivery and the amount of sexual activity, etc. were evaluated. Then, all women were examined by a gynecologist for the color and smell of vaginal discharge and other appearance items such as warts, inflammation, skin rashes, cysts, etc., and sampling was performed in the presence of a physician. It is noteworthy that all pregnant women were cared for until delivery and newborns were also examined and monitored for health status. Also, all the pregnant women studied gave birth to their babies naturally.

For sampling of this statistical population, first two swabs were soaked in 1.5cc of distilled water in a test tube and then placed in an autoclave machine to be sterilized. Actually the posterior wall of the vagina was determined for sampling.

For laboratory examination of the samples, one of the swabs taken for PCR test was transferred to the virology laboratory in less than 20 minutes and the

other swab was placed on Transport Medium Agar for assessing bacterial contamination and then transferred to the bacteriology laboratory.

In general, to study and distinguish the type of bacterial species, some non-specific and specific (selective) culture medias comprising: Nutrient agar, Blood agar, Chocolate agar, MacConkey, EMB and Martin Tire culture Agar were used. The cultivated samples were then placed in an incubator at 37 ° C for 24 to 48 hours. After passing this time, the plates were evaluated for colonization and bacterial growth. For this purpose, the scientific bacteriological methods such as Gram staining and other diagnostic methods such as catalase, oxidase and hemolysis were used.

After identifying bacterial isolates, the necessary measures were taken to detect the contamination load of microorganisms in CFU. Regarding the contamination criterion, samples with more than 0.1×10^6 CFU were considered as infected and pathogenic. Samples containing 0.05×10^6 to 0.1×10^6 CFUs were also re-evaluated and samples containing less than 0.05×10^6 CFUs were removed from the samples.

RESULTS

In this article, a total of 1550 women were sampled, which includes two groups of pregnant women (529 samples, 34.129%) and non-pregnant women (1021 samples, 65.87%), and all people under the age of 20 to over 50 are classified in the 10-years age groups (Table 1). 1535 of all samples (99.036%) were infected (Table 2), which is distributed among non-pregnant women as 1006 infected samples and among pregnant women as 526 infected samples (Tables 3 and 4).

Infections recorded in this article are classified into 4 general categories of bacterial infections (*Staphylococcus sp.*, *Streptococcus sp.*, *E.coli*, *Gardnerella vaginalis*, *Chlamydia* and *Neisseria gonorrhoeae*), fungal (*Candida*), viral (vaginal herpes and genital warts) and protozoa (*Trichomonas*).

According to statistics, in both pregnant and non-pregnant women, *Candida* infections accounted for the highest

number of infected cases, followed by *Gardnerella vaginalis* and *Staphylococcus sp.*, respectively (Table 5 and 6).

In the next step and the study of infections in pregnant women, 192 deliveries with complications were observed, among which the share of ocular and gastrointestinal complications of the baby was higher than the rest (Table 7).

As expected, the majority of samples are related to pregnant women in the age range of 20-39 years (519 out of 529, 98.11%), while in the case of non-pregnant women (493 out of 1021, 48.28%) this ratio is quite different and also all samples recorded in people over 50 years of age are related to non-pregnant people (Tables 3 and 4).

Finally, based on the recorded data related to the time of referral and sampling of pregnant women, the period of the second and third trimesters witnessed the most referrals (443 out of 529, 83.74%) (Table 8).

DISCUSSION

As mentioned earlier, in order to evaluate and review the amount and type of infections related to the female genital area, individuals were divided into two groups: pregnant women and non-pregnant women. Each group, which includes women and girls under the age of 20 to over 50, was placed in the 10-year age groups for more detailed statistical analysis.

The types of infections obtained in each group of pregnant and non-pregnant women are specified in Tables 5 and 6. These women were evaluated for infection with four microbial groups, including bacterial, viral, fungal and parasitic infections.

As it is known, hygiene in pregnant women is very important compared to non-pregnant women in every way. Therefore, we first examine and discuss the infection related to pregnant women.

Pregnant women :

In this study, 529 pregnant women were studied, the majority of whom (46.69%) were in the second trimester of pregnancy. Many studies on pregnant

women and their infants have provided evidence of the effect of different maternal conditions on the infant. In this study, while examining the types of infections and problems of mother, it was found that most of the pregnant women studied were infected with fungal infections (*Candida*) (66.16%).

In a study conducted on 1026 pregnant women (11), two characteristics of preterm delivery and low birth weight were evaluated and it was found that in comparison to contaminated mothers with this fungus in the first trimester of pregnancy, the pre-term delivery and the mean birth weight of newborns was respectively more and reduced in contaminated mothers in second trimester. On the other hand, in another study (12), it was revealed that Chorioamnionitis which would come after vaginal Candidiasis, may has a substantial role in fetal pollution and abortion. In the above study, pregnant women are divided into three categories: Positive Vulvovaginal Candidiasis (VVC) people who have symptoms such as burning, itching, and white discharge; asymptomatic individuals who had colonization in their cultivated samples; and healthy people who have no VVC at all. Although statistically the results in this article on the rate of infection and candidiasis are similar to our results, but the researchers of this study have suggested independent relationship among women's vaginal health, the trimester of pregnancy and fetus

contamination. However, in our study, while examining the problems of childbirth in women and the complications that occur in the fetus (infant), we assume that this issue is not irrelevant.

The most common species of *Candida* that was prevalent among pregnant women in our study was *C.albicans*. A similar result has been reported in studies (13–15). According to a questionnaire obtained in our study from women with candida, it was found that people with VVC mainly had symptoms such as burning, inflammation, itching and discharge. Several cases of dyspareunia (painful intercourse) have also been reported. In agreement with other studies,, candidiasis has occurred in pregnant women as a secondary infection after immoderate use of antibiotics. Due to the fact that the amount of blood plasma and total body fluid increases during pregnancy, more doses of antibiotics are needed to treat urinary tract infections, sexually transmitted diseases and etc. Therefore, short-term use of these drugs in pregnant women can cause abnormalities in the baby, especially in the first trimester of pregnancy when organogenesis occurs (e.g.: Musculoskeletal System Malformation - VSD – ASD - Circulatory system malformation) and its long-term use actually causes changes in the microbiome and natural flora of the mucous membranes in mother and baby

Table 1: The Absolute & Relative Frequency table of pregnant and non-pregnant women classified on each Age group.

Age Group	pregnant women		non-pregnant women		Total	
	N	%	N	%	N	%
0-19	6	0/39%	80	5/16%	86	5/55%
20-29	29	19/22%	147	9/48%	445	28/71%
30-39	8	14/26%	346	22/32%	567	36/58%
40-49	1	0/26%	321	20/71%	325	20/97%
50-	4	0%	127	8/19%	127	8/19%
Total	52	34/13%	102	65/87%	155	100%
	9	%	1	%	0	

Table 2: The Absolute & Relative Frequency table of contaminated and non-contaminated women classified on each Age group.

Age Group	Contaminated Women			Non-Contaminated Women			Total	
	N	% In Total	% In Age Group	N	% In Total	% In Age Group	N	%
-19	84	5/42%	97/68%	2	0/13%	2/32%	86	5/55%
20-29	441	28/45%	99/10%	4	0/26%	0/90%	445	28/71%
30-39	563	36/32%	99/29%	4	0/26%	0/71%	567	36/58%
40-49	322	20/77%	99/80%	3	0/20%	0/20%	325	20/97%
50-	125	8/07%	98/43%	2	0/12%	1/57	127	8/19%
Total	1535	99/03%	*	15	0/97%	*	1550	100%

Table 3: The Absolute & Relative Frequency table of contaminated pregnant women

Age Group	Pregnant Women		Contaminated Pregnant women	
	N	% In Total	N	% In Age Group
-19	6	1/13%	6	100%
20-29	29	56/33%	29	99/33%
30-39	8	41/78%	6	99/55%
40-49	22	0/76%	22	100%
50-	1	0%	0	0
Total	52	100%	52	*
	9		6	

Table 4: The Absolute & Relative Frequency table of contaminated Non-pregnant women

Age Group	Non-Pregnant Women		Contaminated Non-Pregnant women	
	N	% In Total	N	% In Age Group
-19	80	7/93%	78	97/50%

20-29	147	14/40 %	143	97/28%	14%
30-39	346	33/89 %	342	98/84%	33/50 %
40-49	321	31/44 %	318	99/07%	31/15 %
50-	127	12/44 %	125	98/43%	12/24 %
Total	1021	100%	1006	*	98/53 %

Table 5: The Absolute & Relative Frequency table of isolated Microorganisms from Non-pregnant women

Age Group	Non-Pregnant Women		Contaminated Non-pregnant women		Staphylococcus spp.		E.coli		Gardnerella vaginalis		Candida spp.		Trichomonas vaginalis		HPV		Herpes simplex		Chlamydia spp.		Neisseria gonorrhoeae		Streptococcus spp.	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
-19	80	7/83%	78	7/64%	1	0/10%	1	0/10%	2	0/20%	74	7/06%	1	0/10%	1	0/10%	1	0/10%	0	0%	0	0%	0	0%
20-29	147	14/40%	143	14%	23	2/29%	12	1/19%	21	2/09%	64	6/36%	8	0/80%	3	0/30%	4	0/40%	3	0/30%	2	0/20%	3	0/30%
30-39	346	33/89%	342	33/50%	26	2/58%	21	2/09%	53	5/27%	204	20/28%	15	1/49%	5	0/50%	6	0/60%	6	0/60%	3	0/30%	3	0/30%
40-49	321	31/44%	318	31/15%	16	1/89%	22	2/18%	41	4/07%	210	20/87%	14	1/39%	3	0/30%	4	0/40%	1	0/10%	2	0/20%	2	0/20%
50-59	127	12/44%	125	12/24%	11	1/09%	10	1%	17	1/69%	81	8/05%	2	0/20%	1	0/10%	2	0/20%	0	0%	0	0%	1	0/10%
Total	1021	100%	1006	98/53%	80	7/95%	66	6/56%	134	13/32%	630	62/62%	40	3/98%	13	1/30%	17	1/70%	10	1%	7	0/70%	9	0/90%

Table 6: The Absolute & Relative Frequency table of isolated Microorganisms from pregnant women

Age Group	Pregnant Women		Contaminated Pregnant women		Staphylococcus spp.		E.coli		Gardnerella vaginalis		Candida spp.		Trichomonas vaginalis		HPV		Herpes simplex		Chlamydia spp.		Neisseria gonorrhoeae		Streptococcus spp.	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
-19	6	1/13%	6	1/13%	0	0%	0	0%	1	0/19%	3	0/57%	1	0/19%	0	0%	0	0%	0	0%	0	0%	1	0/19%
20-29	298	56/33%	296	55/95%	22	4/18%	19	3/61%	45	8/56%	184	34/98%	9	1/71%	4	0/76%	6	1/14%	2	0/38%	1	0/19%	4	0/76%
30-39	221	41/78%	220	41/59%	8	1/52%	6	1/14%	23	4/37%	160	30/42%	14	2/66%	1	0/19%	2	0/38%	3	0/57%	2	0/38%	1	0/19%
40-49	4	0/76%	4	0/76%	0	0%	0	0%	1	0/19%	1	0/19%	0	0%	0	0%	1	0/19%	0	0%	1	0/19%	0	0%

50-	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%								
Total	529	100%	526	99/43%	30	5/70%	25	4/75%	70	13/31%	348	66/16%	24	4/56%	5	0/95%	9	1/71%	5	0/95%	4	0/76%	6	1/14%

Table 7: The Absolute & Relative Frequency table of clinical congenital disorders which have been observed in newborns.

Age Group	Pregnant Women	Contaminated Pregnants	Defective Parturitions	Preterm Labor	Low Birth Weight	Neonatal conjunctivitis	Neonatal Otitis	NRDS	Abortion	Neonatal Microcephaly	Neonatal Hydrocephalus
	N %	N %	N %	N %	N %	N %	N %	N %	N %	N %	N %
-19	6 1/13%	6 1/13%	3 1/56%	0 0%	0 0%	2 1/04%	1 0/52%	0 0%	0 0%	0 0%	0 0%
20-29	298 56/33%	296 55/95%	101 52/60%	8 4/17%	14 7/29%	45 23/44%	18 9/37%	13 6/77%	2 1/04%	1 0/52%	0 0%
30-39	221 41/78%	220 41/59%	84 43/75%	6 3/12%	10 5/21%	36 18/75%	12 6/25%	12 6/25%	6 3/12%	1 0/52%	1 0/52%
40-49	4 0/76%	4 0/76%	4 2/08%	0 0%	0 0%	2 1/04%	1 0/52%	1 0/52%	0 0%	0 0%	0 0%
50-	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Total	529 100%	526 99/43%	192 100%	14 7/29%	24 12/50%	85 44/27%	32 16/66%	26 13/54%	8 4/16%	2 1/04%	1 0/52%

Table 8: The Absolute & Relative Frequency table of each trimester in which the pregnant women are in.

Age Group	Total	Pregnant Women	First Trimester	Second Trimester	Third Trimester
		N %	N %	N %	N %
-19	86	6 1/14%	1 0/19%	3 0/57%	2 0/38%
20-29	445	298 56/33%	45 8/51%	132 24/95%	121 22/87%
30-39	567	221 41/77%	39 7/37%	111 20/98%	71 13/42%
40-49	325	4 0/76%	1 0/19%	1 0/19%	2 0/38%
50-	127	0 0/00%	0 0/00%	0 0/00%	0 0/00%
Total	1550	529 100/00%	86 16/26%	247 46/69%	196 37/05%

Of course, nowadays it has been proven that some antibiotics such as *Cephalexin*, *Nitrofurantoin* and etc. can be prescribed in the second and third trimesters to treat problems such as UTI in the mother.

Based on the results obtained in our research and during the review of Tables 5 and 6, it can be concluded that the rate of fungal and parasitic infections in pregnant women was significantly higher than non-pregnant women. However, the rate of bacterial infections, including *Gardnerella*, *streptococcus* and *E.coli* infections, was higher in non-pregnant women.

According to (16), the researchers said that *Gardnerella* attach to the vaginal wall epithelium, release the enzyme *vaginolysin*, and by destroying the epithelial cells, they feed on them and damage the vaginal tissue. As a result, the vaginal environment or birth canal becomes more disposed to infection. For the treatment of bacterial vaginosis (BV), Metronidazole is usually used topically or orally. Generally, the first line of treatment for *Gardnerella* infection is the use of Ampicillin and Metronidazole antibiotics. Referring to pharmacological references, Metronidazole is contraindicated for pregnant women, however, no clear link has been found between the drug and fetal abnormalities (17).

As the results obtained by questionnaires, the bacterial vaginosis is more prevalent among people living in slums, low-income and more unaware of health issues. Indeed bacterial vaginosis can accommodate vagina for incidence of other microbial infections which should be regarded seriously for screening of cases and actually well-timed diagnosis and treatment. Therefore, BV by providing favorable conditions for the growth of bacteria such as *Staphylococcus sp.* and *E.coli*, causes abnormalities in delivery process. According to Table 6, about 5/70% and 4.75% of pregnant women are respectively infected with *Staphylococcus sp.* and *E.coli*.

As mentioned in (4,18), researchers have concluded that these microorganisms by causing complications such as Intrauterine infection and *Chorioamnionitis*, make problems such as Necrotizing Enterocolitis (NEC), Retinopathy of Prematurity (POR), Respiratory Distress Syndrome (RDS) and

Intraventricular Hemorrhage (IVH) and can cause irreparable problems for the fetus and baby.

As reported by previous researches, the transmission of bacterial from rectum and urinary tract to the birth canal would be accompanied with contamination of amniotic fluid and even the fetus skin flora which eventually can lead to inflammatory responses beside different crucial complications such as premature delivery, miscarriage (in the first twenty-two week of pregnancy), stillbirth (loss of fetus after 22nd week of gestation), Premature Rupture Of Membrane (PROM), low birth weight and sepsis (18).

The same is true for *Staphylococcus* bacteria, except that unlike *E. coli*, there are many antibiotic treatments. However there are many controversial viewpoints about this kind of treatment in which the bacterial resistance for antibiotics Methicillin/Penicillin and utilization of Aminoglycoside suppositories as advanced treatment are intended (5).

Regarding the condition of the infants themselves, it is noteworthy that in addition to the mentioned and predictable problems, there is a possibility of infection through breast milk or even by way of pollutant surgical instruments in operating room in the process of delivery/Cesarean. This adds to the importance of diagnosing and treating this bacterium, especially in people with weaker immune systems or underlying diseases such as diabetes (4,7).

After above cases, *Trichomonas* has the highest rate of infection (4.56%). Researches have shown that transmission of *Trichomonas* infection occurs through contact with the vaginal or urinary tract secretions of infected people, often through sexual intercourse. However, the transmission of this microorganism through asexual items such as showering, washing clothes, toilets and shared soap has also been reported (19).

Also in Article (20), the researchers examined the prevalence of *group B Streptococci* and *Trichomonas vaginalis* infection and the effect of the two on each other in pregnant women. According to the results of this study, Streptococcal bacterial infections are not associated with *Trichomonas*. In (19), the researchers concluded that *Trichomonas*

protozoa could increase the risk of carcinogenic viruses or cancer-producing metabolites such as HPV through intraepithelial neoplasia of the cervix. According to (20), this infection similar to the infection of *Mycoplasma hominis* and *Corynebacterium* can lead to Preterm labor. As reported by (21), *Trichomonas* infection can cause severe complications such as Endometritis, Salpingitis, inflammation and infection of the pelvis and damage to the upper genital tract in women. Therefore by considering the side effects of using Metronidazole to treat this infection, the personal hygiene issues, especially in pregnant women, are doubly important.

In the following discussion, we examine the viral infections that are statistically close to the prevalence in our studies: genital warts (0.95%) and vaginal herpes (17.11%) in pregnant women.

Compared to these two infections, HPV has a higher transmission rate than HSV and if we do not pay attention and control this infection, the prevalence of this two diseases in the community would be increased which should be regarded seriously due to the complications caused by them (For example, in a study, 70% of cervical cancers were caused by HPV, which also works by destroying the cervical epithelium.) (23)

In the case of HSV, well-timed treatment is also a serious challenge in the face of this infection, because most of the cases observed in this disease are asymptomatic and are not taken seriously by the patient. So every shortcoming in this process has been associated with the aggravation of complications such as urinary retention and the creation of secondary problems (4). It should be noted that in the issue of the occurrence of symptoms, we see similar conditions for HPV and only 1% of infected people develop their disorder as visible warts

According to the general information about *Streptococcus* bacteria, this species is considered as a normal flora, but referring to the results obtained in our study, in some pregnant women the level of infection was too high, so that the pathogenic conditions of this organism have occurred.

According to Table 6, pregnant women in the group age of 20 to 29 years were more infected with this bacteria, and in general, 1.14% of pregnant women in this study were contaminated too. The pathogenicity of *Streptococcus* bacteria is mainly caused by group B streptococci and we classify and study them in three categories.

Firstly, it causes complications such as Cystitis, Amnionitis and Endometritis for pregnant women. Secondly, it causes complications such as stillbirth for the fetus. Also in the third stage, infection is divided into late onset and early onset. If the baby develops streptococcal infections in the first 7 days of life, especially the first 12 hours, it shows symptoms such as Pneumonia, Meningitis and decentralized Sepsis and if after this period until the 89th day of life, especially on days 17 to 32, decentralized bacteremia and meningitis will occur (24).

In many studies, the infection of *Neisseria gonorrhoeae* (Ng) and *Chlamydia trachomatis* (Ct) along with their related complications have been studied. In a comparison, the cause of neonate conjunctivitis and the subsequent blindness has been attributed to prenatal Ng/Ct transmission (25). In our results, neonatal eye infections have the highest percentage among cases of delivery with complications (44.27%). Clearly the statistics have been emphasizing on proper attention to the issue. Cases such as abortion (4.166%), preterm delivery (7.291%) and low birth weight (12.5%) have been considered for both microorganisms. More specifically, the probability of low birth weight was 40% higher among NG-positive mothers which could also be due to the ability of Ng in crossing the placenta and to limit the intrauterine growth (26). In another study, it was stated that NG and CT lead to neonatal mortality with a pattern of preterm delivery (for NG, especially before the 32nd week of pregnancy) (27, 29) but in the case of abortion, several findings are indicative for the role of other variables and even the impotency of Ct (28). It is also considerable that 75% of Ng positive pregnant women were asymptomatic and according to statics the prevalence of this infection was higher in 2nd & 3rd trimester of pregnancy (27,29)

Non-pregnant women:

In the study of non-pregnant women, according to Table 5 and the questionnaire of the clients in this article, we divided people into three categories: people under 20 and over 20 years (age classification), use or not use contraceptives, and having or not having sex. It should be noted that candidiasis is the most common infection in non-pregnant women as well as in pregnant women.

In the first category, due to cultural issues in Iran, it is expected that due to the lack of marriage registration at a young age and lack of sex, the rate of microbial contamination should be low. Our results confirm this and the remarkable point is the high rate of candidiasis infection compared to other ages which can be due to wearing tight clothes and not removing excess hair. Also, cultural issues and the level of public awareness of health issues have affected the statistics of people referring to health centers at this age. In women over the age of 20, in addition to candidiasis being the most common infection, bacterial, viral, and parasitic infections are significantly increased. According to the prepared questionnaire, among non-pregnant women over 20 years old, 89.65% (832 out of 928 cases) had sexual intercourse and the rest did not have sexual relations due to reasons such as non-marriage, divorce, death of spouse and etc. Referring to studies, no positive cases of vaginal herpes, genital warts and Trichomoniasis have been found in asexual pregnant women. On the other hand, bacterial infections have been less prevalent. However, by comparing these people with non-pregnant and sexually active women, many infections have been reported, which may be due to non-observance of personal health issues.

In another category, according to a questionnaire obtained among non-pregnant and sexually active women, the uses of contraceptive methods respectively include the use of condoms (37.8%), birth control pills (19.3%), injectable ampoules or other methods such as Intrauterine Device (IUD) (8%). The rest of the people due to infertility problems or generally not using contraceptive methods, no data have been recorded for them. According to (30), the use of spermicides in women has been associated with an increase in *E.coli*, *Enterococci*, and anaerobic gram-negative

bacilli, while the use of contraceptive pills shows a decrease in these cases.

Also, the researchers concluded that nevertheless the high risk of unwanted pregnancy by using condom than the other methods, but the results show that infections such as *Candida*, *Gonorrhea*, *Trichomonas*, HIV, HSV and HPV are less transmitted through condoms and in fact, other methods are largely incapable of preventing the transmission of infections (31).

CONCLUSION

In this study, we examined the types of infectious diseases in women and the effects of each one on different factors. According to studies, unfavorable economic situation, low level of education, marriage, having multiple sexual partners, lack of personal and social hygiene and sometimes migration have a serious impact on women's well-being. Most sexual diseases affect a person's pregnancy and even if a baby is born, it can lead to neonate contamination.

Therefore, to prevent the epidemic of RTI diseases, it is recommended to take precautions such as raising the level of social knowledge, health education in sexual behaviors and etc.

According to the results, 80 to 90 percent of the samples were taken in the second trimester of pregnancy and for reducing the probable problems in gestational period, delivery and avoiding congenital disorders in baby, it is suggested to take clinical checkups regularly before and during the first trimester of pregnancy .

As discussed in detail in the previous sections, pregnant and non-pregnant women were evaluated for the microbial contamination. By regarding to results of prepared tables, the high level of contamination are recognized. Normally, conditions of vagina is very ideal for pollutions to grow, especially during menstruation in which the setting would be more suitable for bacterial accumulation. So that not paying attention to it may lead to problems such as fertility disorders, Vulvovaginitis, Salpingitis, Pelvic Inflammatory Disease (PID) and etc. It can also cause problems for the fetus in pregnant women including low birth weight,

Chorioamnionitis, Systemic complications and even miscarriage.

ACKNOWLEDGEMENT

First and foremost, praised and thanks to the God, the Almighty, for his showers of blessings throughout our research work to complete the research successfully. Secondly, we would like to express our deep and sincere gratitude to various people for their contribution to this project.

REFERENCE

1. Desai B, Kosambiya JK, Patel B, Barve A, Kumar A, Wells KJ. Health Care for Women International Knowledge about reproductive tract infections and sex work among female textile workers in Surat , India. Health Care Women Int [Internet]. 2019;0(0):1–16. Available from: <https://doi.org/10.1080/07399332.2019.1597873>
2. Tansarli GS, Skolidis T, Legakis NJ, Falagas ME. Abnormal vaginal flora in symptomatic non-pregnant and pregnant women in a Greek hospital : a prospective study. Eur J Clin Microbiol Infect Dis [Internet]. 2016; Available from: <http://dx.doi.org/10.1007/s10096-016-2787-5>
3. Schellenberg JJ, Patterson MH, Hill JE. Gardnerella vaginalis diversity and ecology in relation to vaginal symptoms. Res Microbiol [Internet]. 2017;168(9–10):837–44. Available from: <https://doi.org/10.1016/j.resmic.2017.02.011>
4. Vishwakarma D, Puri P, Sharma SK. Interlinking menstrual hygiene with Women ' s empowerment and reproductive tract infections : Evidence from India. Clin Epidemiol Glob Heal [Internet]. 2021;10(November 2020):100668. Available from: <https://doi.org/10.1016/j.cegh.2020.11.001>
5. Thomas MA, Narayan P. Health Care for Women International Reproductive tract infections : Attitude and barriers among marginalized fisher women in. Health Care Women Int [Internet]. 2017;38(4):361–78. Available from: <http://dx.doi.org/10.1080/07399332.2017.1279616>
6. Thi A, Le K, Thi H, Tran D, Duong TK, Nguyen CC. Health Care for Women International Reproductive tract infection and related factors among female migrants working in industrial zones in Vietnam 2013 – 2014. Health Care Women Int [Internet]. 2018;0(0):1–15. Available from: <https://doi.org/10.1080/07399332.2017.1411915>
7. Torondel B, Sinha S, Mohanty JR, Swain T, Sahoo P, Panda B, et al. Association between unhygienic menstrual management practices and prevalence of lower reproductive tract infections : a hospital-based cross-sectional study in. 2018;1–12.
8. Abbas MA, El Badrey SM, ElDeeb AM, Sayed AM. Effect of aerobic exercises on the thyroid hormones in treated hypothyroid pregnant women. J. Adv. Pharm. Educ. Res. 2019;9(4):49-53.
9. AL-Shakhshir SM, Sulaiman SA, Alhaddad MS, Ismail MP. Assessment of the Effectiveness of a Pharmacist-Led Digital Educational Program on Knowledge, Perception, and Practice of Pregnant Women at a Tertiary Care Teaching Hospital. Arch. Pharm. Pract. 2019;10(1):5-11.
10. Aboud SA, El Sayed HA, Ibrahim HA. Knowledge, Attitude and

- Practice Regarding Prevention of Iron Deficiency Anemia among Pregnant Women in Tabuk Region. *Int. J. Pharm. Res. Allied Sci.* 2019, 8(2):87-97
11. Holzer I, Farr A, Kiss H, Hagmann M, Petricevic L. The colonization with *Candida* species is more harmful in the second trimester of pregnancy. *Arch Gynecol Obstet.* 2017;295(4):891–5.
 12. Waikhom SD, Afeke I, Kwawu GS, Mbroh HK, Osei GY, Louis B, et al. Prevalence of vulvovaginal candidiasis among pregnant women in the Ho municipality , Ghana : species identification and antifungal susceptibility of *Candida* isolates. 2020;3:1–14.
 13. Zisova LG, Chokoeva AA, Amaliev GI, Petleshkova P V, Krasteva MB, Uchikova EH, et al. ORIGINAL ARTICLE , MEDICINE Vulvovaginal Candidiasis in Pregnant Women and its Importance for *Candida* Colonization of Newborns. 2016;58(2):108–14.
 14. Josefina M, Luján M, Fernanda M, Teresa M, Candida P De. Revista Iberoamericana de Micología Prevalence of *Candida albicans* , *Candida dubliniensis* and *Candida africana* in pregnant women suffering from vulvovaginal candidiasis in Argentina. *Rev Iberoam Micol* [Internet]. 2017;34(2):72–6. Available from: <http://dx.doi.org/10.1016/j.riam.2016.09.001>
 15. Martinez T, Baracy G, Gonc CV, Riffel L, Maria A, Martinez B De, et al. Prevalence of *Candida* spp . in cervical-vaginal samples and the in vitro susceptibility of isolates. 2016;8:145–50.
 16. Brannon JR, Dunigan TL, Beebout CJ, Reynolds WS, Hadjifrangiskou M, Ross T, et al. Invasion of vaginal epithelial cells by uropathogenic *Escherichia coli*. *Nat Commun* [Internet]. (2020):1–11. Available from: <http://dx.doi.org/10.1038/s41467-020-16627-5>
 17. Katzung BG. Basic & Clinical Pharmacology.
 18. Cools P. SC. Res Microbiol [Internet]. 2017; Available from: <http://dx.doi.org/10.1016/j.resmic.2017.02.002>
 19. Mielczarek E, Blaszkowska J. *Trichomonas vaginalis* : pathogenicity and potential role in human reproductive failure. *Infection.* 2017;44(4):447–58.
 20. Ahmadi A, Farhadifar F, Rezaii M, Zandvakili F. Group B Streptococci and *Trichomonas vaginalis* infections in pregnant women and those with spontaneous abortion at Sanandaj , Iran. 2018;10(3):166–70.
 21. Hosny AEMS, El-khayat W, Kashef MT, Fakhry MN. ScienceDirect Association between preterm labor and genitourinary tract infections caused by *Trichomonas vaginalis* , *Mycoplasma hominis* , Gram-negative bacilli , and coryneforms. *J Chinese Med Assoc* [Internet]. 2017;1–7. Available from: <http://dx.doi.org/10.1016/j.jcma.2016.10.007>
 22. Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Expert Reviews Sexually transmitted diseases and infertility. *Am J Obstet Gynecol* [Internet]. 2017;216(1):1–9. Available from: <http://dx.doi.org/10.1016/j.ajog.2016.08.008>
 23. Patel R, Kennedy OJ, Clarke E, Geretti A, Nilsen A, Lautenschlager S, et al. 2017 European guidelines for the

- management of genital herpes. 2017;
24. Smith DS, Editor C. Bacterial Infections and Pregnancy. 2021;1–18.
25. Kreisel K, Weston E, Braxton J, Llata E, Torrone E, Hepatitis V. HHS Public Access. 2018;44(6):356–8.
26. Heffron R, Hawes SE. HHS Public Access. 2018;44(5):266–71.
27. Warr AJ, Pintye J, Kinuthia J, Drake AL, Unger JA, McClelland RS, et al. Sexually transmitted infections during pregnancy and subsequent risk of stillbirth and infant mortality in Kenya: a prospective study. 2018;1–7.
28. Olaleye AO, Babah OA, Osuagwu CS, Ogunola FT, Afolabi BB. European Journal of Obstetrics & Gynecology and Reproductive Biology Sexually transmitted
31. Library C. Simultaneous prevention of unintended pregnancy and STIs : a
32. challenging compromise. 2014;20(6):952–63.
- infections in pregnancy – An update on Chlamydia trachomatis and Neisseria gonorrhoeae. Eur J Obstet Gynecol [Internet]. 2020;255:1–12. Available from: <https://doi.org/10.1016/j.ejogrb.2020.10.002>
29. Garcia R, Ana T, Neves L, Grazielle M, Montes C, Jácome J, et al. Syphilis in Pregnancy : The Reality in a Public Hospital Sí fi lis na gestação : a realidade em um hospital público. 2019;90–6.
30. Castro J, Machado D, Cerca N. Unveiling the role of Gardnerella vaginalis in polymicrobial Bacterial Vaginosis bio fi lms : the impact of other vaginal pathogens living as neighbors. ISME J [Internet]. 2019;24–6. Available from: <http://dx.doi.org/10.1038/s41396-018-0337-0>