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Original Research Article

Microbiology for Medical care

Microbiological Study Of Vaginosis And Predisposing Factors In Women In Kalamoon Region, Syria

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Abstract: Vaginal infection is one of the diseases of female lower genital tract, which is caused by various types of microorganisms such as bacteria, fungi and parasites. Our study aims to investigate the prevalence of vaginal infections in Kalamoon region, Syria. (vaginal swabs) were randomly collected from 46 women (18 pregnant and 28non-pregnant) aged 18 - 50 years. Specimens were obtained from the Medical City of Kalamoon University and al-Nabek Hospital between the period of February 2017 and July 2017. Five vaginal swabs were taken from each woman after using speculum. Microorganisms were detected by cultivation and biochemical tests.. Results showed that pregnant women had higher infection rate than non-pregnant ones. It is noted that the highest prevalence was of bacteria (76.75%) than fungi (23.25%) with the absence of Trichomonas vaginalis and Gardnerella vaginalis. This was proved by applying Gimsa test and direct microscopic test. Higher rates of infection were noticed in the age group of 36-50. The isolated microorganisms were gram-negative streptococci (11.62%), gram-positive streptostreptococci (97.67%), gram-negative bacilli (32.55%), gram-positive bacilli (39.53 %) and fungi (23.25%). The isolated streptococci were 32.55% Streptostreptococci and 65.11% Staphylococcus, amongst them there were (35.71%) for Staphylococcus aureus. No more investigation was done for gram negative streptococci. The prevalence of Candida Albicans was 2.32% and 20.93% for candida non- albicans. We had noticed an increase in fungal infections with pregnancy. We also studied the referred to the relationship between isolated microbes and methods of contraception; also we had determined microbes that were isolated in conjunction to the color of vaginal secretions. The relationship between each of bacterial infections and symptoms with presence of WBCs were also separately studied.

Keywords: Genital Tract, Vaginosis, bacteria, fungi, parasites, staphylococcus aereus.

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1. INTRODUCTION

The Female Genital Tract (FGT) anatomically is divided into internal and external genitalia. The internal FGT is divided into three compartments: the lower genital tract (vagina and ecto-cervix), the endo-cervix and the upper genital tract (Endometrium and Fallopian tubes), fig 1.[1] The lower FGT is accordingly defined by the non-sterile areas. The vagina plays a broad range of roles: it receives the erect penis during sexual intercourse, it transports uterine secretions and menstrual flow and it provides a passage for delivery of a fetus during birth [2].

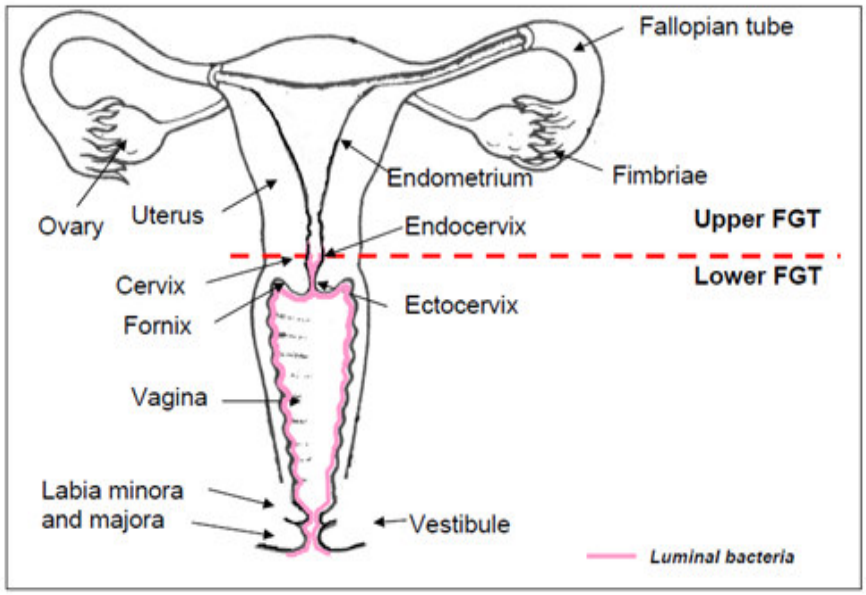

Fig 1. Organs and the area of microbial colonization of the FGT[3]

The vaginal epithelium consists of four layers: basal layer, para-basal layer, intermediate layer and superficial layer. The superficial layer has variable thickness of squamous cells containing large amounts of glycogen, which is necessary for the production of lactic acid by resident bacteria [4]. The vaginal secretion is a mixture of several components, including ions (Na^+, $Ca2^+$, Cl^-), proteins/peptides, glycoproteins, lactic acid, acetic acid, glycerol, urea and glycogen. *Lactobacillus*, which are Gram-positive bacilli, present in the normal biota of healthy women and absent in most women with bacterial vaginosis[5]. These bacteria degrade glycogen and create an acidic environment, which restricts the growth of pathogenic microorganisms. The normal pH in the vagina ranges from 3.5 to 4.5 with a typical value of 4.2. *Lactobacilli* have been shown to interfere with the colonization of pathogenic bacteria by blocking attachment to the vaginal epithelium [6]. Endogenous and exogenous factors, including menses, douching, use of contraceptives, vaginal medication use, number of sexual partners, frequency of intercourse, methods of menstrual protection have been suggested to be associated with shifts in the vaginal microbiota[7]. Lower genital tract complaints, such as abnormal discharge, odour, vaginal itching and vaginal burning, among women may be the result of bacterial vaginosis (BV), vulvovaginal candidiasis, trichomoniasis, gonorrhea and chlamydia infections [8]. Symptoms of discharge and itching were shown to be more frequent due to BV than *Candida vaginitis*, while vaginal burning was equally common among the two infections. Furthermore, in a quite high frequency (20-^{3}4%), infections cannot be identified by the symptoms given above [9]. Viral diseases of the reproductive system are difficult to treat, and so they represent an increasing health problem[10]. A much-publicized STI is genital herpes, usually caused by H*erpes simplex virus* type 2 *(HSV-2)*. Less effective, Warts are an infectious disease, known as papillomaviruses. *Trichomonas vaginalis* is anaerobic protozoan; it is transmitted sexually and, in addition, it causes trichomoniasis [11]. Our aim is to investigate the prevalence of vaginal infections in women in Kalamoon region , to isolate and identify infection causing lower female genital tract (Fungi, Bacteria and Trichomonas vaginalis) , to compare isolation rate using direct microscopic examination and culture examination , to study the relation between vaginal infections pregnancy and contraception methods ,to determine the percentage of vaginal infections associated with symptoms, presence of white blood cells in smears and to compare our study with other reported studies and reports.

2. MATERIALS AND METHODS

The study was conducted in The Medical City of Kalamoon University and Al-Nabek Hospital in the period of time between February 2017 and July 2017. Ethical Approval was taken as the hospital is a part of the university. We also had the patients consent for this study.

2.1 Collection of Samples

A sterile cotton swab was used for collecting vaginal secretions, after using a speculum. A total of 46 women (18pregnant + 28 non-pregnant) were clinically examined and five samples were taken from each patient. One of them was for Gram staining, another one was for Giemsa staining evaluation. The other three samples were inoculated on Blood Agar BA (general media for bacteria cultivation)(EMB(Eosin Methylene Blue Agar) (gram negative bacilli cultivation media) and on Sabouraud Dextrose Agar SDA (Fungi cultivation media), chabman agar (aureus cultivation media)subsequently. The patients were specified according to their ages to these sub-groups (18-25, 26-35,36-45,<50),

2.2 Direct Microscopic Examination

Specimens of vaginal secretion subjected for direct examination were done by placing two swabs on clean slides, the first swab for Gram stain and the second swab for Giemsa stain. Then the slides warmed gently (but not allowed over heat) and examined under the microscope for presence of any bacteria, fungi, red blood and white cells, clue cells and*Parasite* using 100X magnification

2.3 Culture growth of Specimens

Specimens intended for culturing were inoculated on Sabouraud Dextrose Agar (SDA), Blood Agar Base BA and EMB Agar. The SDA plate was inoculated at (25±1) ° C for 48-72 hours. The Blood Agar Base and EMB Agar were incubated at 37° C for 24 hours. The SDA agar is provided from Himedia Company (India), EMB from Biomark Laboratories (india), BA from SISCO research laboratories, India. Microscope identification of the positive cultures were made. The following criteria were taken into consideration in the identification

1. Colony morphology (color, smell and hemolysis).
2. Microscopic characteristic (bacteria: size, shape and arrangement; fungus: shape and fungal buds). Lactophenol cotton blue was used for fungi.
3. Biochemical tests: Only Hydrogen Peroxide to distinguish between streptococcus and staphylococcus.
4. Germ tube test for identification Candida albicans.
5. Cultivation on Mannitol Salts Agar for identification Staphylococcus aureus.

2.4 Data Analysis

We used Microsoft Excel 2010 for charts and graphs for the percentages for each subdivided group.

3 STATISTICAL ANALYSIS

The statistical analysis software was SPSS. The Chi square plus Fisher's exact test were used for the relationship between infections and pregnancy case results. The relationship between pregnant or un-pregnant contraceptive methods and infections were studied and datas were resulted in figures below.

3.1 Relationship between infections and pregnancy case.

Table 1. Pregnancy: infections cross tabulation in Chi squred test

pregnancy : infections Cross tabulation							
			infections				
			2	3	1	pos	Total
pregnancy	1	Count	1	13	3	3	20
		Expected Count	1.3	10.9	3.0	4.8	20.0
		% within pregnancy	5.0%	65.0%	15.0%	15.0%	100.0%
		% within infections	33.3%	52.0%	42.9%	27.3%	43.5%
		% of Total	2.2%	28.3%	6.5%	6.5%	43.5%
	0	Count	2	12	4	8	26
		Expected Count	1.7	14.1	4.0	6.2	26.0
		% within pregnancy	7.7%	46.2%	15.4%	30.8%	100.0%
		% within infections	66.7%	48.0%	57.1%	72.7%	56.5%
		% of Total	4.3%	26.1%	8.7%	17.4%	56.5%
Total		Count	3	25	7	11	46
		Expected Count	3.0	25.0	7.0	11.0	46.0
		% within pregnancy	6.5%	54.3%	15.2%	23.9%	100.0%
		% within infections	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	6.5%	54.3%	15.2%	23.9%	100.0%

Table 2. Results of chi square test for the relationship between pregnancy and infections

Chi-Square Tests				
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	2.041[a]	3	0.564	0.587
Likelihood Ratio	2.097	3	0.553	0.587
Fisher's Exact Test	2.126			0.587
N of Valid Cases	46			

[a]5 cells (62.5%) have expected count less than 5. The minimum expected count is 1.30

Table 3. Symmetric measures for pregnancy infections chi square test

Symmetric Measures				
		Value	Approx. Sig.	Exact Sig.
Nominal by Nominal	Phi	0.211	0.564	0.587
	Cramer's V	0.211	0.564	0.587
N of Valid Cases		46		

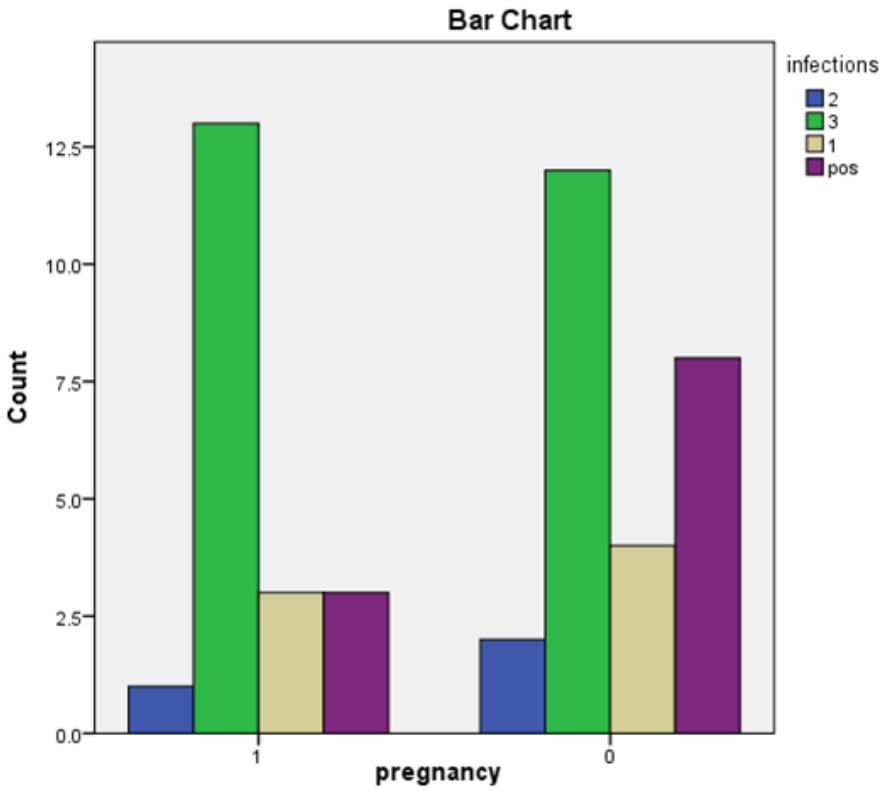

Fig 2. Bar chart for the relationship between pregnancy and infections

As can be read from the previous tables (1,2,3) chi square value is 2.041 and 6 cells count less than five. The asymp sig 0.564 is bigger than a value 0.05 . This shows us there is no statistically significant association between pregnancy and infections. From phi and cramer's V value 0.211 indicates that the strength of association between variables is week (Figure 2).

3.2 Relationship between pregnancy \ unpregnant (using contraceptive methods) and infections

Table 4. Contraceptive methods: infections cross tabulation in chi square test

Contraceptive methods infections Cross tabulation							
			infections				Total
			0	1	3	2	
Contraceptive methods	0	Count	0	7	1	7	15
		Expected Count	1.0	8.2	2.3	3.6	15.0
		% within contraceptive methods	0.0%	46.7%	6.7%	46.7%	100.0%
		% within infections	0.0%	28.0%	14.3%	63.6%	32.6%
		% of Total	0.0%	15.2%	2.2%	15.2%	32.6%
	2	Count	0	3	1	1	5
		Expected Count	.3	2.7	.8	1.2	5.0
		% within contraceptive methods	0.0%	60.0%	20.0%	20.0%	100.0%
		% within infections	0.0%	12.0%	14.3%	9.1%	10.9%
		% of Total	0.0%	6.5%	2.2%	2.2%	10.9%
	3	Count	2	2	2	0	6
		Expected Count	0.4	3.3	0.9	1.4	6.0
		% within contraceptive methods	33.3%	33.3%	33.3%	0.0%	100.0%
		% within infections	66.7%	8.0%	28.6%	0.0%	13.0%
		% of Total	4.3%	4.3%	4.3%	0.0%	13.0%
	1	Count	1	13	3	3	20
		Expected Count	1.3	10.9	3.0	4.8	20.0
		% within contraceptive methods	5.0%	65.0%	15.0%	15.0%	100.0%
		% within infections	33.3%	52.0%	42.9%	27.3%	43.5%
		% of Total	2.2%	28.3%	6.5%	6.5%	43.5%
Total		Count	3	25	7	11	46
		Expected Count	3.0	25.0	7.0	11.0	46.0
		% within contraceptive methods	6.5%	54.3%	15.2%	23.9%	100.0%
		% within infections	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	6.5%	54.3%	15.2%	23.9%	100.0%

Table 5. Results of chi square test for the relationship between contraceptive methods and infection				
Chi-Square Tests				
	Value	**df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**
Pearson Chi-Square	16.555[a]	9	0.056	0.052
Likelihood Ratio	15.304	9	0.083	0.123
Fisher's Exact Test	12.761			0.087
N of Valid Cases	46			

[a]14 cells (87.5%) have expected count less than 5. The minimum expected count is .33.

Table 6. Symmetric measures for chi sqaure test for contraceptive methods and infections relationship				
Symmetric Measures				
		Value	**Approx. Sig.**	**Exact Sig.**
Nominal by Nominal	Phi	.600	.056	.052
	Cramer's V	.346	.056	.052
N of Valid Cases		46		

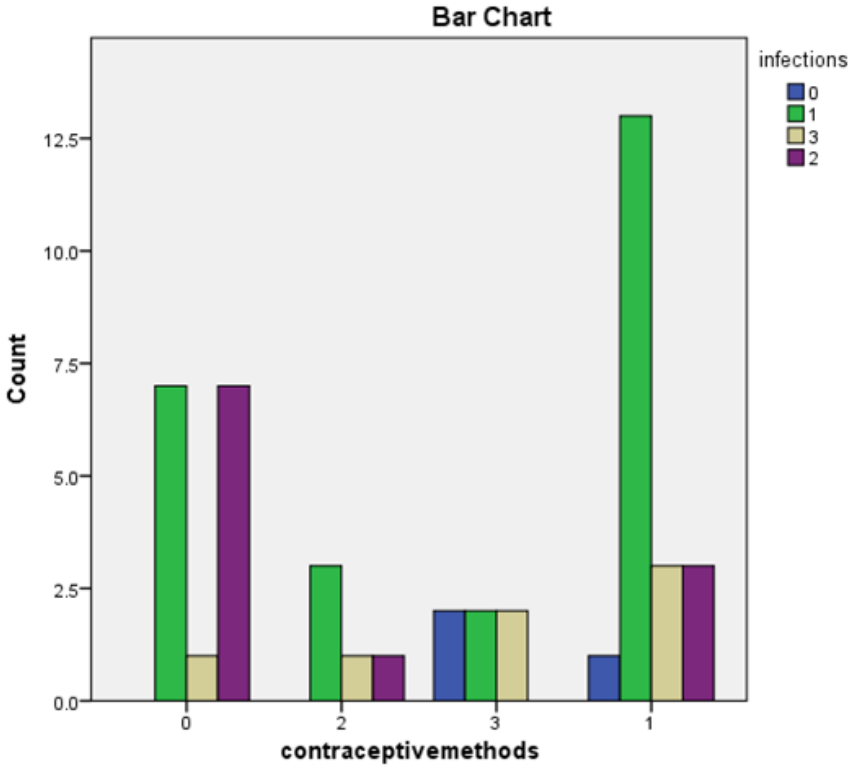

Fig 3. Bar chart for contraceptive methods and pregnancy relationship

From the above tables (4,5,6), chi square value is found to be 16.555 and 14 cells count less than five. The asymp sig 0.56 is less than a= 0.05. This indicates that there is no statistically significant association between contraceptive methods and infections. From phi and cramer's V value, 0.6 and 0.346 indicates that the strength of association between variables is strong (Figure 3).

4 RESULTS AND DISCUSSION

The investigation of the prevalence of vaginal infections in Kalamoon Region in Syria has not been described previous literatures and studies. In our study, among the 46 tested women, there were 26 infected pregnant women (88.23%), and 20 infected non-pregnant women (76.92%). Results agree with a study made in Iran (2009) [12], which demonstrated that pregnant women have a higher rate of infections (90,76%) than non-pregnant women (82.3%). However, our findings were higher than the percentage reported in Brazil (2014)[13]. The highest incidence of vaginal infections showed in age ranged between (36-50) years old, (As can be seen in fig 4). On the contrary, a study in Damascus, Syria (2007) [14],demonstrated that the highest vaginal infections were in age group <30.

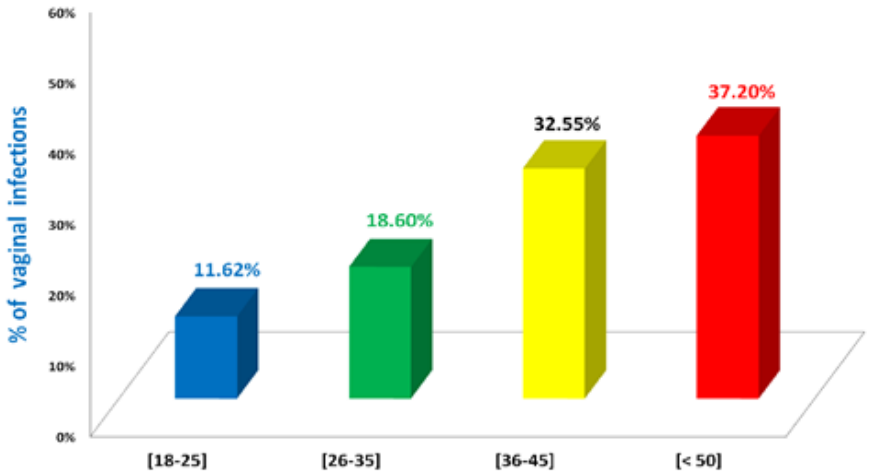

Fig 4. Percentages for vaginal infections according to age sub groups

The prevalence of bacterial infections was higher than fungal infections, and the accuracy of isolation from plates (culturing) was higher than smears for both bacterial and fungi (23.25% from culturing while 11.6% from smears for fungi). The incidence of fungi were isolated at Kalamoon region was (10%) C*andida albicans* and (90%) *C.*non-*albicans*. Our findings came in accordance with the study in Ghana (2013)[14-15], which demonstrated that the prevalence of *C. albicans* was 15.6% and 84.4% for *C.*non-*albicans*. While we disagree with the study in India (2014) [16] which found *C.albicans* in 56% cases and 44% for *C.*non-*albicans,* variant degree of prevalence rate among people of different communities may be seen due to certain factors such as hygiene behaviors, moisture and sociodemographic characteristics. The rate of fungal infections was higher in pregnant women (52.94%) than non- pregnant women (3.84%). A study In India (2014)[16], Vulvo Vaginal Candidosis VVC was 30 (10%) in pregnant women and 23 (7.7%) in non-pregnant women and this result agreed with our result. In the present study, the increased incidence of *Vaginal candidiasis* in pregnant women may be due to elevated levels of progesterone and estrogen. Progesterone has suppressive effects on the anti-*Candida* activity of neutrophils. Estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida*. The highest frequency of isolated bacteria in smears was for gram-positive bacilli (74.41%) while the lowest percentage was for gram-negative streptococci (6.97%), whereas, by inoculation, the highest percentage was for gram-positive streptococci (97.67%) and the lowest percentage was for gram-negative streptococci (11.62%). In our study, the highest isolation rate for gram-negative bacilli was for Enterobacter(42.85%), *Klebsiella* (28.75%), *Escherichia coli* (21.42%) and *Proteus*(7.14%) discerningly as shown in fig 5. A study performed in Nigeria (2008) showed that (8.3% *Escherichia coli* was isolated, whereas 50% for *Enterobacter* and 10% for *Proteus*.[17] By comparing our results, we found that *Enterobacterium* had the highest rate in both studies, but different frequency was obtained for other mentioned gram negative bacilli. In a related study in Iraq (2011) [18], the results were (2%) for *E. coli*, 4.6% for *Klebsiella*, 3.3% for *Proteus. However,* these results were in disagreement to our results for isolation percentage.

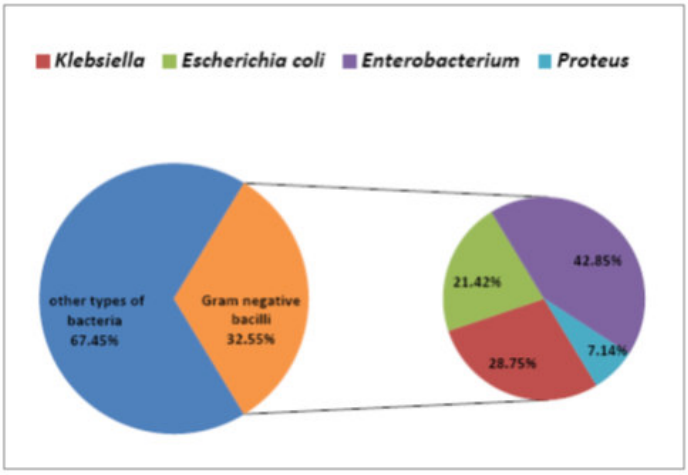

Fig 5. Types of Gram-negative bacilli

The isolation rate of *Staphylostreptococci* was 65.11% and it was higher than *Streptostreptococci* (35.71%). According to the type of hemolysis it was found that β-hemolytic has the highest percentage in both *Staphylostreptococci* and *Streptostreptococci*. A study in Iraq (2010)[19] showed β-hemolytic *Streptococcus* (11.3%) and *Staphylococcus aureus* (6.3 %) while a study in Nigeria (2008) [20] showed *Staphylococcus aureus* (3.6%), β-hemolytic *Streptococcus* (1.2%). We noticed that the percentage of *S. aureus* was less than other species and the higher frequency of hemolysis was the type β hemolysis for both *Staphylostreptococci* and *Streptostreptococci* and these results were really different from the previous results of other studies. Our study showed that (53.33% of women with bacterial infections used IUDs while 16.66% of

women had fungal infections and have used oral contraceptives. A study in Iran (2009) included 500 non-pregnant women showed that 16.2 % had bacterial vaginosis (BV)[12] and they preferred to use IUDs, 4.8% to Vulvovaginal candidiasis (VVC). They also have used oral contraception. Another study in Syria (2007) showed that a percentage of women who used oral contraceptives (4.2%) had fungal infections. [14] Our study showed high percentage of bacterial infections in women who used IUDs but a low percentage for women with fungal infections who used oral contraceptives. We conclude that the result from the previous studies' differ from our study. Our results showed that 32.55% of women had WBCs in their smears whereas 67.75% without WBCs in their smears. A related study was also done in Syria (2004) showed contrasting results. The percentages were 50.71% for women who had WBCs in their smears and 49.29% for the opposite case. [21] Our study also showed that 32.55% of women had WBCs in their smears and all of them were infected with bacteria. On the other hand, 51.16% of the women had symptoms of infections. Among these women, there were 31.81% women who had WBCs (fig 6). In the previous study which was performed in Syria (2004) 50.71% women had WBC among them 85.84% had symptoms and 27.35 % had WBC with fungal infections. [21] Our study showed that all women were infected by bacteria and had WBCs in their smears while other studies showed a weak relationship between incidence of WBCs and fungal infections instead of bacterial infections. For symptoms, in Syria (2004),[21] the majority of women had symptoms in conjunction with WBCs but we suspect on that as we had a minor percentage of this conjunction.

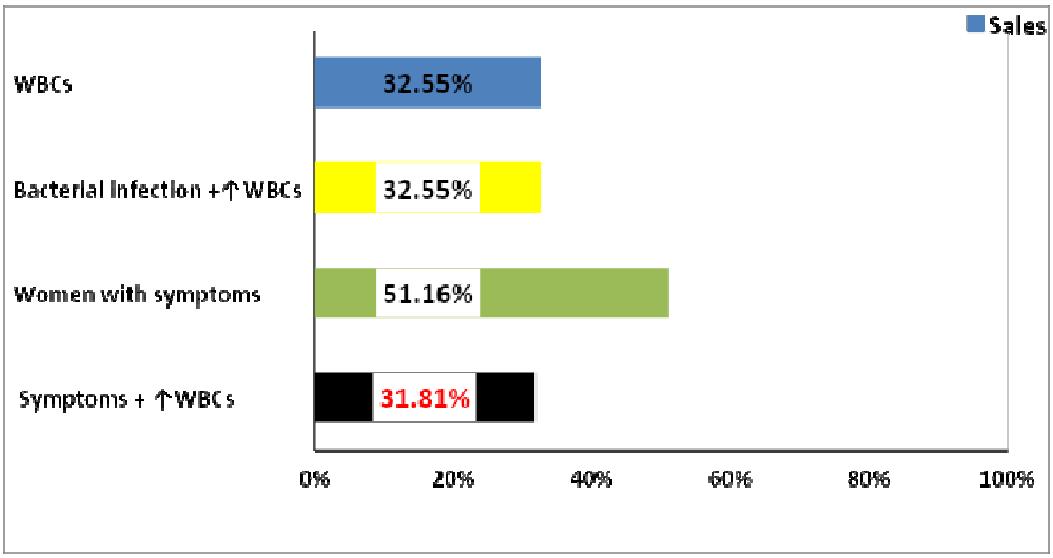

Fig 6. Relationship between both bacteria and symptoms with WBCs.

The vaginal secretions were white, yellow and green in colour 70 (81.39%), 10 (11.64%), 6 (6,97%) respectively. The isolation rate of fungi for the 70 (81.39%) women with white vaginal secretions was 20 (28.57%), *Streptococcus* 26 (37.14%), and *Staphylococcus* 42 (60%). The highest isolation rate for 10 (11.62%) women with yellow vaginal secretions was *Staphylococcus* 10 (100%) and the lowest was for *Streptococcus* 4 (40%). On the other hand, the highest isolation rate for 6 (6.97%) women with green vaginal secretions was *Staphylococcus* 6 (100%) and *Streptococcus* 2 (33%) as it is shown in figure 7.

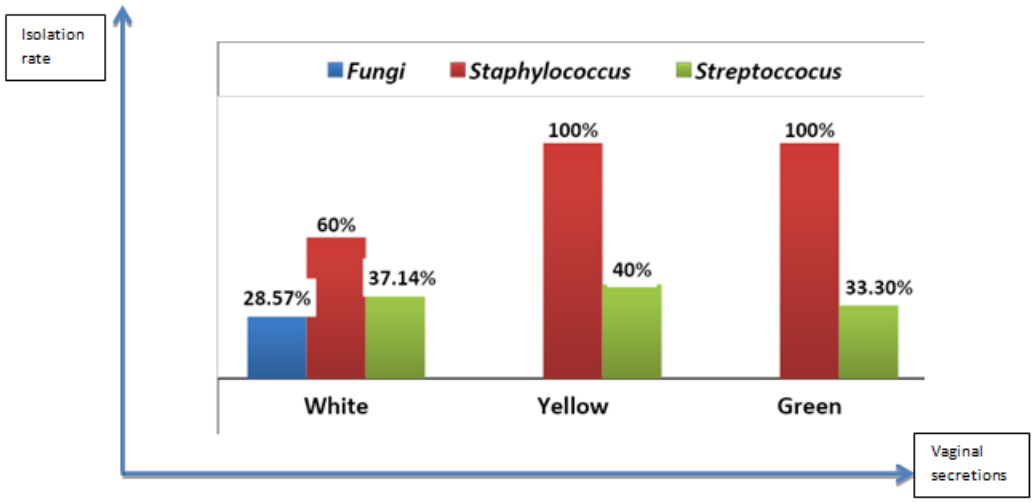

Fig 7. Chart of isolation rate and vaginal secretions color

In our study, direct examination by using Giemsa technique showed that no sample contains *Trichomonas vaginalis*. In other studies performed in Iran (2009)[12], Nigeria (2008) [17] and in (2014)[22] *Trichomonas vaginalis* had the percentage 6.6 % - 3.4% - 0.2% respectively. Other study in Syria showed that the percentage of *Trichomonas vaginalis* was 2%. For clue cells, the percentage was 0% in our study as their presences refer to infection by *Gardnerella vaginalis*. Many studies reported

the prevalence of *Gardnerella vaginalis* as in Iran (2014)[22 21], *it* was 0.6% and in Iraq (2010) [19]it was 57.9%.The different results may be due to the larger number of collected samples and sociodemographic characteristics.

5 CONCLUSION

This study has identified, for the first time, vaginal infections in the women population in Kalamoon region and it has indicated an influence of many factors on prevalence and species of infections in these women.

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8 AUTHOR CONTRIBUTION STATEMENT

Dr. Sundus Yaseen : designed and conducted the study. Dr. Maissa Abd Alkareem: designed and conducted the study. Dr. Farah Yousefcoordinated, edited, and rectified the manuscript for publishing issues as corresponding author. She also has done the statistical studies.

9 CONFLICT OF INTEREST

Conflict of interest declared none

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