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Correlation between Vaginal Yeast Infection and *Interleukin -10* gene Polymorphism in Women within Mosul City

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Keywords: *Candidas spp*, vaginal yeast infection, Interleukin-10.

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Abstract:

Vaginal candidiasis is a common condition among women due to genetic and environmental factors, which are associated with the causative agents of Candida species sp, leading to physical symptoms in affected women. Sixty-five samples (45 samples from women with vaginal candidiasis and 20 samples from healthy women) were collected from vaginal swabs and blood samples. Microscopic diagnostic tests were performed to investigate and identify Candida species. Tetra-ARMS PCR was also conducted to determine the association of the interleukin-10 gene polymorphism with vaginal candidiasis in women from the city of Mosul, northern Iraq. Furthermore, genetic variation results were analyzed using five molecular tests. The microscopic examination results of vaginal swabs revealed four types of yeast: Candida albicans represented 67% of sample frequency, C. glabrata 21%, C. tropicalis 7%, while C. krusei represented 5% of sample frequency. Moreover, the results of Tetra-ARMS-PCR of blood samples from infected women showed a higher percentage of the wild CC genotype (70%) compared to the mutant TT genotype (12%), while the heterozygous CT genotype was (18%) compared to the healthy group (55%, 5%, 40% respectively). The frequency of the (C) allele in infected samples was high (80%) compared to the mutant (T) allele (20%), whereas

in the healthy group, the frequencies of the C and T alleles were (75% and 25% respectively). The Hardy–Weinberg equilibrium test showed an environmental factor effect and the disappearance of the genetic factor.

Keywords: *Candidas spp*, vaginal yeast infection, Interleukin-10.

الارتباط بين عدوى الخميرة المهبلية وتعدد اشكال جين Interleukin -10 لدى النساء في مدينة الموصل

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المستخلص:

يعدّ داء المبيضات المهبلي حالة شائعة بين النساء بسبب العوامل الوراثية والبيئية، والتي ترتبط بالعوامل المسببة لأنواع المبيضات، مما يؤدي إلى أعراض جسدية لدى النساء المصابات. أُخِذَت خمسة وستين عينة (٤٥ عينة من النساء المصابات داء المبيضات المهبلية و ٢٠ عينة من النساء السليمات) من مسحات مهبلية وعينات دم. أجريت الاختبارات التشخيصية المجهرية للتحقيق وتحديد أنواع المبيضات. كما تم إجراء تفاعل البوليميراز المتسلسل Tetra-ARMS لتحديد علاقة تعدد أشكال جين Interleukin-١٠ مع داء المبيضات المهبلية للنساء في مدينة الموصل، شمال العراق. علاوة على ذلك، تم تحليل نتائج التباين الجيني باستخدام خمسة اختبارات جزيئية. أظهرت نتائج الفحص المجهري للمسحات المهبلية وجود أربعة أنواع من الخميرة؛ مثلت المبيضة البيضاء نسبة تكرار العينة ٦٧٪، و*C.glabrata* نسبة تكرار العينة ٢١٪، و*C.tropicalis* نسبة تكرار العينة ٧٪، بينما مثل *C.Krusei* نسبة تكرار العينة ٥٪. فضلا عن ذلك، أظهرت نتائج تفاعل البوليميراز المتسلسل رباعي التكافؤ (Tetra-ARMS-PCR) لعينات الدم المصابة نسبة أعلى من النمط الجيني CC البري (٧٠٪) مقارنةً بالنمط الجيني المتحور TT (12%)، بينما كانت نسبة النمط الجيني المتغايرCT(١٨٪)مقارنةً بالمجموعة السليمة (٥٥، ٥، ٤٠٪) على التوالي. كان تكرار الأليلات (C) في العينات المصابة مرتفعًا (٨٠٪) مقارنةً بالأليل المتحور (T) (20%)، بينما في المجموعة السليمة كان تكرار الأليلين C وT(٧٥٪, 25%) على التوالي. أظهر اختبار توازن هاردي-وينبرغ تأثير العامل البيئي واختفاء العامل الوراثي. تعدُّ فطريات المبيضات السبب الرئيسي لعدوى الخميرة المهبلية لدى النساء. إضافةً إلى ذلك، لوحظ تأثير العامل البيئي، كما لوحظ اختفاء التأثير الجيني لجين الإنترلوكين-١٠.

الكلمات المفتاحية: أنواع المبيضات، داء المبيضات المهبلية، الانترلوكين-١٠

1. Introduction:

The abnormal growth of the female genital system's mucous membrane is a hallmark of vaginal yeast infection which results in the infection of the vagina with *Candida* sp. [1]. As it is able to inflict the disease, it possesses the relevant enzymes and metabolic systems that enable it to survive in the changing temperature of the host according to the epidemic and to overcome its defense mechanisms [2]. As *Candida* sp. has virulence factors that help it to stabilize and inflict the disease and confronting the host's defense mechanisms, including the morphological change and the formation of the biofilm, secreting the external enzymes and fungal toxins, the damage of the epithelial cells, resisting the phagocytosis and evading the immune cells and all this contribute to the development of the disease, the persistence of the epidemical factors and defeating the defense mechanisms of the host [3]. Moreover, depending on the species, the infection kind, and the host's response [4]. The most typical symptoms include intense itching in the vagina or around the vulva, thick, white discharge that resembles cottage cheese and is frequently odorless, burning or irritation when urinating or having sex, redness and swelling in the vaginal or vulvar area, vaginal pain or a pressure-like sensation, and in some extreme cases, skin cracking around the vagina [2].This is due to factors including long-term use of antibiotics, hormonal changes, a weak immune system, uncontrolled diabetes, tight or non-cotton underwear, and psychological stress [4]. Recent research has shown that the host's genetic variety is crucial in determining the severity of the invasive fungal infection and its susceptibility to infection, indicating that genetic variances play a crucial role in fungal infections .The gene *IL-10* is located on the ٣٢-٣١q١ chromosome and this gene encodes the cytokine protein, which adjusts the fungal infection through anti-inflammatory practices against the infection and it is produced mainly by the monocytes and to a lesser degree, it is produced by the lymphocytes. The cytokine has multiple effects in terms of immunological regulation, because it controls the expression of Th1 cytokines, MHC class 11 Antigens and macrophage costimulatory molecules. Additionally, it increases the persistence and frequency of B-cells. This cytokine also inhibits NF-Kappa B activity and helps to coordinate the JAK-STAT signal pathway [5,6]. As the occurrence of the mutation in the gene IL-10 makes it vulnerable to the infection with the *Candida sp*. due to the defect or the absence of immunity to the fungi [7]. In addition to that, the increase of the infection of with the opportunistic fungal diseases could be due to the multiple genetic morphologies that control the diseases and the

environmental and genetic factors as well [8]. Research objectives are: to investigate the prevalence of vaginal yeast infections among women in Mosul, Iraq, isolate and identify the Candida species causing the infection using microscopic examination ,study the relationship between interleukin-10 gene polymorphisms and vaginal yeast infections using Tetra-ARMS PCR ,analyze the genetic distribution of the different types (CC, CT, TT) and compare them between infected and healthy women, assessing the frequency of alleles (C and T) and determining their relationship to the risk of infection ,determining the influence of environmental and genetic factors on the spread of infection using the Hardy-Weinberg equilibrium test.

2. Materials and methods:

2.1. Specimen collection

Sixty-five specimens were collected from women at Al-Batoul Teaching Hospital in Mosul, Iraq, during the period from 22/1/2024 to 8/4/2024 after obtaining ethical approval from the College of Environmental Sciences, University of Mosul, under Protocol No. 62 dated 17/1/2024. (45) specimens of the specimens were taken from women with vaginal yeast infection (experimental group) and (20) who were infection from healthy women (control group) from vaginal swabs and blood, where (5) ml of each specimen was taken from venous blood and preserved in EDTA anticoagulant tubes. The ages of the women ranged between 20-50 years. [9].

2.2. Isolation and identification of fungi:

Vaginal swabs were cultured on Sabouraud Dextrose Agar and incubated at 37°C for 48 hours. Fungal colonies were examined microscopically using Lactophenol blue stain. Identification of *Candida species* was based on colony morphology and microscopic features [10,11].

2.3. Tetra ARMS-PCR:

After extracting the DNA (blood) of 65 specimens utilizing the [12]. Modified technique (Allele-specific amplification is a straightforward and effective technique for detecting single nucleotide polymorphisms SNPs), four microliters of DNA and one microliter of each IL-10 gene primer, supplied by Macrogen Company were added, as displayed in Table 1[13].

Table 1: Primers that were utilized to determine genetic variation in the *interleukin-10* gene using PCR technology

Primer	Sequence	Band size	Annealing
F-outer	5- CCTCTTACCTATCCCTACTTCCACC -3	639 bp	61
R-outer	5- GACAACACTACTAAGGCTTCTTTGGTAA -3		
F-inner	5- GTAGTCTGCACTTGCTGAAAGCTT -3	393 bp	
R-inner	5- TCAGTGTTCCTCCCAGTTACAGTC -3	299 bp	

Table 2, the primers were added to the Master mix's contents before reaction tubes were put within the thermocycler to start reaction chain. utilizing an electrophoresis device, specimens were put onto 2% agarose gels, Red Safe dye was added, and DNA bands were then viewed under a UV lamp **[14].**

Table 2: the approved program in PCR

Number	The stage	The temperature	Duration	Number of cycles
1	First denaturation	95	5 min.	1
2	Denaturation	95	45 sec.	35
3	Annealing	61	1 min.	
4	Extension	72	1 min.	
5	Final extension	72	7 min.	1
6	Stop reaction	4	3 min.	1

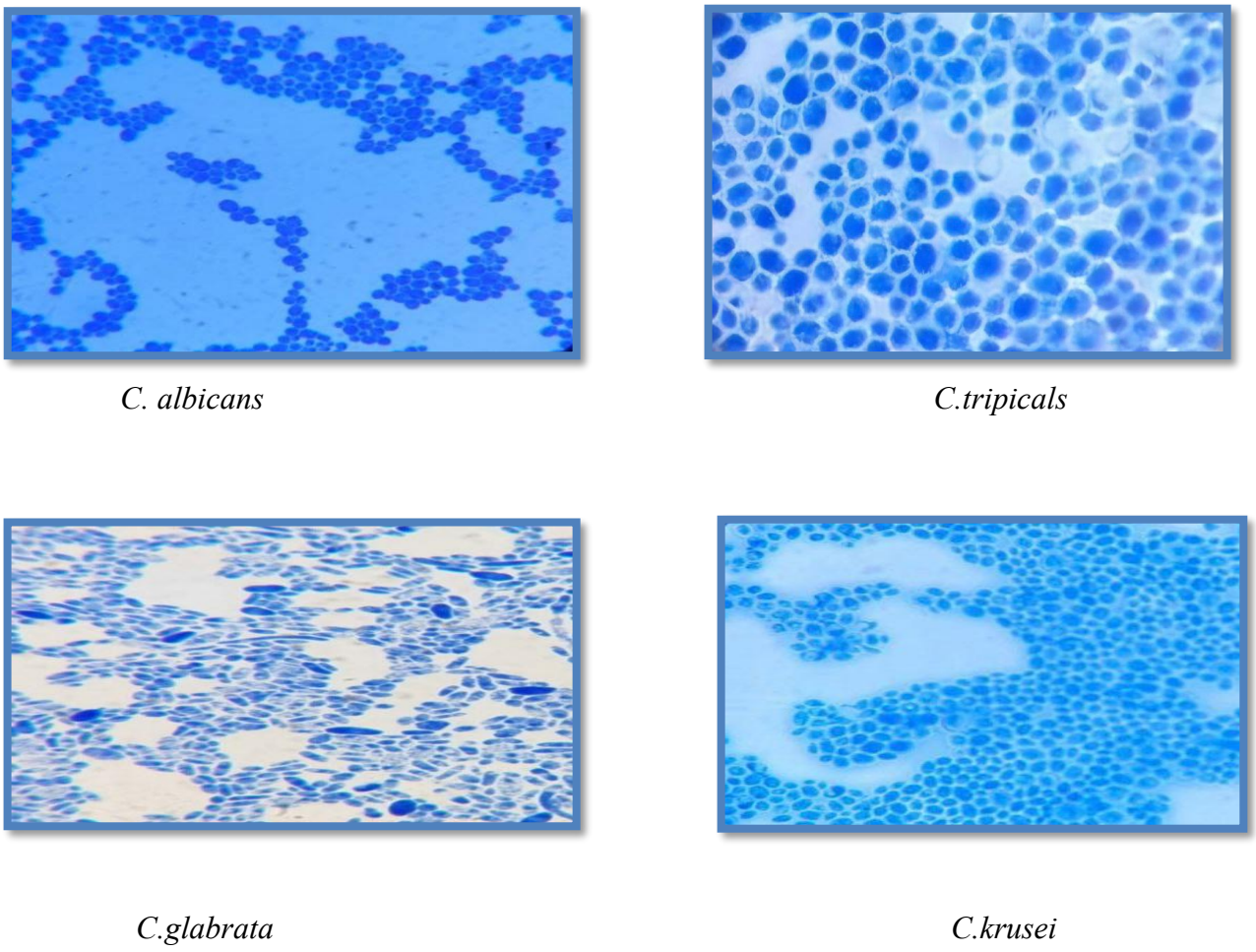
2.4. Analysis of statistics

The statistical analysis was conducted using the T-test (SPSS), and the acceptable level of statistical distinction was (P 0.05>). Additionally, the Cl, P value, and Odds Ratio were computed.

3. Results:

3.1. Isolation and Identification of Candida

The results of the clinical specimens collected from the female genital system indicate that four types of *Candida* sp. were identified depending on the diagnostic characteristics in cultures and by the microscope as shown in the Figures 1-2. The percentage of C.*albicans* was the frequency (67%) then C.*glabrata* the frequency (21%), *C. tropical* the frequency (7%) and finally *C.krusei* the frequency (5%) .



C. albicans *C.tripicals*

C.glabrata *C.krusei*

Figure 1: *Candida spp* under a microscope using lactophenol blue under 100X.

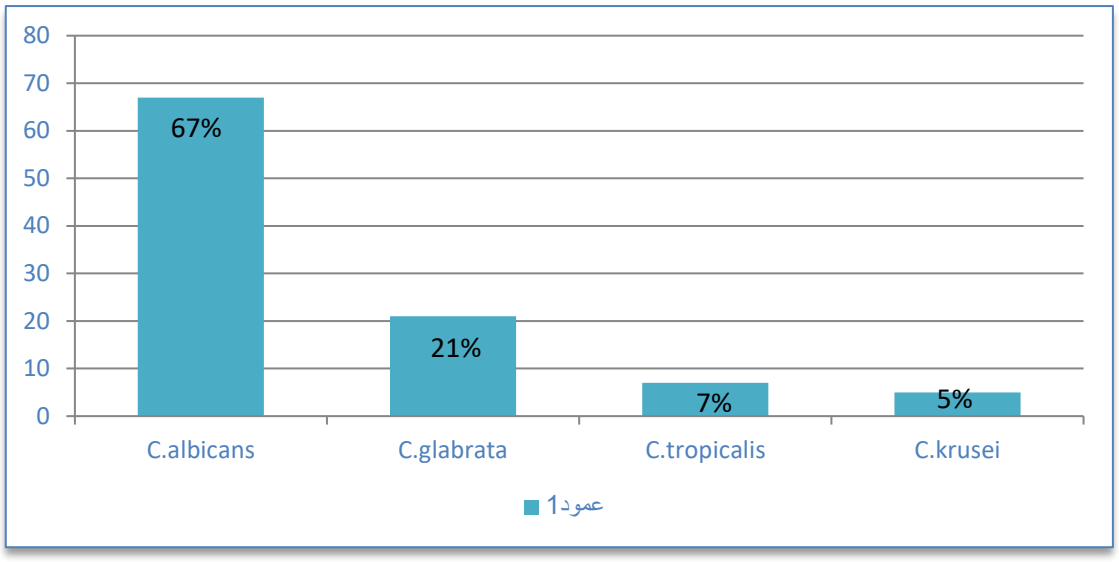


Figure 2: the percentages % of each type of *Candida spp*.

3.2. Results of Analysis of the genetic Contrast of the *Interleukin -10* and determining its relationship with the events of vaginal yeast infection

Five molecular tests were used to analyze the genetic variation

3.2.1. Identifying the proportion of observations of the different genotypes

The results of the TARMA PCR, demonstrated in Figure 3, showed that there is not a relationship between the patients who suffer from the frequent vaginal infections and the IL-10 gene's genetic variation.

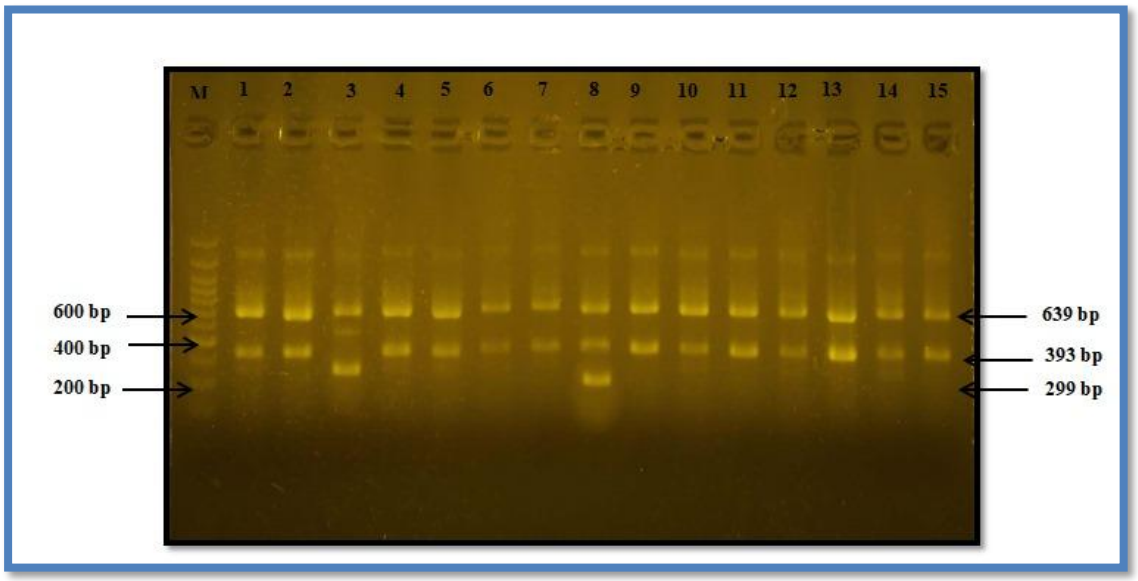


Figure 3: The product of the TARMS-PCR reaction for the genetic variation of the *IL-10* gene

From the PCR results, it was clear that there were three different genetic patterns: TT, CC and CT and the normal pattern is CC (٦٣٩) bp and the hetero genotype (393) bp CT, while the mutant genotype (299) bp and (100) bp Marker the volume index supplied by Biolabs Company and it was isolated by the agarose gel with a concentration of 2. Also, Table 3 shows that the allele observation and the frequency of the various genetic patterns of IL-10 were evident in the women infected with the vaginal yeast infection and the frequency of the pattern (wild genotype) CC was (70%) compared to the (mutant genotype) TT (12%). As for the (hetero genotype) CT the percentage was (18%), which is low in contrast to the control group, which had the value of (55%) for the typical genotype, (40%) for the hetero genotype and (5%) for the genotype of the mutation. Also, the OR value of the genotype was (1.71) and so, it is higher than (1.0) at a likelihood of p (0.05)

Table 3: Distribution Interleukin -10 gene alleles and genotypes as a percentage

SNP1	**Sick women**		**Healthy women**		**P-value**	**Odds ratio**	**Confidence interval 95%**
	NO.	%	NO.	%			
CC	32	70	11	٥٥	P = 0.6376	1.7188	0.1805 to 16.3676
CT	8	18	8	٤٠			
TT	5	12	1	٥			

3.2.2. Determining the allele frequency and the observation level

The research current results, for the women infected with the vaginal yeast infection, showed a frequency of the normal allele C (80%), which is considered high compared to the mutant allele T (20%). Regarding the control group, the normal allele's frequency was (75%) and for the mutant allele was (25%) as displayed in Table 4.

Table 4: the allelic frequency ratio

Allele	Sick women		Healthy women		P-value	Odds ratio	Confidence interval 95%
	No	%	No	%			
C	36	80	15	75	P = 0.5229	0.7500	0.3103 to 1.8129
T	9	20	5	25			

3.2.3 Analyzing the genetic variation results by Hardy Weinberg equilibrium test

The distribution of the genetic patterns of the patients were dealt with using Hardy Weinberg equilibrium test, which is the third analysis of variation in question in order to identify the severity of cases according to the recommended equation. When the results were compared, as shown in Table 5, between the observation percentage obtained and the observation percentage of HWE equilibrium, the value obtained was: p value = 0.002869, which is lower than $p < 0.05$, and this denotes that there is a variation in the within the study groups and that it is not covered by the equilibrium law.

Table 5: shows the results of the HWE balance test

Genotype	CC	CT	TT
Genotype observed	32	٨	٥
Anticipated genotype	28.8	14.4	1.8
P value = 0.002869		Chi squared value X^2= 8.88	

Also, there was a variation in the recorded and the predicted genetic percentages and this indicates the effect of the environmental factor and the disappearance of the genetic factor in the cases studied.

3.2.4. Verify that the inheritance of this variation recessive in the study groups.

This indicates that the genetic variant apparent an apparent pattern as in the case of the normal genotype, as shown in Table 6.

Table 6: The study group's distribution of IL-10 polymorphisms using the recessive model

Genotype	Sick women	Healthy women	Odds ratio	Confidence interval	P-value
CT-CC	40 (88%)	19 (95%)	0.4211	0.0459 to 3.8591	P = 0.4441
TT	5 (12%)	1 (5%)			

3.2.5. Verify that the inheritance of this variation dominant in the study groups.

According to Table 7, this indicates that the variant genotype produces a protein similarly to the mutant genotype, and that this is dependent on the P value.

Table 7: IL-10 polymorphism distribution in the research group using the dominant model

Genotype	Sick women	Healthy women	Odds ratio	Confidence interval	P-value
CC	32 (75%)	9 (80%)	0.4965	0.1667 to 1.4792	0.2087
TT+CT	13 (25%)	11 (20%)			

When examining how the experimental group's and the control group's genotype percentages, when comparing the findings in the dominance distribution, it was evident that P value = 0.2087, which is lower than P > 0.05, differs from P value = 0.4441, which is greater than P > 0.05 in the recessive distribution test, i.e. the mutant genotype has a recessive effect on the disease case and its effect is not dominant .

4. Discussion:

A fungal infection of the vagina and vulva is called vaginal yeast infection, or vaginal candidiasis. It is brought on by an overabundance of a fungus known as *Candida spp*., which is found in the body naturally but in balanced amounts. The infection typically arises when the vagina's normal bacterial and fungal balance is upset, allowing the *Candida spp*. fungus to proliferate [15,16]. Its virulence factors, which include adhesion, the secretion of decomposition enzymes, and the ability to transform from yeast to hyphal forms the epidemic filamentous shape—allow it to invade mucus tissues, which is essential for the disease to develop and for the formation of biofilms on the host's surfaces. This is why the percentage of

Candida albicans was found to have increased significantly [17]. The study's findings concurred with those of multiple other investigations, which indicated that the infection with *C.albicans* was the highest in terms of spread when vaginal swaps were taken from women, who are infected with vaginal yeast infection [18,19]. It was also noted that *Candida krusei* decreased to 5%. These results were similar to the results of Ahmed and Khan's study [20], who attributed the reason for its decrease compared to other species to the fact that the vaginal environment is acidic and unsuitable for its growth.

So far, there are no studies that link SNPs IL-10 gene to the hazard of vaginal yeast infection. This was the first research between polymorphisms in the Interleukin-10 gene and vaginal yeast infection [21]. When a genetic mutation (SNP) occurs in the IL-10 gene, the level or function of this cytokine may change, potentially affecting the body's ability to control *Candida spp*. infections. Some mutations may result in increased production IL-10, which may suppress the natural immune response against fungi, increasing the risk of infection. The anti-inflammatory cytokine interleukin-10 (IL-10) is crucial for controlling the immune response, inhibiting excessive inflammatory responses [22, 23, 24]. This study found the absence of factors genetic as well as the influence of environmental factors, some of the most important environmental factors include consuming foods high in sugar, using antibiotics, disrupting the circadian rhythm, stress, and depression, which weaken the immune system and make it harder for the body to fight off fungi. Hormonal changes during pregnancy, or the use of hormonal contraceptives affect vaginal acidity and facilitate yeast growth [25,26 ,27]. despite the high genotype CC, TT in women with vaginal yeast infection (70%-12%) compared to healthy women (55%-5%). This demands conducting many studies, such as examining known SNPs (-1082, -819, -592) of the Interleukin-10 gene or measuring the level of Interleukin-10 in vaginal secretions and linking it to genes, analyzing genetic interaction with other genes such as (IL-4 and TLR-2).

5. Conclusions:

Women in Mosul frequently get vaginal yeast infections, which are probably brought on by a mix of environmental and hereditary causes. The results of the microscopic analysis indicated that the most prevalent species was *Candida albicans*, which was followed by *Candida glabrata* and then, to a lesser degree, *Candida tropicalis* and *Candida krusei*. Genetic variations in the Interleukin-10 gene are linked to heightened vulnerability to vaginal yeast infections, particularly in women with the CC genotype, according to tetra-ARMS PCR

data. Hardy-Weinberg equilibrium analysis demonstrated that environmental factors had an impact on infection prevalence, with a discernible decrease in the genetic role. This study highlights the importance of molecular analysis and genetic polymorphisms in understanding the mechanism of susceptibility to infection, clearing the path for the future creation of individualized preventative or treatment plans. To further understand the immune gene interaction, future research should examine other immune genes linked to vaginal yeast infection, examine the relationship between genotypes and treatment response, which could aid in the development of tailored treatment plans based on the genetic profile of the patient, examine the impact of behavioral and environmental factors (such as personal hygiene, nutrition, and antibiotic use) on the frequency and severity of Candida infections, and confirm the overlap between fungal infections and the vaginal microbiome using contemporary genetic sequencing technologies (e.g., NGS).

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