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**College of Education for Pure Sciences**  
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# **Bacterial Detection and Genetic Polymorphisms of Some Cytokines in A Sample of Iraqi Women with Bacterial Vaginosis**

**A Thesis**

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## 1.1 Introduction

Bacterial vaginosis is an environmental disparity of the vaginal micro biota the three most ordinary causes of vulvo vaginitis are bacterial vaginosis ,being the most prevalent one , tagalong by candidiasis and trichomoniasis (Ranjit, *et al.*,2018 ). Bacterial vaginosis is the most common vaginal infection in women of puberty and causes significant physical, psychological and social symptoms as well as many adverse reproductive health effects (Russo *et al.*,2019 ).

However, evidence from recent studies of the pathogenesis of BV suggests that this bacterium forms a biofilm in the vaginal epithelium that serves as a “scaffolding” to which other bacterial species adhere in a symbiotic fashion, colonizing the vagina, Though asymptomatic in at least half of affected women, thispolymicrobial condition can produce a thin, white, homogenous discharge with a distinct “fishy” odor. The changes in the vaginal flora seen in BV are associated with serious sequelae, such as preterm delivery, spontaneous abortion, postpartum endometritis, and increased susceptibility to HIV and other sexually transmitted infections(Hussien *et al.* ,2020)

Aerobic vaginitis (AV), a term coined in a hallmark by (Donders *et al.*,2003 )to emphasize its difference from BV, is characterized by inflam mation of the vaginal epithelium and also characterized by abnormal vaginal microflora containing aerobic and enteric bacteria like *Escherichia. coli*, *Klebsiella .spp.*, *Acinetobacter spp.*, *Staphylococcus spp.*, *Enterococcus spp.*, and group B *Streptococcus* (GBS). However, both AV and BV are vaginal dysbioses characterized by the reduction in lactobacilli (Son *et al.* ,2018)

Studies show that the proportion tends to increase significantly in sub-Saharan Africa and decreases in eastern and western Europe (Lakshmi *et al.*,2013) The phases of response to both innate and adaptive immunity and physiological defense mechanisms that target foreign microorganisms such as virus or bacteria, are regulated by peptide molecules known as cytokines. Many of these cytokines are synthesized by white blood cells in the bloodstream, such as monocytes, neutrophils, and Eosinophils and basophils (Mitchell,*et al.* ,2015).

The altered levels of some pro-inflammatory cytokines were found in females with bacterial vaginosis (Beigi, *et al.*, 2007). Several genes that encode different cytokines may play critical roles in host susceptibility to brucellosis, as the proportion of cytokine production varies between individuals and depending on the genetic polymorphism of the cytokine (Budaka *et al.*, 2007). Several studies have found that, modifying the reproductive health risks associated with bacterial vaginosis due to the Presence of genetic polymorphisms in the genes associated with the inflammatory response, in females heterozygous for the allele of the IL1ra and IL1 $\beta$  gene was observed, its levels were low, but in females homozygous for the wild-type allele, high levels were observed . Whereas, women carrying the TLR4 polymorphism were associated with a lower response to LPS when colonized with bacterial species associated with BV, and with 10-fold higher amounts of *Gardenrella vaginalis* colonization compared to women without the polymorphic allele. Note that women with IL1 $\beta$  and IL8 genetic polymorphisms accompanied by an increase in cytokine response led to a lower prevalence of BV, while women with IL6 gene polymorphisms associated with a lower response had a higher prevalence of BV ( Ryckman, *et al.*, 2009). One study found the localized cytokines IL-2, IL-12, IL-6, TNF- $\alpha$ , and IFN- $\gamma$  to represent the T helper type 1 (Th1) lymphocyte response that regulates toxic T lymphocytes and is responsible for clearing inflammation. Macrophages in combination with soluble antimicrobial agents in the reproductive tract In addition, the presence of interleukin IL-10 whose function is anti-inflammatory and IL-10 is a general regulatory cytokine. With bacterial vaginosis or HIV (Campos, *et al.*, 2012). Several genetic polymorphisms have been identified, particularly a single nucleotide polymorphism (SNP) in cytokines that serves as a DNA sequence biomarker, helping to identify disease-related genes . Therefore, this study came to investigate some local cytokines (IL-13, IL-5, IL-7, IL-9, IL-3) and their relationship to bacterial vaginosis disease.

الطراز الوراثي / الاليل C/CC قد اظهر نسبة عالية من (2.30, 2.20) على التوالي مشيرا الى انه يشكل عامل خطورة (RRs) لمرض (BV) بينما الطراز الوراثي / الاليل TT/T فقد اظهر نسبة منخفضة من (OR) (0.30, 0.45) على التوالي مشيرا الى انه يعتبر عامل وقائي PFS بالنسبة لمرضى BV ولا يوجد هناك فروق معنوية توزيع عينات المرضى والسيطرة بالنسبة لل (IL-3rs40401).

بالإضافة الى (IL-3 rs31480) اظهر فيه الطراز الوراثي / الاليل CC/T نسبة عالية من (OR) (1.09, 1.07) على التوالي مشيرا الى كونه عامل خطورة بالنسبة لمرض BV بينما كان الطراز الوراثي / الاليل TT/C و CT يظهر نسبة منخفضة من (OR) (0.56, 0.77) و 0.93 على التوالي مشيرا الى كونه عامل وقائي بالنسبة لمرض BV ولا توجد فروق معنوية في التوزيع بين عينات المرض والسيطرة.

النتائج أظهرت ان تحليل تسلسل الجينات لل IL-5 كان في 31 عينة مرضى و 12 عينة سيطرة تم تأكيد التشخيص بواسطة (NCBI)، بالإضافة الى انه (IL-5 rs 2096812) قد أظهر التغيرات الجينية الى انه الطراز الوراثي / الاليل T/TT كان ذو نسبة عالية من (OR) (2.64, 1.54) على التوالي مشيرا الى انه يشكل عامل خطورة (RRs) بالنسبة للمرض BV بينما كان الطراز الوراثي / الاليل CC/C والطراز TC يظهر نسبة منخفضة من (OR) (0.67, 0.94) و 0.65 مشيرا الى انه يشكل عامل وقائي PFs لمرض BV، ولم توجد هناك فروق معنوية في التوزيع بين عينات المرض والسيطرة.

النتائج أوضحت ان تحليل تسلسل الجينات للانترلوكين 7 كان في 45 عينة مرضى و 18 عينة سيطرة، وقد تم تأكيد التسلسل المضخم بواسطة (NBCI) بالإضافة الى ان التغيرات في (IL-7 rs9643739) أشار الى ان الطراز الوراثي الاليل CC/C والطراز الوراثي CT قد اظهر نسبة عالية من (OR) (2.13, 2.27) و 2.18 على التوالي مشيراً الى انه يشكل عامل خطورة (RRs) بالنسبة لمرضى BV اما الطراز الوراثي TT/T فقد اظهر نسبة منخفضة من (OR) (0.37, 0.46) على التوالي مشيراً الى انه يكون عامل وقائي من مرض BV ولا توجد فروق معنوية في التوزيع بين عينات المرضى والسيطرة.

النتائج كشفت انه تحليل تسلسل الجينات كان في 49 عينة للمرضى و 18 عينة سيطرة، وتم تأكيد التسلسلات المضخمة بواسطة NBCI بالإضافة الى انه اظهر (IL-9 rs 13137072) ان الطراز الوراثي / الاليل CA/A اظهر نسبة عالية من (OR) (1.39, 1.49) على التوالي مشيرا الى انه يمثل عامل خطورة RRs بالنسبة للمرض BV اما الطراز الوراثي CC/C فقد اظهر نسبة منخفضة من (OR) (0.66, 0.67) على التوالي مشيرا الى انه يكون عامل وقائي (PFs) ولا توجد فروق معنوية في توزيع العينات بين المرضى والسيطرة.

بالإضافة الى ان (IL-9 rs (1799962) كشفت النتائج الى ان الطراز الوراثي / الاليل AC/C اظهر نسبة عالية من (OR) (1.49, 1.51) على التوالي مشيرا الى كونه عامل خطورة RRs بالنسبة لمرضى BV بينما الطراز

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## 1.2 Aims of study

Due to the prevalence of bacterial vaginosis among women in Iraqi society and the lack of studies on this disease, this study came to shed light on some immunological, genetic and pathological factors among Iraqi women. Such association study may help to understand the immunogenetic predisposition for the development of bacterial vaginosis, as well as their protective effects can also be determined.

- 1- Isolation and diagnosis of the common bacterium that causes bacterial vaginosis and comparing it with healthy controls.
- 2- genetic polymorphisms in in some cytokines (IL-3, IL-5, IL-7, IL-9 and IL-13) genes using the polymerase chain reaction (PCR) device and using the SNP technique.