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A GENETIC POLYMORPHISM OF INTERLEUKIN 9 GENE (RS1313970720) IN BACTERIAL VAGINITIS S OF IRAQI WOMEN PATIENTS

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ABSTRACT:

Bacterial vaginitis (BV) is the most prevalent alteration of vaginal microflora worldwide We aimed to investigated between the polymorphism of the single nucleotide of the gene interleukin-9(SNPrs1313970720) and BV in Iraqi women. Where samples were collected blood from Pregnant women patients with BV Genomic DNA was obtained from 75 BV positive patients, and 25 not Pregnant women as a control. Analysis of Hardy-Weinberg equilibrium (HWE) in BV patients and controls revealed that the *IL-9* genotypes were agree with the equilibrium, and no significant differences (p = 0.76, 0.90) were observed between the observed and expected genotype frequencies. We demonstrated that women who were heterozygous CA and A allele were risk for BV, with an OR =(1.39) and (1.49) respectively compared to controls. While homozygous CC and allele C were preventive factor for BV, with OR (91.8), (0.96) respectively compared to controls in the rs1313970720 .These data show that host genetic variants at the IL-9 SNP predispose to BV among Iraqi women. The results of the study indicate that IL-9 SNP showed associations (positive and negative) with BV patients and may be have a role in the mechanism of etiopathogenic of BV in the samples of Iraqi patients.

Keywords: Bacterial Vaginitis, IL-9 Gene Polymorphism.

I. INTRODUCTION

Bacterial vaginitis BV is the most widespread vaginal infection amidst women of sexual age and is correlated with significant physical and psychosocial soreness as well as several important adverse reproductive health Prevalence of BV is consistently high worldwide, but can vary by bgeographic location, socio-demographic factors, and health behaviors. Studies show that ratio tend to be highest in sub – Saharan Africa and lowest in East and western Europe. (Kenyon et al., 2013; Peebles et al., 2019). The physiological defense mechanisms targeting foreign microorganisms such as virus or bacteria, the drift phases of both innate and adaptive immunity are regulated by peptide molecules known as cytokines .many of these cytokines are crafted by population of leukocytes as well in the blood stream, including monocytes, neutrophils, or eosinophils. Evidence exists that demonstrates altered levels of certain pro inflammatory cytokines in women with bacterial vaginosis (Lisa et al., 2017).

In humans, several genes encoding different cytokines may play crucial roles in host susceptibility to brucellosis, since cytokine production capacity varies among individuals and depends on cytokine gene polymorphisms. In several studies, the reproductive health risks associated with BV are modified by the presence of genetic polymorphisms in genes associated with the inflammatory response, showed that in women heterozygous for an allele of the IL1ra gene and IL1β but women homozygous for the wild type allele showed increased levels. Women carrying a TLR4 polymorphism associated with lower response to LPS had no change in IL1β when colonized with BV-associated bacterial species, and 10-fold higher quantities of G. vaginalis colonization compared to women without the polymorphic allele. Therefore, theres relationship between vaginal pH and and to measure cytokine levels in endocervical secretions of women with BV or HIV (Campos et al., 2012). Many gene polymorphism, especially the cytokines, have been identified single nucleotide polymorphism (SNP) avariation at asingle nucleotide position in DNA sequence act as biological marker, helping in locating genes that

are conjucated with disease. So we decide to examine the localized cytokines (IL-9 because function is an inflammatory molecules and there is cooperation between that bacteria and increased cytokines Interleukin-9 (IL-9) is a pleiotropic cytokine whose gene is located on the long arm of chromosome 5 . Specific secretion of IL-9 is observed not only in T helper type 2 Th2cells but also in activated Th9 cells, Th17 cells, regulatory T cells, and mast cells, natural killer T cells, and dendritic cells. The IL-9 receptor consists of two subunits the alpha-chain (IL-9Rα), which is bound to Janus Kinase 1 (JAK1), and the common gamma chain (γc), which is bound to JAK3. When IL-9 binds to its receptor, activation of JAK1 and JAK3 kinases is induced, and as a result, signal transducer and activator of transcription 1 (STAT-1), STAT-3, STAT-5 pathways are triggered (Asao et al., 2001; Goswami, and Kaplan, 2011). It has been clarified that IL-9 plays an important role in different inflammation processes and autoimmune diseases (Karagiannis and Wilhelm., 2017). Interestingly, recent research has suggested that IL-9 can have both a protumorigenic and anti-tumorigenic role in the pathogenesis of neoplasia (Vargas., 2017). Gene expression of IL-9 is controlled by phosphorylated STAT molecules in the nucleus, where they bind to regulatory sequences and provide pleiotropic biological functions of IL-9 (Gerlach and Weigmann., 2019; Lee et al., 2020).

II. MATERIAL AND METHODS

Subjects

A total of 75 married pregnant patients, and 25 non-pregnant women as a control were included in this study, subjected for sampling which included high vaginal swabs for each woman. Clinical samples were collected from female patients admitted to the out-patient clinics of Gynecology and Obstetrics, in Al-Batoul Teaching Hospital\Baquba for Gynecology and pediatrics in Diyala Governorate during the period from December 2019 to June 2020. All woman were underwent-detailed history regarding age (the age of women ranged from 16 to 68 years old), symptoms of infection, jobe, abortion, house and unmarried women were excluded from sampling. The high vaginal swab was collected by a cotton swab collector from the posterior fornix after the vault of the vagina was exposed to a sterile non-lubricated vaginal speculum. The speculum may be moistened with warm water before use but antiseptics or gynecological exploration cream should not be used, since these may be lethal to gonococci. Amies and Stuart transport media are convenient for transport of cervical and vaginal samples (Jozef et al., 2003). From each woman, two swab samples were collected. The first swab was used for a direct wet-mount examination and, whiff test, as well as, Gram stain examination.

Detection of IL9 Polymorphism

Single nucleotide polymorphisms (SNPs) of 1 cytokine and cytokine receptor genes IL-9 were detected by PCR-SSP (polymerase chain reaction-sequence specific priming).Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood gDNA Miniprep System, Promega .The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was allowed to solidify at room temperature for 30 minutes. The comb was carefully removed, and the gel was placed in the gel electrophoresis tank. The tank was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel For PCR product, 5µl was directly loaded to well Electrical power was turned on at 100 volt/ 50mAmp for 60 min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system.

Statistical Analysis

PCR product were sent for Sanger sequencing using ABI3730XL, automated DNA sequences by Macrogen Corporation – Korea. The results were received by email then analyzed using geneious software. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com.

III. RESULT AND DISCUSSION

The current study indicated that electrophoresis of agarose gel for PCR amplified products (rs1313970720) of the *IL-9* gene were showed a single a band approximately of 810 bp molecular size, as Showed in- Figure.1.

The SNP rs1313970720 (C/A; Chromosome5) was presented with three genotypes (CC, CA and AA) and two alleles (C and A). HWE in BV patients and healthy individuals detected that the genotypes were concordant with the equilibrium and there are no obvious differences (p >0.7658-0.9035) were watched between the observation and expected genotype frequencies (Table 1).

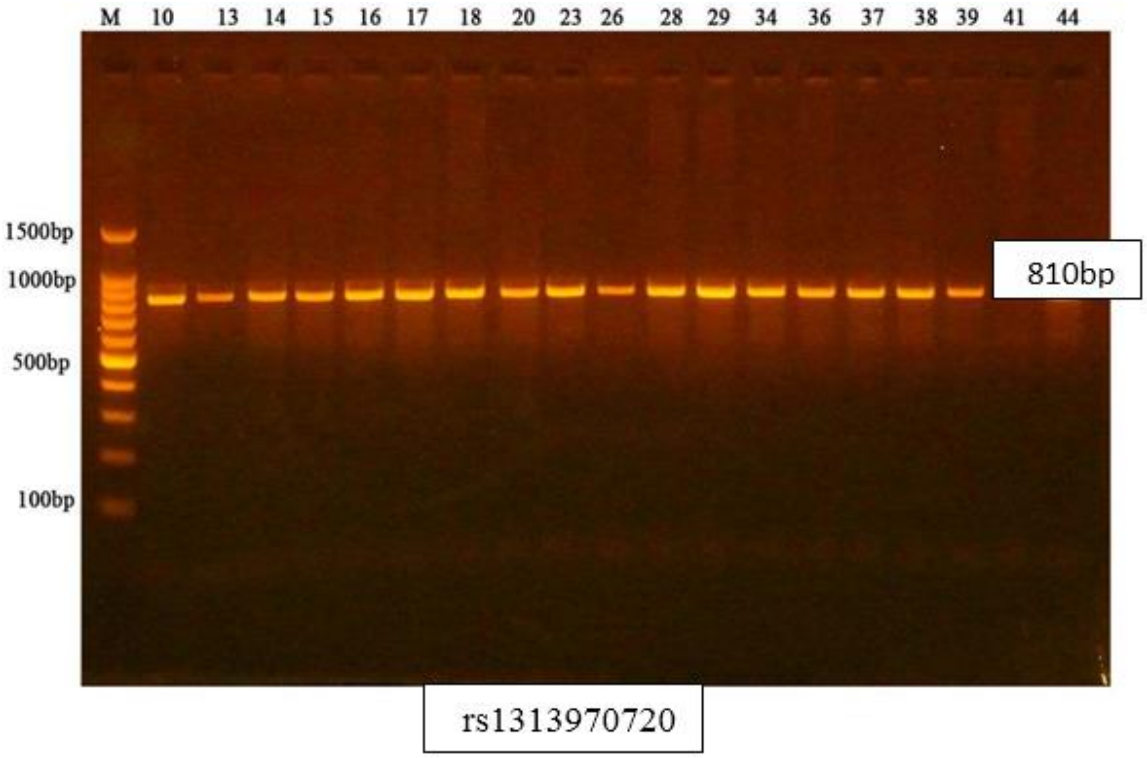

Figure 1.

Table 1. Statistical analysis of associations between IL9 SNP rs1313970720 genotypes or alleles in bacterial vaginosis patients and controls

Genotypes	Patients No. (%)		Control No. (%)		X^2	OR (95% CI)	Fisher's exact probability (2-tailed)
	Observed	Expected	Observed	Expected			
C	94 (0.96)	95.92	35 (0.97)	35.71	0.125	0.67 (0.07-6.05)	1.0
A	4 (0.04)	4.08	1 (0.03)	2.78		1.49 (0.17-13.42)	
CC	45 (91.8)	45.1 (92.0)	17 (94.4)	17.01 (94.5)	0.130	0.66 (0.07-6.01)	1.0
CA	4 (8.2)	3.8 (7.8)	1 (5.6)	0.97 (5.4)	0.081	1.39 (0.15-12.58)	1.0
AA	0 (0.0)	0.1 (0.2)	0 (0.0)	0.01 (0.1)	-	-	-
Total	49 (100.0)	49 (100.0)	18 (100.0)	18 (100.0)			
P-HWE	0.7658		0.9035				

The result explian that heterozygous CA genotype and A allele were significantly increased in women (8.2 and 0.04 %, p=1.0 and p=1.0 respectively) were significantly rise in patients compared with controls (5.6 and 0.03%, p=1.0 and p=1.0 respectively). The associated EF values were 1.39 and 1.49, respectively. In contrast, CC genotype and C allele frequencies were significantly decreased in infected women (91.8 and 0.96 %, p=1.0 and p=1.0 respectively) frequencies were significantly decreased in patients compared to controls (94.4 and 0.97%, respectively p=1.0 and p=1.0). Interrelated PF values were 0.66 and 0.67, respectively, The results in presented study were considered the first kinds in Iraq, Arab world and the world and there were no similar previous studies to the results presented study. However, One of studies mentioned that conducted in Switzerland patients, On the role of interleukin-9 polymorphisms with cancer , However, they did not detect any significant differences between genotypic distributions of IL-9 rs1859430 rs2069870, rs11741137, rs2069885, and rs2069884 SNPs in the LSCC and control groups our study was in contrast to this study they determined that patients carrying AA genotype at IL-9 rs1859430 have a higher risk of death (2.503 , p = 0.015) than those carrying AG heterogenotype (Pasvenskaite et al., 2021).

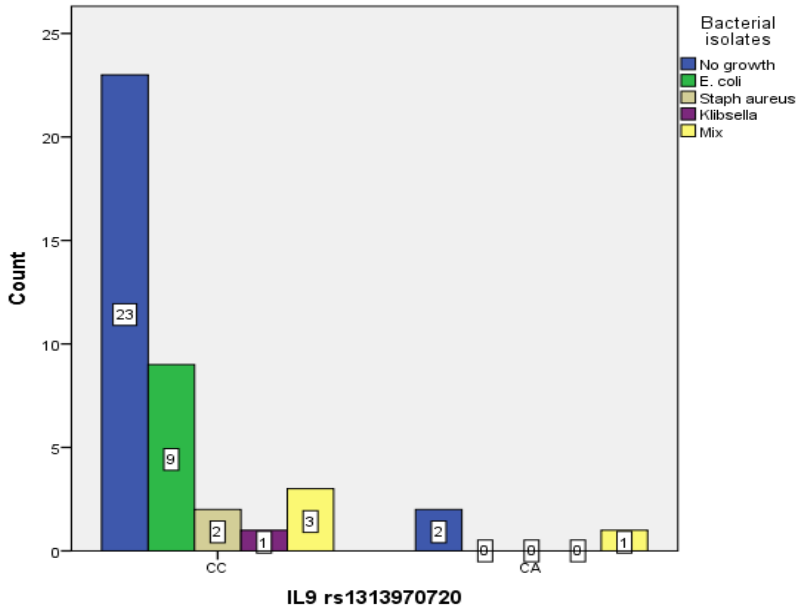

Figure 2. Showed bacterial vaginosis that be isolated from infected wemon of IL-9 (rs1313970720)

The result showed that two genotype (CC,CA) show high frequencies of the patient their culture for them was negative, if we compared between these genotype, the homozygous CC genotype show high frequencies of bacteria E coli and mix bacteria also it has been showed low frequencies of Staphylococcus spp and Kliebsella so the patient have this type of genotype is more dangerous of infection with E coli and mix bacteria whereas the heterozygous CA genotype investigation aperenced low frequencies for mix bacteria so this genotype a protective role for patient from bacteria E coli and Staphylococcus spp and Kliebsella.

In this study, we stratified our bacterial vaginitis -infected patients by IL-9 genotypes, and we evaluated the progression of bacterial vaginitis, which constitutes a critical element of the management of patients with BV The major finding of our study was that the rs1313970720 allele were associated with a higher risk of BV progression in women -infected patients. Some preclinical studies have previously shown a potential benefit for interleukins during bacterial infections. For instance, immunomodulatory effect of *IL1*β polymorphisms was shown to be associated with susceptibility of acquiring BV. Another study showed increased susceptibility to RVVC due to reduced levels of vaginal anticandidal factors in *IL-4* polymorphism homozygotes carriers (Babula et al., 2005; Cauci et al., 2007). Also, polymorphisms in *IL-6* were associated with reduced cytokine responses, conferring increased risk to BV and premature deliveries (Gómez et al., 2010). Other studies carried out On the other hand, Schuurhof et al. suggested that the IL9 genetic polymorphism (rs2069885) has an opposite effect on the risk of severe respiratory syncytial virus infection bronchiolitis in boys and girls and IL9 is upregulated in the nasal mucosa during the pollen season and correlates with tissue infiltration by eosinophils. (Nouri-Aria et al., 2005; Fatahi et al., 2016) . However, *IL-8* polymorphism was shown to be associated with increased cytokine responses and decreases BV risk (Goepfert et al., 2005). Presence of polymorphisms in tumor necrosis factor-α (*TNF*-α) in BV women were associated with increased vaginal TNF-α levels and preterm deliveries (Genç MR et al., 2007). Studies have shown association of *TLR2* polymorphisms with 3-fold increased risk of acquiring BV and increased colonization of BVAB (Taylor et al., 2014). In consonance, a non-synonymous SNP (nsSNP) in *TLR2* was linked with defective protein function, which subsequently reduced the production of pro-inflamatory cytokines and predisposition to RVVC Rosentul et al., 2014). Moreover, it was shown that sensing of BV associated bacteria is facilitated *in situ via* TLR4 signaling, through NF-κB pathway leading to lymphocytes enrolment by cytokines secretion, thus causing genital inflammation (Anahtar et al., 2015).

In our proposed model of, IL-9 can exert various effects on both adaptive and innate immune cells. These effects include (1) stimulating the differentiation and proliferation of Th17 cells, (2) promoting immune suppressive functions of Tregs, (3) enhancing cytotoxicity of Tc cells, (4) inducing activation and accumulation of MCs, (5) activating and maintaining ILCs, (6) activating DCs toward type 2 responses, (7) promoting allergic inflammation of NKT cells, and (8) regulating memory B cell development. After being stimulated by IL-9, the immune cells secrete cytokines that, in turn, may also act as feedback responses to promote the expansion of IL-9-producing cells. (Roediger et al., 2015; Ross et al., 2018). In conclusion, the results indicated that IL-9 gene

SNP (**rs1313970720**) is a risk factor with BV might have a role in the etiopathogenic mechanism which interrelated (positive and negative) with BV in Iraqi population.

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