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GENETIC INVESTIGATION OF THE RISK OF INTERLEUKIN-13 LOCUS RS200196698 IN DIYALA WOMEN WITH VAGINITIS

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Abstract

Bacterial vaginosis (BV) is considered as the most prevalent vaginal disease worldwide. The current study aimed to detect the risk relative of genotypes of interleukin-13 locus rs200196698 in Iraqi women who diagnosed with BV. Genomic DNA was extracted from 70 blood samples, which were split between pregnant women who tested positive for BV and control (non-pregnant women) in the ratio of 57 to 13 respectively. The study has demonstrated that the women with BV exhibited a higher frequency of the homozygous genotype GG and G alleles which mirror a higher chance of developing BV than control women, with OR values of 3.97 and 3.59, respectively. On the other hand, homozygous genotype AA and A alleles in the IL13-3596 SNP rs200196698 were protective agents against BV with OR (0.00) and (0.28) respectively compared to controls. The findings of this study have shown that host genetic variations at the IL13 SNP have roles in increasing risk relative to genotypes and increasing the ability to BV among pregnant Iraqi women. In conclusion, Individuals with homozygous GG genotypes were at increased risk of developing BV. It's probable that this predisposes women to developing BV.

Keywords: Bacterial vaginosis, Genotypes, Interleukin-13.

INTRODUCTION

The inflammatory condition known as bacterial vaginosis (BV) is brought on by a polymicrobial infection that includes pathogenic bacteria that replace the vaginal normal flora. As a result, women of all ages experience a variety of physiological and clinical symptoms as a result of this replacement (Mohammed-Amin *et al*., 2021). Women who have been diagnosed with bacterial vaginosis are more likely to acquire additional sexually transmitted infections, and pregnant women are more likely to give birth before their due date (Russo *et al*., 2019: Deese *et al*., 2018: Javed *et al*., 2019). The majority of vaginal infections in women of reproductive age are caused by bacteria, and estimates range from 5 to 70% of women acquire them (Javed et al., 2019). It's interesting to consider that, globally, this illness is shown to be least frequent in Asia and Europe and most common in several regions of Africa. Although percentages vary between ethnic groups, non-white women "(51% African Americans, 32% Mexican Americans)" are more likely to experience them than white women (Jain *et al*., 2018; Eastment and McClelland, 2018).

Innate immunity and adaptive immunity are the two main categories of immune systems in humans. In contrast to adaptive immunity, which develops after exposure to a particular kind of pathogen and persists in the body (memory cells store knowledge by generating antibodies to the pathogen), innate immunity is present at birth. The immune cells that make up innate immunity are called monocytes. They have developed into macrophages and are mostly found in the blood circulation and surrounding tissues, where they combat harmful organisms and dead cells (Thumoju and Mohan, 2018). Cytokines, which are peptide molecules produced by leukocytes (monocytes, neutrophils, or eosinophils) in the blood stream, control these mechanisms known as the adaptive and innate immune systems. (Mitchell, *et*

al.,2015). Nearly every cell produces cytokines, which are tiny proteins (40 kDa) that are released to control and impact immune response (Kany *et al*., 2019). Gene polymorphism may result in changes in gene expression, with cytokines playing a key role. Interleukin-13 (IL-13) has been implicated in the immunopathogenesis of the disease (Haider *at al*., 2023). The IL-13, which was released by activated T-helper 2 (Th2) cells and associated with the control of humoral immunity and the type II cytokine-mediated immune response, served a variety of biological purposes, such as stimulating an inflammatory immune response and preventing the production of inflammatory cytokines. Numerous illnesses, including mycobacterial infection, type 1 diabetes, periodontitis, viral infections, and autoimmune disorders, have been linked to genetic variations in the interleukin-12, interleukin-13, and TNF- proteins (Jang *et al*., 2016; Loures *et al*., 2019; Santos *et al*., 2017). The expression of cytokines can be altered by polymorphisms in the genes, which can change the prognosis for either acute or chronic illness (Kozak *et al*., 2020).

MATERIALS AND METHODS

This section In this study, vaginal swabs from each of the 57 pregnant women as well as 13 non-pregnant women who served as controls were collected. The investigation was done between January and June 2022 in the department of gynecology and pediatrics at the Al-Batoul Teaching Hospital in the Baquba city which is a center of Diyala Governorate. Patients who attended outpatient clinics were asked to provide clinical samples. All of the participating women ranged in age from 16 to 68 years. EDTA tubes to collect of blood samples were used and all tubes were kept either frozen at -20 or at 8C in appropriate storage vials until required.

Detection of IL13 SNP rs200196698 Polymorphism

The "Wizard Genomic DNA Purification Kit" (Promega, USA) and blood samples kept in EDTA tubes were used to purify the genomic DNA. To find polymorphism at promoter region locations, the "Polymerase Chain Reaction-Specific Sequence Primer (PCR-SSP) assay" was utilized (IL13 SNP rs200196698). We used the CTS-PCR-SSP Tray Kit from Macrogen Company in Korea to demonstrate 2% agarose-gel electrophoresis. "Initial denaturation at 94 °C for 2 minutes, followed by denaturation at 94 °C for 15 seconds, and then 10 cycles of annealing and extension at 65 °C for 60 seconds" were the thermocycling conditions. Following 20 cycles of annealing (61 C for 50 seconds) and extension (72 C for 30 seconds), the denaturation condition was (94 C for 15 seconds). Reactions were halted by cooling at 4C.

Table 1: Primer used to amplify Gene *IL13*

Primer Name	Seq.	Annealing Temp. (°C)	Product size (bp)
IL13 rs200196698 F	5-GGGAAGCTGGCTGAATATC-3`	**65**	**1000**
IL13 rs200196698 R	5`-CTGGTGTCCACTGCTTTAG-3`		

Statistical Analysis

The IL13 SNP rs200196698 genotypes were detected using percentage frequencies. Using "two-tailed Fisher's exact probability (P)," significant differences were used to compare the distributions of these variables in BV-infected and healthy women. In order to estimate the genotype linked with the infection, "relative risk (RR), etiological fraction (EF), and preventative fraction (PF)" were also used. The estimates were calculated using the "WINPEPI computer programs for epidemiologists" application, which is free to download from http://www.brixtonhealth.com.

RESULTS AND DISCUSSION

RESULTS

Table 2: Numbers and percentage frequencies (observed and expected) of *IL13* gene (rs200196698 SNP) genotypes and their (HWE) bacterial vaginosis patients and healthy individuals

Groups			IL13 rs200196698					H-W X_2 P ≤
			GG	GA	AA	G	A	
BV. No.57	Observed	NO.	53	4	0	110	4	N.S.
		%	92.98	7.01	0.00	96.49	3.51	
	Expected	NO.	53.07	3.86	0.07	Not Estimated		
		%	93.11	93.11	0.12%			
Controls No.13	Observed	NO.	10	3	0	23	3	N.S.
		%	76.92	23.08	0.00	88.46	11.54	
	Expected	NO.	10.17	2.56	0.17	Not Estimated		
		%	78.25	20.41	1.33			

Table 3: Statistical analysis of associations between IL-13(rs200196698 SNP) genotypes or alleles and bacterial vaginosis patients and healthy individuals.

Type of comparison bacterial vaginosis	Statistical Evaluation *IL-13* Genotype or Allele	Relative Risk	Preventive or Etiological fraction	Fisher's Exact Probability	95%Confidence Intervals
Disease versus Controls	GG	69.6%	3.97	0.066	0.80 - 19.63
	GA	17.3%	0.25	0.113	0.05 - 1.24
	AA	0.00	0.00	0.00	0.00
	G	69.6%	3.59	0.119	0.77 - 16.77
	A	8.3%	0.28	0.119	0.06 - 1.30

DISCUSSION

This The results of the investigation show, the IL-13-3596 rs200196698 SNP contains three genotypes, GG, GA, and AA, which correspond to the two alleles G and A. As shown in tables 2 and 3, these genotypes are consistent with "Hardy-Weinberg equilibrium (HWE)". Where the study found that the IL13-3596 SNP is considered a significant genetic marker for bacterial vaginosis etiology, particularly when we consider the RR values of (69.6) and (69.6.). Because it was found that the frequency of the GG genotype and the G allele (92.98 vs. 96.49%; P = 0.066, respectively).The associated EF was considerably higher in patients than in controls, (76.92 vs. 88.46%; P = 0.119).The values were 3.97 and 3.59, respectively. In contrast, AA genotype and A allele (0.00 vs. 3.51%, P = 0.00 respectively). In comparison to controls, frequencies were markedly lower in patients (0.00 vs. 11.54%;P = 0.119), and the related PF values were 0.00 and 0.28, respectively. Therefore, these results indicate that IL13-3596 SNP may have an important role in protection against BV. Therefore, because it is considered as an anti-inflammatory, it may be linked to both a raised and reduced risk of bacterial vaginosis in Iraqi women. In addition, a novel SNP (rs1881457C) in the IL13 regulatory area has been linked to a higher risk of coronary artery disease in a Chinese Han co-hort (Zha *et al*., 2018). It may also aid in a more accurate clinical diagnosis of bacterial vaginosis. Nevertheless, other research examining polymorphisms of the promoter region of the IL13 gene have had roughly contradictory results because of racial differences, but they agree that IL-13 is a significant interleukin involved in immunity and that its polymorphisms are essential in preventing the development of bacterial vaginosis disease (Iwaszko, *et al*., 2021 ; Abdulla and Mahmood, 2022). There is convincing evidence from both humans and mice that IL-13 is involved in modulating the inflammatory response as well as contributing to the pathophysiology associated with illness (Hoving, 2018). Also, IL-13 might be a component of the apparatus operating in both acute and chronic neurological and psychiatric disorders and could offer fresh avenues for simultaneous therapeutic modulation of both (Li *et al*., 2023).

Conclusions

The results of this investigation have demonstrated the role that host genetic variants at the IL13 SNP play in elevating risk in relation to genotypes and enhancing the susceptibility of pregnant Iraqi women to BV. Those with homozygous GG genotypes had a higher chance of developing BV.

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