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The Impact of MBL-2 and HLA-G Insertion/Deletion Gene Polymorphism in Vulvovaginal Candidiasis in Iraqi Patients Women

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ABSTRACT

Objectives: MBL (Mannose-binding lectin) is a protein made predominantly inside of the liver, which linked mannose, N-acetyl glucosamine, and focus residues on the cell walls of the microorganism. We saw that the polymorphisms of (MBL2 gene) affect host sensitivity to Candida infection. Human leucocyte antigen-G (HLA-G) are an insertion/deletion polymorphism play a role as immunotolerance, stimulate apoptosis of activated CD8+ T cells, interactions with the (T regulatory cells), modification of the action of dendritic cells and natural killer cells. The polymorphisms in these genes have been reported in several diseases. To evaluate the impact of MBL-2, HLA-G (Ins/Del) polymorphisms in Vulvovaginal candidiasis (VVC) Iraqi patients women.

Material and Method: Fifty four blood samples from each (VVC)patients women and healthy control group was collected, then DNA was extracted and analyzed for MBL-2 and HLA-G genotypes and Alleles frequencies with (PCR) and by Gel electrophoreses using 2.25%, 3% Agarose concentration (respectively) was examined.

Results: The result revealed that 6(12%) patient women infected with vulvovaginal candidiasis has bb allele, and 36(72%) patients infected with vulvovaginal candidiasis has aa allele which referred as wild type, while 8(16%) patients has Ab allele which consider as mutant. There was significant increasing in the bb genotype and Ab genotype frequency in candida patients and increase infection with vulvovaginal candidiasis and b allele consider as a risk factor. *P 0.008*, 0.02*; OR= 0.42(0.33-0.54), 5.33(1.06-26.64). For HLA-G gene polymorphism has 13(26%) patients has Deletion / Deletion genotype, 20(40%) patients has Insertion/ Deletion genotype while 17(34%) patients has Insertion / Insertion genotype, there was significant relation between HLA-G genotype and vulvovaginal candidiasis infection p=0.01,, we found significant increase of D/D genotype in controlled group when compared with patients p=0.03, OR=0.34 CI;(0.12-0.98) which suggests an increase protective effect of D/D genotype against the vulvovaginal candidiasis infection.

Conclusion: There was significant relation between MBL-2 and vulvovaginal candidiasis patients women it consider as a risk factor, so there was also significant relation between HLA-G and vulvovaginal candidiasis patients women it consider as a protective effect.

Key words: Vulvovaginal Candidiasis (VVC), MBL-2, HLA-G Polymorphism.

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INTRODUCTION

Candida albicans is a common opportunistic pathogen yeast, oral and genital mucosal infections are commonly found in medical practice and invasive candidiasis continues to pose a threat to the hospital environment. It is widely recognized that weakness or host resistance defects predispose to infection with *Candida*; however, individuals with cell-mediated immunity defects appear to be prone to mucocutaneous candidiasis, while systemic infections are more frequently associated with neutropenia or neutrophil function defects. [1] *Candida* is typically a genus of fungal known to be non-pathogenic species that reside in healthy individuals as natural flora in the Oral mucous membranes [2].

Candida is a diploid fungus have (8) chromosomes, with (2) pairs. The size of its genome is approximately (16 Mb). It has (6,158) genes of coding. This yeast is the main cause of fungal invasive pathogen. Species of *candida* are the important reason of opportunistic fungal pathogen around the world. *Candida* is the first human fungal infection causing pathogens ranging from surface mucosal infections to spreaded, systemic diseases those also face life threats [3]

Vulvovaginal candidiasis is characterized as symptomatic vaginitis (vaginal inflammation), which often involves the vulva, caused by a *(Candida* yeast) infection. Predominant signs are the itching of the vulva and irregular vaginal discharge (which may be thin, a fluid "cheese-like," or watery secretion). Differentiation from other types of vaginitis requires microscopic presence of leasts of vaginal fluid. [4]

When there is clinical symptoms and signs, (vulvovaginal candidiasis) diagnosed by a group of microscopic examination seeing yeasts, hyphae, budding, culture or a group of both from a (vaginal swab) *Candida albicans* are the most prevalent pathogen, but many species of non-*albicans Candida* can be detected that could cause symptoms. [5]

(MBL) is a big macromolecule with a structure same as a bouquet. MBL's standard structural unit is an MBL polypeptide chain has four domains: (1) a region of 21-amino acid N-terminal cysteine, (2) a collagen-like domain of 59 amino acids, (3) a domain of 30-amino acid α-helical, hydrophobic coiled-coil neck, essential for oligomerisation, and (4) a domain of 188-amino acid C-terminal carbohydrate recognition; [6]

Mannose-binding lectin act a big part in innate immune response of human. It can show the polymorphisms in the *MBL2* gene affect host sensitivity to *Candida* infection, particularly in codon number (54), (the variant (B) allele; wild allele called: A). [7]

The innate system of immunity, as the complement system, play role as a first line of defence against diseases. (*MBL*), a part of the collect in group in the superfamily C-type lectin, is a significant component in innate immunity. MBL recognizes a number of agents for infection, including the yeasts, the fungi, the bacteria, the parasites, and so on. C-terminal domains through their Carbohydrate-recognition (CRD). *MBL* enables the pathogens binding complement mechanism in an antibody- and C1-independent process involving *MBL*-associated serine proteases, thereby facilitating pathogen elimination. *MBL* also promotes cell debris phagocytosis, and can thus prevent autoimmune. [8]

(*MBL)*,were encoded as 10q21.1 by MBL2, was widely referred to as an acute phase protein that raises serum levels after infection. It is an ideal pattern recognition receptor which binds different sugars to the pathogen surface and thus induces pathogen removal through complementary activation, opsonization and/or phagocytosis. [9]

The *HLA-G* gene, is found on chromosome six (6p21.31), consist of 14 bp (IS / DL) and +3142G > C (rs1063320) polymorphism in a translated *HLA-G* region (3UTR). In the promoter region, Polymorphism affects *HLA-G* rate of expression and level of plasma as well as by 3 variants of a translated region (UTR). [10]. A non-classical *HLA* stage (Ib) molecule, (*hla-g*), can stop the functions of natural killer (NK) cells, CD4 ;+ G3, -G4) and three secret soluble proteins (*HLA-G5,*-G6, and -G7 *.(HLA-G* show tolerance functions for immunity that induce apoptosis of increased CD8 + T cells, interactions with T regulatory cells, modulation of natural killer cell activity and dendritic cells, and stopping of the allo-cytotoxic T lymphocyte response.[11]

SUBJECT MATERIAL AND METHOD

Subjects

This study includes 100 patients women, 50 patient infected with vulvovaginal candidiasis and also include 54 women were diagnosed for the (*Candida*) free by physician and after asked them about there is no previous *candida* infection. The age of these women is ranging from 15to 45 years old. These specimens were taken from (Maternity and children of Babylon hospital). The study was conducted during a period from 1st August of 2019 to the end of October 2019, about three ml of patients women and control group blood obtained and putted in EDTA tubes and stored in refrigerator until DNA extraction.

Extraction of DNA

The extraction of DNA was managed from 200µL of patient's women blood, by using Intron DNA extraction kit and following the manufacturer's instructions. The analyses were done in the laboratories of Science collage / Babylon University.

DNA Genotyping

The genotyping for *MBL-2* and *HLA-G* was completed using the Polymerase Chain Reaction technique (PCR) and Green master mix from (promega). PCR volume of reaction for MBL-2 was 25 µl consist of 12.5 µl master mix mixed with 7 µl from DNA and 1 µl from both forword and reverse and 3.5 from D.W. The PCR Program for amplification of the Mbl-2 primer was amplified using the following PCR programme; first denaturation 94C° for 4 min, and 38 cycle for 94C° for 21 sec, 60C° for 45 Sec,72 C° for 25 Sec, and the final step 72 C° for 2 min. Convential PCR was used to amplified the target site of DNA samples for HLA-G primers, the total PCR mixture (25 µl) was consist of 12.5 µl master mix mixed with 6 µl from DNA and 1.25 µl from both forword and reverse and 4 µl from D.W. The PCR Program for amplification of the *HLA-G* primer was amplified using the following PCR programe; first denaturation 94C° for 4 min, and 37 cycle for 94C° for 20 sec, 6058C° for 25 Sec,72 C° for 30 Sec, and the final step 72 C° for 120 sec.

Analyzing

(Gel electrophoresis) was prepared, 2.25% agarose gel for *MBL-2* gene polymorphism and 3% agarose gel for *HLA-G* gene polymorphism, with 5 µl red safe. By using the trans illuminator, gel were analyzed and genotypes were determined.

Statistical Analyses

The potential associations of *MBL-2* and *HLA-G* gene polymorphism with the risk of vulvovaginal candidiasis infection were analyzed by comparing the *MBL-2* and *HLA-G* insertion / deletion in Patients women and the group of control using [chi-square] test (*P* value < 0.05 consider as significant), and [odd ratio] (OD) test, CI 95% for estimate the impact of this mutation with the infected group compared with the control group, this analysis were completed by using [SPSS] program.

RESULTS

The molecular investigation of *Mbl-2* gene polymorphism in the patients women infected with vulvovaginal candidiasis was done for 50 patient women, and 50 control group without any history of infection with candidiasis to find the association of the present of the gene polymorphism and allele frequencies with the vaginal infection with this fungi. The PCR results for amplification of the Mbl-2 gene gave the result: The B allele was the result of undigested product mutant type of PCR (349bp) (homozygote). And A allele was the result of digested product wild type with 2 fragments (260bp, 89bp). While the AB genotype was the result of 3 fragments (349bp, 260bp, 89bp) (heterozygote).as seen in the figure(1), The result revealed that 6(12%) patient women infected with vulvovaginal candidiasis has bb allele, and 36(72%) patients infected with vulvovaginal candidiasis has a allele which referred as wild type, while 8(16%) patients has Ab allele which consider as mutant, compared with the control group, 0(0%) containing the bb allele and 48(96%)containing the aa allele, and 2(4%) contain the Ab allele, there was statistically significant increasing in the bb genotype and Ab genotype frequency in *candida* patients and increase infection with vulvovaginal *candidiasis* and b allele consider as a risk factor. *P 0.008*, 0.02*; OR= 0.42(0.33-0.54), 5.33(1.06-26.64) as seen in the table (1)

The molecular investigation of *HLA-G* by using the PCR program for insertion /deletion polymorphism, the PCR results shown an amplicon of (224bp) for *HLA-G* insertion homozygote and (210) for *HLA-G* deletion homozygote and both bands for insertion / deletion genotype figure(2) From PCR analyzing these results were obtained ; for the patients infected with vulvovaginal candidiasis 13(26%) patients has Deletion / Deletion genotype, 20(40%) patients has Insertion/ Deletion genotype while 17(34%) patients has Insertion / Insertion genotype, when we compared this results with the result of control group which were 22(44%) patients with Deletion /Deletion, 18(36%) patients with Insertion /Deletion and 10(20%) patients with Insertion/Insertion genotype, there was significant relation between *HLA-G* genotype and vulvovaginal candidiasis infection p=0.01, table (2), we found significant increase of D/D genotype in controlled group when compared with patients p=0.03, OR=0.34 CI;(0.12-0.98) which suggests an increase protective effect of D/D genotype against the vulvovaginal candidiasis infection, the results shown in Tables (1,2).

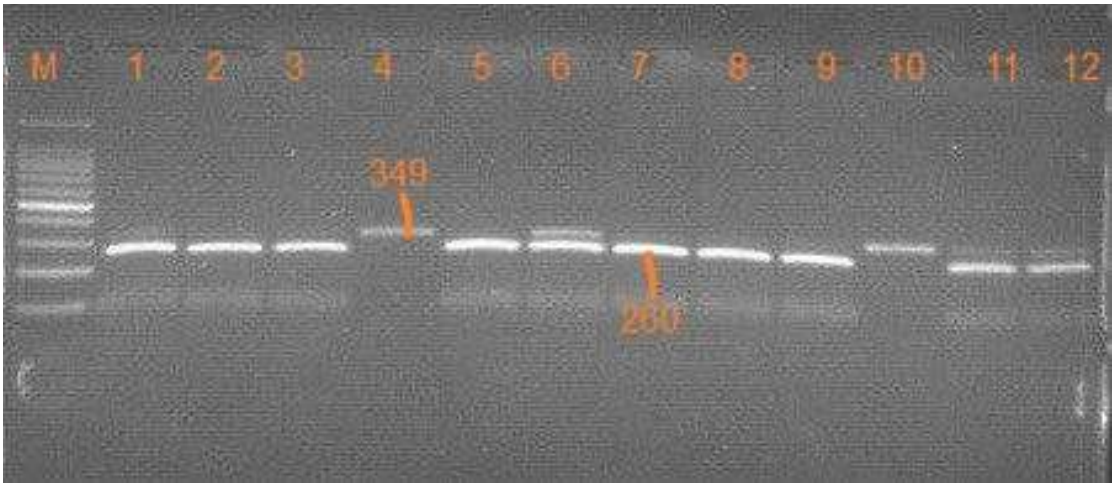

Fig. 1: MBL-2 gene polymorphism M: (100- bp) DNA ladder, the piece BB homozygote (4-10) without cutting sized 349 bp. And the piece AA heterozygote (1-2-3-5-7-8-9) appeared 2 pieces sized 260,89bp. The piece AB(6-11-12) appeared 3 pieces sized 349,260-89..concentration of agarose (2.25%).

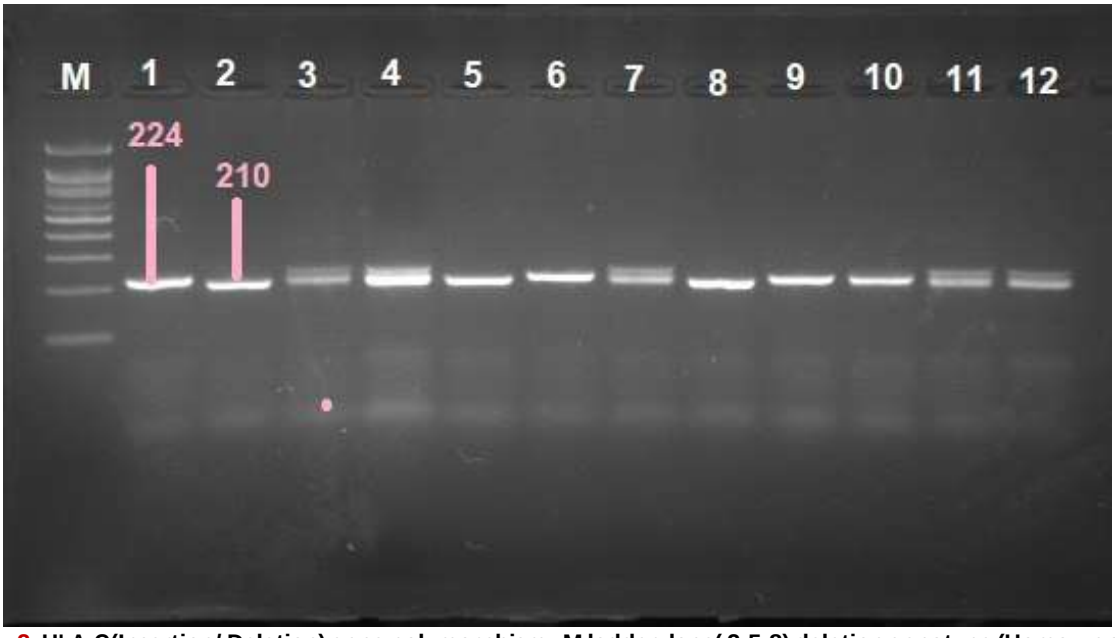

Fig. 2: HLA-G(Insertion/ Deletion) gene polymorphism : M ladder, lane(2-5-8) deletion genotype (Homozygote for Deletion) with size 210bp, lane(3-4-7-11-12) insertion /deletion genotype (Heterozygote), lane (1-6-9-10) insertion genotype (Homozygote for Insertion) with size 242bp, agarose concentration: (3%).

Table 1: MBL-2 gene polymorphisms (Allele and genotype frequencies) between vulvovaginal candidiasis positive patients women and healthy controls:

	Control (50)	Patients(50)	P value	OR(CI95%)
aa genotype	48(96%)	36(72%)		
bb genotype	0(0%)	6(12%)	0.008*	0.42(0.33-0.54)
Ab genotype	2(4%)	8(16%)	0.02*	5.33(1.06-26.64)
A allele	98	80		
B allele	2	20	<0.001*	12.25(2.78-53.98)

*$P \leq 0.05$; OR= (95%CI)

Table 2: HLA-G (IS/ DL) gene polymorphisms (Allele and genotype frequencies) between vulvovaginal candidiasis positive patients women and healthy controls:

	Control(50)	Patients(50)	P value	OR(CI95%)
ins genotype	10(20%)	17(34%)		
del genotype	22(44%)	13(26%)	0.03*	0.34(0.12-0.98)
heterozygote genotype	18(36%)	20(40%)	0.28	0.65(0.23-1.79)
ins allele	38	54		
del allele	62	46	0.01*	0.52(0.29-0.91)

*$P \leq 0.05$; OR= (95%CI)

DISCUSSION

This result agree with the result : The women that have the "B" variant allele of *MBL2* codon 54 polymorphism that more dangerous of developing RVVC. For this reason,the *MBL* genotypic research can be used as surrogates for *MBL* serum levels to classify women with *MBL* deficiencies for alternative therapeutic options[6] also similar to the study that said Carriage of the variant *MBL2* codon 54 allele B was more frequent in women with recurrent vulvovaginal candidiasis.[12] In another study we saw the important role for *MBL* on the regulation of cellular responses induced by *C. albicans*-, *MBL* can affect the expression of proinflammatory cytokine and chemokine by modifying *C. albicans*-/TLR-signaling pathways.[8].In another study another disease *MBL* gene polymorphism is linked with the evolution of (primary vulvar vestibulitis syndrome) and a miniature the ability for TNF-alpha output in response to microbial strains.[13]

HLA-G expression was linked with many autoimmune and inflammatory pathogens, and have the ability to made viral infections. Homozygosity for the 14-bp DL(D/D genotype) in the children were a dangrous factor for hepatitis C virus that transmitted from mother to baby and high level of soluble *HLA-G* in amniotic fluid associated with congenital *Toxoplasma gondii* transmission, for this reason *HLA-G* is necessary in modulation of immunity for keeping the fetus.[14]

Exploring possible relationships among the *HLA-G* 14-bp IS/DL polymorphism and AID dangerous, it has published several case-control studies. For SLE, the results achieved have been inconclusive and even contradictory. Studies of juvenile idiopathic arthritis, ulcerative colitis, cohn 's disease, idiopathic dilated cardiomyopathy, vulgaris pemphigus and non-segmental vitiligo showed big linking between the 14-bp IS/DL and the dangerous of pathogen, while RA and multiple sclerosis showed no linking.[11]

In another studies in Iraq, there is no significant alteration between heterozygous and homozygous of the *HLA-G* 14bp (deletion/ insertion) polymorphism amongst husband's strange and husbands relatives related with abortion of pregnant women.[15]. The *HLA-G* gene have a relation with recurrent abortions by the antiphospholipid syndrome.[16] And we saw in another study *HLA-G* genotype is not a cause of recurrent pregnancy loss in these women.[17]

CONCLUSION

There was significant relationship between volvovaginal candidiasis and *(MBL-2),* gene polymorphism they consider as a risk factor to increase vulvovaginal candidiasis infection. Also there was significant relationship between volvovaginal candidiasis and (*HLA-G*) gene polymorphism they increase protective effect against the vulvovaginal candidiasis infection.

CONFLICT OF INTEREST

None

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