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RESEARCH ARTICLE

The Impact of FLG Mutation and TRBV Insertion/deletion Gene Polymorphism in *Molluscum Contagiosum* and *Papillomavirus* Iraqi Patients

Saba Amer Mughir Al-Ammar[1*], Israa Adnan Ibraheam[2], Manar Mohammad Hasan Al-Murshidi[1]

[1.] *Biology Department /College of Science for Women/ Babylon University / Babylon / Iraq.*

[2.] *Biology Department /College of Science for Women/ Babylon University / Babylon / Iraq.*

***Corresponding Author: Saba Amer Mughir Al-Ammar**

Abstract

Objective: Filaggrin (FLG) has very important role in the skin terminal differentiation and formation, which encoded by FLG gene. TRBV4-3, TRBV3-2 are an insertion/deletion polymorphism in human T cell receptor beta locus (TRBV).The polymorphisms in these genes have been reported in several diseases. To evaluate the impact of FLG (R501X), TRBV (I/D) polymorphisms in *Molluscum contagiosum*(MCV) and *Papillomavirus* (HPV) Iraqi patients. Material and Method: thirty one blood samples from each MCV and HPV patients and healthy control group was collected, then DNA was extracted and analyzed for FLG and TRBV genotypes and Alleles frequencies with (PCR) and by Gel electrophoreses using 1.5%, 2% Agarose concentration (respectively) was examined. Results : FLG mutations have been detected in 35% of MCV patients and in 26% of HPV patients while only 10% in the control group , in addition mutant allele (T allele) has been detected in 18% in MCV patients , 13% in HPV patients and only in 5% in the control group ,when we compared with the control group, we found that the genotype and allele frequency have significant association with MCV infection group $P<0.05$, and have no significant relation with HPV infection group. For TRBV (TRBV4-3, TRBV3-2) gene polymorphism has no significant association with MCV and HPV infection groups when compared with the control group $P>0.05$.Conclusion: FLG mutations are associated with MCV infection while there is no significant associated with the HPV. Moreover the TRBV gene polymorphisms have no significant association with the MCV and HPV infection.

Keywords *Molluscum contagiosum, Human Papillomavirus, FLG (R501X), TRBV Polymorphism.*

Introduction

The main functions of the skin is the Protection and defense against several stimuli , the Regulation of the trans-epidermal water losses (TEWL), the defense against several of external physical / chemical agents and protection from entry of microorganisms are the important function of the skin barrier [1].

Skin immune system composed of two structural compartments: the epidermis and the dermis, the major immunological cells which are resident in the skin include Langerhans cells (LCs), and melanocytes which produce melanin, occupy epidermis, while the dermis is populated by Macrophage, DCs subpopulation and several types of T cells, The effectiveness of this system depends on the communication between these resident immunological cells and the outer environment e.g., neighboring fibroblast and keratinocyte [2]. Keratinocytes form stratum corenium and produce Filaggrin which bind to Keratin fibers

forming a tight barrier, which important for skin function preventing entry of several pathogens [3].

The impair of skin function cause several infection, the most of this infection caused by *Molluscum contagiosum* , *Papillomavirus* and Herpes virus [4].*Molluscum contagiosum* (MCV) belong to the family *poxviridae* [5].MCV transmitted directly through skin to skin contact and cause lesions which spread to other part by autoinoculation [6].

MCV replicate only in skin keratinocytes [7] and produces small, benign, multiple lesions that can persist for long periods of time [8] which are yellowish white , lobulated and amorphous central structure which surrounded by fine linear vessels and sometime this vessels blurred and it doesn't cross the lobule's center [9].

With size range 3-5mm or it can be giant ,the incubation period range from two to eight weeks or it could persist for six months [9]. Human Papillonavirus (HPV) is small, non-enveloped, have circular double-stranded DNA (ds-DNA) genome with size 8 kb, belong to Papillomaviridae family, more the 120 different HPV has been identified [10]. HPV infect Keratinocytes at different part of the body and cause several different squamous benign or malignant skin lesions [11].

The life cycle of HPV depending on the differentiation program of skin Keratinocytes [12], to stimulate its proliferation , some of viral proteins are suppresses the mechanism control the cell cycle , include E6 viral protein which bind to tumor suppresser protein (P53), which interfere with the normal cell function and enhance its degradation [13] .the infection of HPV occur directly (skin to skin contact) , incubation period range from 1-6 months [14].

FLG protein is essential for skin formation and function, [15] this protein is encoded by FLG gene which located on chromosome 1q21, composed of 3 exons and 2 introns, Exon 3 is coded for pro-filaggrin polyprotein, [16] pro-filaggrin first expressed in stratum granulosum in epidermis then its photolysis to produce Filaggrin monomers which bind to keratin fibers producing skin barrier against several different pathogens [17].

FLG mutation cause impair skin function because its cause loss of Filaggrin protein which results in decrease of keratohyalin granules in addition to impaired of keratinization process, and therefore increase risk for *Molluscum contagiosum* and *Papillomavirus* infections and several other diseases [18]and[15].

T cells are very important role for intracellular pathogens recognition and initiating pathways causes infected cells destruction, it recognize pathogens bonded to MHC molecules by receptors called T cell receptors (TCRs) [19].

TCRs (α and ϐ) are the main receptor for immunological responses, TRBV consist of 48 functional gene and 19 pseudogene, it located in chromosome 7, these TCRs are diversified by random rearrangement in V,D and J genes(insertion / Deletion) at TCRϐ locus during T cells maturation [20]. One of these insertion TRBV 4-3 and pseudogene TRBV3-2,Deletion of these functional gene increase either susceptibility or resistance for viral infection [21].

As the role of FLG mutations and TRBV mutation in the in the etiology of *Molluscum contagiosum* and *Papillomavirus* have not been studied previously in Iraq , we investigate the most common FLG mutations,R501X and TCR B locus mutation TRBV 4-3 and pseudogene TRBV3-2, and Analyzing the impact of these mutation on infection with both *Molluscum contagiosum* and *Papillomavirus*.

Subject Material and Method

Subjects

This prospective study includes 62 patients, 31 patient infected with *Molluscum contagiosum* and 31 patients infected with *Papillomavirus*, and also include 31 people without any history for infection with both virus neither *Molluscum contagiosum* nor *Papillomavirus* as control group.

With the clinical diagnosis of *Papillomavirus* and *Molluscum contagiosum* infected patients by Specialist Physician between august- December/ 2018 in Department of Dermatology /Babylon Governorate / Margan teaching hospital, about two to three ml of patients and control blood obtained and putted in EDTA tubes and refrigerated until DNA extraction.

DNA Extraction

The genomic DNA extraction was conducted from 200µL of patient's blood, from each study subject, by using Favrogene DNA extraction kit and following the manufacturer's instructions. The analyses were performed in Laboratories of Faculty of Science / Babylon University.

DNA Genotyping

The genotyping for FLG mutation and TRBV4-3, TRBV 3-2 was performed using the Polymerase Chain Reaction technique (PCR) and using Green master mix (promega).

The genotyping of R501X accomplished by using the primer pair as previously used in study (Visser et al., 2013) [22]. The PCR performed by use 0.8 µL from either R501C or R501T along with 0.8 µl R501 common, 12.5 µL master mix ,5 µL of extracted blood and 5.9 µl of nuclease free water for each sample, Reaction was amplified using the following PCR programme; first denaturation 94C^{o} for 180 sec , and 35 cycle for 94C^{o} for 15 sec , 69C^{o} for 50 Sec ,72 C^{o} for 15 Sec, and the final step 72 C^{o} for 120sec. genotyping of TRBV4-3, TRBV3-2 was performed by using the primer pair as with previous study by [19].

The PCR for TRBV4-3 and TRBV3-2 was accomplished by mixing 1 µl from each primer forward and reverse with 12.5µl , 4.5 µl nuclease free water and 6µl of extracted DNA for each sample .the PCR condition for amplification this reaction was ; first denaturation 94C^{o} for 240 sec , and 35 cycle for 94C^{o} for 30 sec , 60C^{o} for 30 Sec , 72C^{o} for 40 Sec , and final step 72C^{o} for 10 minutes.

Analyzing

Gel electrophoresis were performed on ,1.5% agarose gel for R501X gene polymorphism and 2% age rose gel for TRBV4-3 and TRBV 3-2 gene polymorphism , containing 4.5 µl red safe .by using transilluminator the gel was analyzed and genotypes were determined.

Statistical Analyses

The potential associations of FLG mutation and TRBV4-3 and TRBV3-2 with the risk of *Molluscum contagiosum* and *Papillomavirus* infection were analyzed by comparing the FLG mutation and insertion / deletion in (TRBV4-3 and TRBV3-2) in patients with the control group by using chi-square test (*P* value < 0.05 consider 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 was performed using SPSS program (IPM , version 24).

Results

The genotype of whole 62 patients (31 patients of *Molluscum contagiosum* and 31 patients of *Papillomavirus*) was analyzed for detection the presence of normal or mutant genotype of FLG (R501X) ,we observed bands with size (57) for the wild type (CC genotype) and (58)bands of the mutant (CT) genotype as seen in Figure (1), from these results we found 65% of MCV patients has CC genotype while the 35% has CT genotype , when this results compared with the control group(90% of control group has the CC genotype while only 10% has the CT genotype) we found a significant correlation between the genotyping and MCV infection *P*=0.03 , OD =5.133 ,CI: 95%.

The Allele frequency also investigated, we found a significant increase of the C allele in the control group P=0.04, OD =4.24which provide protection against infection, the results shown in Table(1).The genotyping and allele frequency have no significant association with the HPV infected patients , as seen in Table(2). The TRBV4-3 and TRBV3-2 amplification used to confirm the presence or absence of TRBV4-3 and TRBV3-2, TRBV3-2 is pseudogene located in the same loci (12.5kb insertion/deletion) along with TRBV4-3 [23].

From Gel electrophoresis results, we observed that 52% of patient with *molluscum contagiosum* carrying TRBV4-3,TRBV3-2 (insertion), while 48% didn't use TRBV4-3,TRBV3-2 (Deletion) , and 55% of patient infected with papillomavirus using TRBV4-3,TRBV3-2 (insertion) , while other 45 % of HPV patients didn't used TRBV4-3, TRBV3-2 (deletion) Figure (2), when this finding compared with the control group (68% insertion and 32% deletion) the result show there is no significant association between insertion/deletion polymorphism and MCV and HPV infection *P*=0.196. The allele frequency also investigate for detection its relation to increase or decrease the risk for MCV and HPV infection , we found there is no significant association between allele

frequency and this viral infection *P*=0.29, the results shown in Table (1,2).

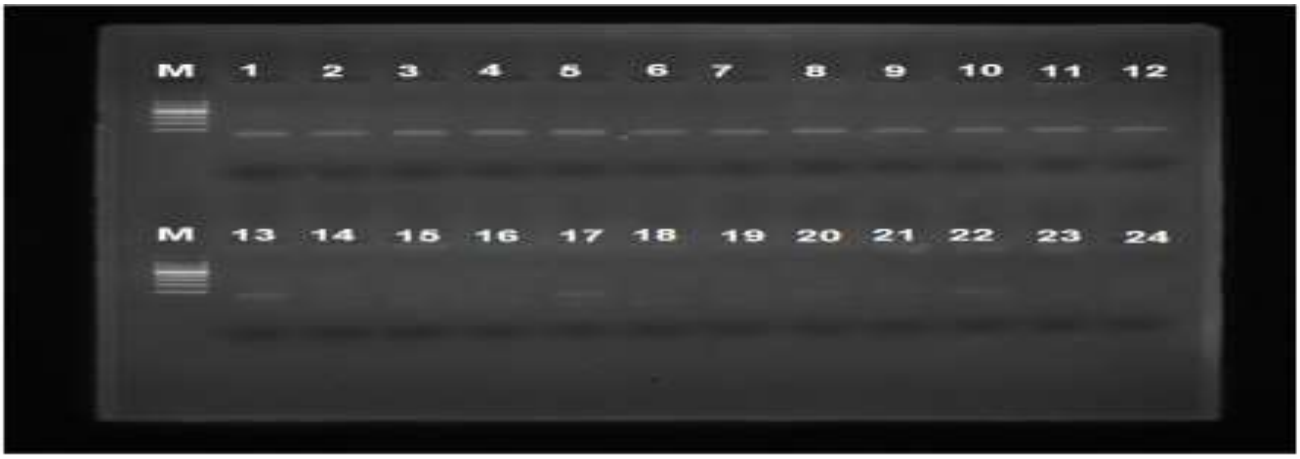

FIG 1: M 100 bp DNA ladder, lane 1-12 positive results for R501X normal allele , lane 13,17,20,21,22 positive results for R501X mutant allele ,lane 14,15, 16, 18,19,23,and 24 negative results for R501X mutant allele

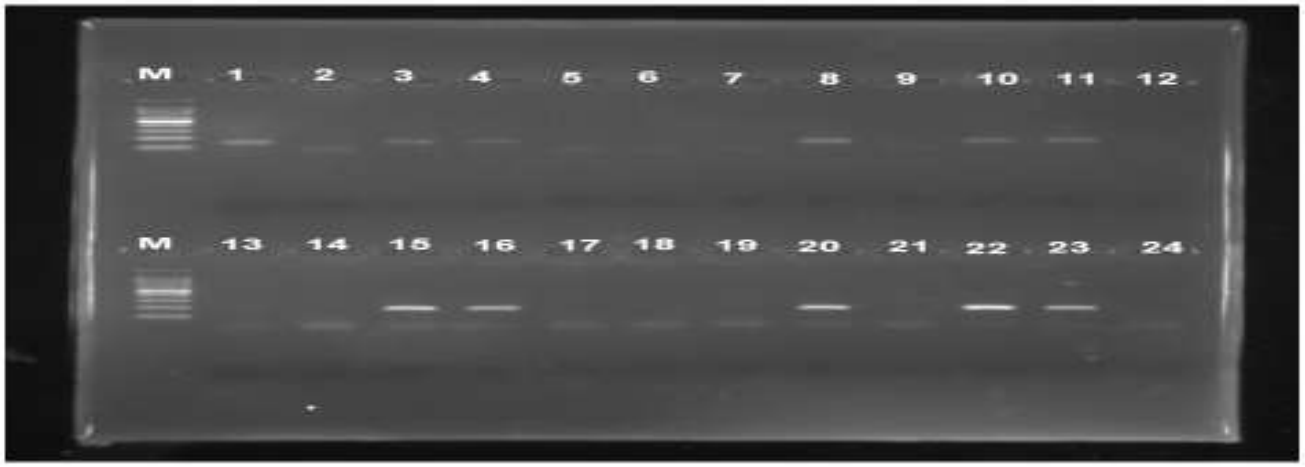

FIG 2: M 100 bp DNA ladder , lane 1,3,4,8,10, and 11 positive results insertion (presence) pseudogene TRBV3-2 , lane 2, 5,6,7,9,and 12 positive results for deletion (absence) pseudogene TRBV3-2 , lane 15, 16, 20, 22, and 23 positive results for TRBV4-3 insertion, lane 13, 14, 17, 18, 19, 21, and 24 positive results for TRBV4-3 deletion

Table 1: Allele and genotype frequencies of FLG (R501X) and TRBV 4-3, TRBV3-2 polymorphisms among Molluscum contagiosum positive patients and healthy (uninfected controls)

Gene	MCV		Control		P value	OD (CI95%)
	no	%	no	%		
FLG						
C allele	51	82%	59	95%	0.04*	Ref
T allele	11	18%	3	5%		4.24(1.121-16.0)
CC Genotype	20	65%	28	90%	0.03*	Ref
CT Genotype	11	35%	3	10%		5.133 (1.26-20.8)
TRBV4-3 TRBV3-2						
II Genotype	16	52%	21	68%	0.196	Ref
DD Genotype	15	48%	10	32%		1.969 (0.702-5.52)

Table 2: Allele and genotype frequencies of FLG (R501X) and TRBV 4-3, TRBV3-2 polymorphisms among Papillomavirus positive patients and healthy (uninfected controls)

Gene	HPV		Control		P value	OD (CI95%)
	no	%	no	%		
FLG						
C allele	54	87%	59	95%	0.2	Ref
T allele	8	13%	3	5%		2.914(0.735-11.55)
CC Genotype	23	74%	28	90%	0.18	Ref
CT Genotype	8	26%	3	10%		3.246 (0.77-13.66)
TRBV4-3 TRBV3-2						
II Genotype	17	55%	21	68%	0.29	Ref
DD Genotype	14	45%	10	32%		1.279 (0.615-4.860)

Discussion

FLG R501X is single nucleotide polymorphism C>T (SNP) that change the (CGA) coding for arginine into (TGA) stop codon. This polymorphism truncated the profilaggrin protein; therefore it cannot process to Filaggrin proteins, thus this polymorphism defined as loss of function mutation or polymorphism [20].

This mutation caused dysfunction of skin barrier that increase risk for several diseases including the viral infection , so we observed that most of the FLG mutation (CT genotype) are significantly increase in MCV patients Table (1), in addition the allele frequency also significantly increase in those patients , this result support the correlation of the FLG mutation with increased risk for MCV infection, and this finding are correlated with the involvement of FLG mutation as risk factor for increase susceptibility of other disease such as rhinitis , atopic dermatitis (AD), asthma, and food allergies [19].

When this results compared with the control group we found that about 90% of controls have the (CC genotype) the wild type or without mutation where it significantly increase, cause it expressed for the functional profilaggrin producing normal skin barrier. For HPV patients we found there is no significant association between HPV infection and FLG R501X genotypes and allele frequencies Table 1. TCR beta locus sequence polymorphism that inactivate otherwise functional (BV) segments is common and cause the variability in functional BV genes expressed through the human population [24]. Brenen [19] found that TRB deletion can skew antigen – specific T cell receptor usage and T cells fine specificity, thus contribute to most inter individual immune responses variability and identification of epitope escape mutation throughout the population. In our study we found that the is no significant association between TRBV4-3, TRBV3-2 gene polymorphism and MCV and HPV infected Iraqi patients.

Although this results but the deep observation of this result we found that most of the control group has the Insertion genotype but only (32%) of them have the Deletion genotype when this result consider as bases for comparison the Deletion genotype are increase in MCV and HPV infected patients, suggesting that the TCR repertoire diversity generated with present of TRBV4-3, TRBV3-2 provide protection against viral infection This is the opposite of what Brennan found in his study [19].

Conclusion

FLG mutations (genotypes and allele frequencies) are associated with MCV infection while there is no significant associated with the HPV infection in addition the CC genotype and C allele (no mutation) significantly increase in control group which confer resistance. Moreover the TRBV gene polymorphisms have no significant association with the MCV and HPV infection.

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