J. Cardiovasc Disease Res., 2020; 11(4): 205 – 209
A Multifaceted Peer Reviewed Journal in the field of Cardiology
www.jcdronline.org | www.journalonweb.com/jcdr
P – ISSN: 0975 – 3583 Online: 0976 – 2833

Interferon Gamma and Mannose Binding Protein: Role in Susceptibility to Cytomegalovirus Infection

[1]Shahad Abd Alrahman, [2]Zainab A. Tolaifeh, [3*]Israa Adnan Ibraheam

[1,2,3]Biology Department, College of Science for Women, University of Babylon, Iraq.
[3]E-mail: israaadnan_ibraheam@yahoo.com*
Corresponding author: Israa Adnan Ibraheam, israaadnan_ibraheam@yahoo.com

ABSTRACT

Objectives: IFN- γ is a cytokine that is essential to the viral innate and adaptive immunity. MBL contributes to activation of the complement system lectin pathway in an antibody-independent mechanism. The polymorphisms in these genes have been reported in several diseases. To evaluate the impact of IFN-γ, MBL polymorphisms in Cytomegalovirus (CMV) Iraqi patients.

Material and Method: Fifty blood samples from CMV patients and healthy control group was collected, then DNA was extracted and analyzed for IFN-γ and MBL genotypes and Alleles frequencies with (PCR) and by Gel electrophoreses using 2.25% Agarose concentration (respectively) was examined.

Results: MBL mutations have been detected in 18% of CMV patients while only 4% in the control group, in addition mutant allele (B allele) has been detected in 14% in CMV patients and in 0% in the control group, when we compared with the control group, Genotype and allele frequency were found to have a significant association with the P<0.05 group of CMV infections. The TT genotype is associated with CMV viral clearance for IFN-α gene polymorphism.

Conclusion: MBL and IFN- γ Linked to cmv infection.

Key words: Health, Viral Clearance, Protein, Infections.

Correspondence:
Israa Adnan Ibraheam
Biology Department,
College of Science for Women,
University of Babylon,
Iraq.
E-mail Address:
israaadnan_ibraheam@yahoo.com

Submitted: 25-09-2020
Revision: 20-10-2020
Accepted Date: 25-11-2020

DOI: 10.31838/jcdr.2020.11.04.36

INTRODUCTION

Human cytomegalovirus (HCMV), a member of the Herpesviridae herpes betaherpesvirus subfamily, is the main viral cause of birth defects and life-threatening complications in immunocompromised people. HCMV is the human herpes virus with the greatest genetic and structural complexity, With a dsDNA genome of 240 kilo-base pairs encoding more than 220 open reading frames (ORFs) and at least 145 specific genes (1). CMV displays a broad variety of host cells that can invade different cell types, such as endothelial cells, epithelial cells (including retinal cells), smooth muscle cells, fibroblasts, bleucocytes, and dendritic cells (2). Cytomegalovirus (CMV) is regarded as the most prevalent human congenital viral infection (3). HCMV is a severe opportunistic infection in immunocompromised individuals like those infected with the human immunodeficiency virus (HIV) and immunosuppressive drug transplant patients due to an impaired immune system (4). Its considered a major cause of morbidity and mortality in immunocompromised hosts (3). The immune response to CMV is a complex collection of immunological processes involving both innate and adaptive immunity (5),(6). HCMV's ability to effectively infect the host and cause disease is likely to be due at least in part to a variety of HCMV genes encoding proteins with immunomodulatory functions (7). Such us: The interferon-γ gene is located on the 12q24.1 chromosome and consists of 4 exons and 3 introns encoding a short 166 amino acid polypeptide (8). It is secreted mainly by T-cells and natural killer (NK) cells and, to a lesser degree, by other cell types such as macrophages, dendritic cells (DC) and B-cells (9). (IFN-π) is a cytokine important for both innate and adaptive immunity to viral and intracellular bacterial infections and the regulation of tumours.. Increased IFN-π development is associated with a variety of inflammatory and autoimmune diseases. IFN-π's significance in the immune system derives from its ability to cause various genes, the majority of which encode immunoregulatory molecules (10). Mannose-binding lectin (MBL) is located on chromosome 10 (10q11.2-q21) and consists of four damaged three-introne exons. Six single nucleotide polymorphisms (SNPs) in the MBL2 gene are known to be associated with variations in the serum volume and/or function of MBL. MBL is mainly developed by the liver but small quantities have been found in the small intestine and testis tissue (11). MBL helps stimulate the lectin pathway of the supplement system in an antibody-independent manner and can promote opsonophagocytosis, modulate inflammation and induce cell lysis. (12).

MATERIALS AND METHODS

Subjects

Current research is case-control study that involves 100 females, divided into two classes, the 50 females with Human Cytomegalovirus and 50 females that are apparently stable. All participants obtained four ml of venous blood using disposable syringe, four ml of blood was obtained for genetic study and slowly pushed into the EDTA tube and given the patient's name at -20 °C.

DNA Extracton

The DNA was extracted from each patent and control group blood using 200ml of whole blood in addition to the human DNA extracton kit of Intron Biotechnology and follow the guidelines of the Manufacturer.

DNA Genotyping

The genotyping of IFN-π gene polymorphism was detected by the use of the amplification refractory mutation system (ARMS) PCR and the use of Green master mix (promega), the genotyping of IFN-π+874 gene polymorphism by using the primary pair as previously used in the study (13). The total volume of the reaction was 25 µl, consisting of 12.5 µl of the master mixture combined with 7 µl of DNA and 100 P mole (1 µl) of the specific A primer with 100 P mole (1 µl) of the specific primary T and 1 µl of the primary antisense and 3.5 µl of the distilled water. The PCR mixer uses this PCR program condition to amplify: frst denaturaton 94 ° C for 4 min, and 39 cycles for 94 ° C for 25 sec, 60 ° C for 25 sec, 72 ° C for 25 sec, and fnal step 72 ° C for 2 min. The genotyping of MBL gene polymorphism was performed using the PCR technique (Polymerase Chain Reacton) and using Green Master Mix (Promega). Genetic engineering of Mbl2 gene polymorphism

Using the priming pair as used in the analysis ((14)). The volume of the reaction was 20 µl, containing 10 µl from the master mix, 0,6 µl from both forward and reverse, 6 µl from the DNA sample and 2,8 µl from the distilled water. The PCR mixer uses amplified with this condition PCR system. : front denaturat on 94 ° C for 4 min, and 39 cycle for 94 ° C for 20 sec, 61 ° C for 25 sec, 72 ° C for 25 sec, and fnal step 72 ° C for 2 min. The restriction fragment length gene polymorphism was performed using the enzyme BanI restriction 10 µl pcr product reaction, 0.5 µl R.E. 0.2 Approx bovine serum albumin, 5 µl smart cutting buffer and 7.3 D.W.

Analyzing

Gel electrophoresis was performed on 2,25 per cent agarose gel for IFN-α gene polymorphism and 2,25 per cent age rose gel for MBL gene polymorphism, containing 4,5 µl red safe. The gel was analyzed and genotypes determined using transilluminator.

STATISTICAL ANALYSES

Potential associations of MBL and IFN-π with the risk of cytomegalovirus infection were analyzed by comparing MBL and IFN-π in patients with the control group using Chi-square test (P value < 0.05 deems important) and oddratio (OD) test CI 95% to estimate the impact of this mutation on the infected group in contrast with the control group.

RESULTS

To detect a presence of normal or mutant genotype of MBL2 the genotype of whole 50 patients was analyzed, the results of PCR showed the polymorphism of Mbl2 showing the presence of A and B alleles and three genotypes (AA, AB, and BB). Figure (1), significant difference in AB, frequency of the BB genotypes between patient and control groups (P-value = 0.013,0.003 respectively) where AB genotype was found in 18% and BB genotype was found in 14% of the group of patients, while AB genotype was found in 4% and BB genotype was found in 0% of the group of controls.

The B allele frequency (i.e., the AB, BB genotypes) increased significantly in the group of patients compared with control group, where two (2.04 percent) of the control compared twenty-three (23 percent) of the patient as shown in table (1). The product of IFN-like +874 PCR amplification as shown in Figure (2). In this study, the results were found as follows: 13(36%) had AA genotype, 19(38%) had AT genotype, and 13(26%) had Cytomegalovirus (CMV patients) TT genotype, when compared to control group 8(16%) with AA genotype, 20(40%) had AT genotype, and 22 (44%) had TT genotype, as shown in table (2).

There is a significant difference between patient and control group in TT genotype (p=0.01), whereas there was no significant difference between patient and control group in AA, AT genotype, and Allel A (p=0.005) is significant.

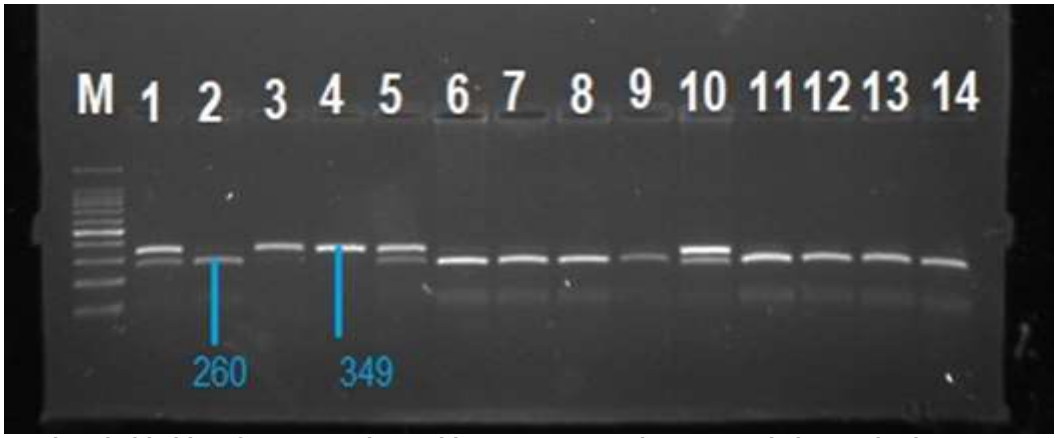

Fig. 1: M: (100-bp) ladder of DNA, Lane (1-5-10) heterozygote AB (349,260,89 bp). Lane (3-4) Homozygotes BB (349bp). Lane (2,6,7,8,9,11,12,13,14) Homozygotes AA (260,89 bp)

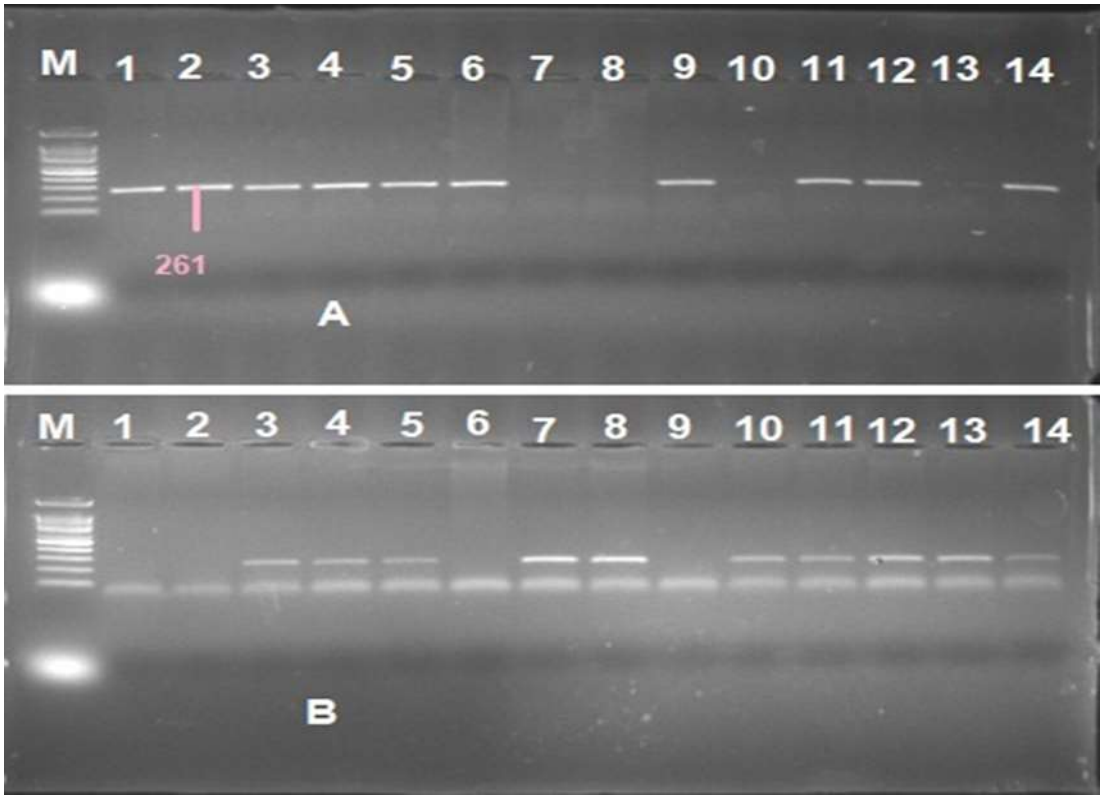

Fig. 2: M: (100-bp) ladder of DNA,, Lane (1-2-6-9) genotype A, Lane (7-8-10-13) genotype T, Lane (3-4-5-11-12-14) genotype AT. (size product 261bp)

Table 1: Allele and genotype frequencies of Mbl2 gene polymorphism among Cytomegalovirus positive patients and healthy (uninfected controls)

Genotype Mbl	Patients	Control	P Value	OR=(95%CI)
AA[a]	34(68%)	48(96%)		
AB	9(18%)	2 (4%)	0.013*	0.15(0.03-0.77)
BB	7(14%)	0(0%)	0.003*	0.41 (0.32- 0.53)
Total	50	50		
Allele				
A	77	96	<0.0001*	0.07(0.01-0.30)
B	23	2		

$P \leq 0.05$; OR=(95%CI); [a] reference

Table 2: IFN allele and genotype frequencies +874 gene polymorphism in active and stable patients with cytomegalovirus (uninfected controls)

Genotype Inf gamma	Patients	Control	P Value	OR=(95%CI)
AA[a]	18(36%)	8(16%)		
AT	19(38%)	20(40%)	0.08	2.36(0.83-6.72)
TT	13(26%)	22(44%)	0.01*	3.80 (1.29- 11.19)
Total	50	50		
Allele				
A	55	36	0.005*	2.17(1.23-3.83)
T	45	64		

$P \leq 0.05$; OR=(95%CI); [a] reference

DISCUSSION

There are many of genetic polymorphisms were associated to elevated risk of infection with CMV. In this study, the effect of polymorphisms of IFN-ɣand MBL2 was assessed in 100 samples (50 cases of CMV-infection (CMV+)) & (50 control samples (CMV-)) (13). Mannose-binding lectin (MBL) regarding essential component for innate immune system of human, which can bind to a big range of pathogens including, herpes simplex 2, influenza A, HIV, SARS-CoV.

Exon 1 has MBL deficiency with single nucleotide polymorphisms (SNPs), And a number of infections have been reported as associated with the MBL2 gene of human promoter region (15). mutations in Exon 1 of the MBL2 gene appeared differ in different populations(16). According to the present study the codon 54 (B allele) mutation of the MBL2 gene was associated with CMV infection. Compared to other studies, the 2011(17) and 2009(18) study by Rooij et.al and Cervera et.al suggests that MBL2 independently increased the risk of CMV, where B allele had a risk association with HCMV in this study. There are association between A allele and elevated risk of CMV infection in IFN-ɣ(+874 A > T). While TT genotype have association to viral clearance of CMV, those results matched D's previous study. Vu et.al 2014 and (17).In another study it was found that the genotype IFN-ɣ(+874 A > T) TT is associated with high IFN-c production of and low production may bind with genotype AA. Previously, allele of IFN-ɣ+874 A has been noted had relationship with various infections, including tuberculosis, human papillomavirus-induced cervical cancer, parvovirus B19 infection, Hbs virus infection, HIV / immunodeficiency syndrome infection, SARS infection, BK virus, revealing its potential function (17).

CONCLUSION

Increase of the Mbl2 gene polymorphism, AB and BB genotype in Cytomegalovirus infected patients showing that the mutant allele is known to be a risk factor for CMV infection, while the AA genotype has been increased in control uninfected community regarded as an infection-protective factor. According to IFN-ɣgene polymorphism, the A allele is substantially associated with an increased risk of CMV infection while the TT genotype is associated with CMV viral clearance.

ACKNOWLEDGMENT

We would like to thank all those who supported me and all the people who helped to finish this job.

CONFLICT OF INTEREST

None

REFERENCES

1. Yu X, Shah S, Lee M, Dai W, Lo P, Britt W, Zhou ZH. Biochemical and structural characterization of the capsid-bound tegument proteins of human cytomegalovirus. *Journal of structural biology* 2011; 174(3): 451-460.
2. La Rosa C, Diamond DJ. The immune response to human CMV. *Future virology* 2012; 7(3): 279-293.
3. A Ross S, Novak Z, Pati S, B Boppana S. Overview of the diagnosis of cytomegalovirus infection. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)* 2011; 11(5): 466-474.
4. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. *Clinical microbiology reviews* 2009; 22(1): 76-98.
5. Badami KG. CMV and transfusions, an old story that's not quite over yet. *International Journal of Clinical Transfusion Medicine* 2014; 2: 7-19.
6. Geist LJ, Hinde SL. Susceptibility to cytomegalovirus infection may be dependent on the cytokine response to the virus. *Journal of Investigative Medicine* 2001; 49(5): 434-441.
7. Avdic S, McSharry BP, Steain M, Poole E, Sinclair J, Abendroth A, Slobedman B. Human cytomegalovirus-encoded human interleukin-10 (IL-10) homolog amplifies its immunomodulatory potential by upregulating human IL-10 in monocytes. *Journal of Virology* 2016; 90(8): 3819-3827.
8. Sarvari J, Norozian H, Fattahi MR, Pirbonyeh N, Moattari A. The role of interferon gamma gene polymorphism (+874A/T,+2109A/G, and-183G/T) in response to treatment among hepatitis C infected patients in Fars Province, Southern Iran. *Hepatitis monthly* 2014; 14(1): e14476.
9. Pollard KM, Cauvi DM, Toomey CB, Morris KV, Kono DH. Interferon-γ and systemic autoimmunity. *Discovery medicine* 2013; 16(87): 123-131.
10. Popadic D, Savic E, Spuran Z, Markovic M, Stojkovic MM, Ramic Z, Pravica V. Distinctive Frequencies of+ 874T/A IFN-γ Gene Polymorphism in a Healthy Serbian Population. *Clinical and translational science* 2012; 5(6): 461-463.
11. Lo RS, Austin AS, Freeman JG. Association of Mannose-Binding Lectin Gene Polymorphisms with Liver Diseases: A Review. *Open Medicine Journal* 2018; 5(1): 39-46.
12. De Morais VMS, De Lima ELS, Cahú GGDOM, Lopes TRR, Gonçales JP, Muniz MTC, Coêlho MRCD. MBL2 gene polymorphisms in HHV-8 infection in people living with HIV/AIDS. *Retrovirology* 2018; 15(1): 75.
13. Vu D, Shah T, Ansari J, Sakharkar P, Yasir Q, Naraghi R, Min D. Interferon-gamma gene polymorphism+ 874 A/T is associated with an increased risk of cytomegalovirus infection among Hispanic renal transplant recipients. *Transplant Infectious Disease* 2014; 16(5): 724-732.
14. Hammad NM, El Badawy NE, Nasr AM, Ghramh HA, Al Kady LM. Mannose-binding lectin gene polymorphism and its association with susceptibility to recurrent vulvovaginal candidiasis. *BioMed research international* 2018.
15. Gelemanović A, Dobberpuhl K, Krakar G, Pataržić I, Kolčić I, Polašek O. Host genetics and susceptibility to

congenital and childhood cytomegalovirus infection: a systematic review. *Croatian medical journal* 2016; 57(4): 321-330.

16. Hu Y, Wu D, Tao R, Shang S. Association between mannose-binding lectin gene polymorphism and pediatric cytomegalovirus infection. *Viral immunology* 2010; 23(4): 443-447.
17. Mitsani D, Nguyen MH, Girnita DM, Spichty K, Kwak EJ, Silveira FP, Abdel-Massih R. A polymorphism linked to elevated levels of interferon-γ is associated with an increased risk of cytomegalovirus disease among Caucasian lung transplant recipients at a single center. *The Journal of Heart and Lung Transplantation* 2011; 30(5): 523-529. http://dx.doi.org/10.1016/j.healun.2010.11.008
18. Cervera C, Lozano F, Linares L, Antón A, Balderramo D, Suárez B, Navasa M. Influence of mannose-binding lectin gene polymorphisms on the invasiveness of cytomegalovirus disease after solid organ transplantation. *In Transplantation proceedings*, *Elsevier,* 2009; 41(6): 2259-2261. http://dx.doi.org/10.1016/j.transproceed.2009.06.056

Cite this article: Israa Adnan Ibraheam Interferon Gamma and Mannose Binding Protein: Role in Susceptibility to Cytomegalovirus Infection. J. Cardiovascular Disease Res., 2020; 11 (4): 205 – 209